

EVALUATION OF MUSCLE METABOLISM IN INDIVIDUALS WITH SPINAL CORD INJURY

by

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(Under the Direction of Kevin McCully)

ABSTRACT

Individuals with a spinal cord injury (SCI) are at risk of cardiovascular disease and diabetes. Impaired muscle oxidative metabolism could contribute to disease risk. The purpose of this study was to evaluate oxidative muscle metabolism in the legs of people after spinal cord injury using the rate of phosphocreatine (PCr) resynthesis after exercise. A second purpose was to evaluate PCr recovery rate following voluntary exercise and electrical stimulation in able-bodied subjects. PCr resynthesis was measured after electrical stimulation or exercise using ^{31}P magnetic resonance spectroscopy with a 10 cm surface coil placed over the right vastus lateralis in a 3 Tesla magnetic resonance spectrometer. Subjects with SCI were electrically stimulated for 60 seconds at 4 Hz. The able-bodied subjects first performed a 39-second maximal voluntary isometric contraction and electrical stimulation at 4 Hz for 60-90 seconds (113). PCr recovery rate was 42.7 seconds mean (SD) and $82.4 \pm (30.6)$ seconds for AB and SCI subjects, respectively. In AB subjects, PCr recovery rate was $25.9 \pm (4.7)$ seconds following voluntary exercise. In conclusion, mitochondrial capacity in paralyzed muscle as measured by PCr recovery rates was about half of the able-bodied subjects. This decrease in mitochondrial capacity may play a role in the decline in insulin sensitivity in people with SCI.

KEY WORDS: Phosphocreatine, spinal cord injury, maximal voluntary isometric contraction

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CHAPTER I

INTRODUCTION

Spinal cord injury is a lifelong condition that currently affects 250,000-400,000 individuals in America (65). The patterns in causes of death have changed over time with increasing survival. Within the first year, respiratory illnesses were the most common cause of death (34, 85, 102). Within the first few years after injury, urinary deaths rank first, heart disease deaths second, and respiratory-related deaths third. As the years go on, this order changes (34). Now, persons with SCI have an increased prevalence and experience an earlier onset of diabetes mellitus and cardiovascular disease.

Although there have been improvements in medical care that allow persons with SCI to live longer lives, the mortality rates are still elevated above the able-bodied population (37). Lifestyle changes could potentially prevent or delay the onset of diabetes in persons with SCI. Because of this increased risk of diabetes and cardiovascular disease, it is important to look at the modifiable risk factors for areas to reduce mortality such as obesity, inactivity, diet, and blood pressure (65).

Because of the restrictions associated with a sedentary lifestyle, individuals with SCI become highly deconditioned. Their paralysis and immobilization may cause individuals with SCI to experience disorders of carbohydrate metabolism and dyslipidemia. Bauman et al (6) reported that individuals with SCI and the greatest neurological impairments have the worst carbohydrate tolerance and highest insulin values. These disorders can be associated with a loss of lean body tissue and a gain in adipose tissue (7). The decrease in total muscle mass negatively affects the health of an individual because of the metabolic changes that occur.

After the denervation of skeletal muscle, it has been shown that insulin resistance occurs (7). There is an increase in plasma glucose levels making glucose intolerance higher in individuals with SCI. Bauman et al (7) reported that there is a reduction in whole body glucose transport that changes in proportion to muscle mass. The glucose transport system is, however, still intact and functional (7).

The denervation of a skeletal muscle causes the cell to go through many other changes as well (7). For example, muscle fiber cross-sectional area has been shown to decline 40-50% after injury (27, 87). Not only is there a decrease in cross-sectional area but an increase in intramuscular fat (IMF) (32). IMF is a good predictor of plasma glucose and may contribute to the onset of impaired glucose tolerance and type II diabetes, especially in SCI (32). There is also a reduction in the activities of metabolic enzymes with a shift towards fast-glycolytic fiber characteristics. There appears to be an increase in Type IIb skeletal muscle fiber types and a decreased proportion of slow oxidative type I fibers (69). Type IIb fibers are less sensitive to insulin action and have a reduced glucose uptake due to reduced capillary density. One of the indicators of oxidative metabolism potential of a fiber is the number of capillaries surrounding a muscle fiber. Martin et al (69) reported that the capillary-to-fiber ratio is significantly lower in persons with SCI (1.7) than able-bodied individuals (2.6).

Spinal Cord Injury (SCI) may lead to a decreased mitochondrial function (46, 66, 69). Succinate Dehydrogenase (SDH) activity has been reported to be 47-68% below those observed in able-bodied population (69). Greater fatigue can be partially attributed to these lower levels of metabolic enzymes. Castro et al (27) found that there was only about a 10% reduction in SDH values in the muscle fibers of a person with SCI within 6 months after injury. It is possible that these reductions in metabolic enzymes leads to insulin resistance, sarcopenia, and exercise

intolerance (68). The negative effects of decreased mitochondrial function might play an important role in the development of cardiovascular and metabolic disease. Mitochondria dysfunction, the reduced function of existing mitochondria, can lead to the impairment of ATP production. The reduction of ATP production leads to a loss of oxidative metabolism. Assessing mitochondrial function, however, has been very difficult in persons with SCI.

Muscle metabolism has been measured using fatigue tests. However, these tests may be misleading and inaccurate, particularly in people with SCI. This is because it can be difficult to separate the decline in force with fatigue from the decline in force from muscle damage. Muscle fatigue is defined as a reduction in force induced by exercise and caused by metabolic byproducts. Muscle damage is due to structural changes in the muscle fiber, not due to metabolic byproducts. Skeletal muscle after SCI becomes highly susceptible to fatigue (15). The higher levels of muscle fatigue might be due to injury not muscular metabolism (67). Muscle injury contributes to the high levels of fatigue during a single bout of isometric exercises. During bouts of EMS separated by a minute, muscle torque fails to recover between the bouts in subjects with SCI. The unloading and long-term inactivity of the muscles in persons with SCI resulted in an increased susceptibility to contraction induced muscle injury (13, 67). There also appeared to be a greater relative area of muscle with increased T2 relaxation times. T2 images may reflect the edema associated with muscle damage. An increase in plasma Creatine Kinase values has also been seen in patients with SCI after a single bout of exercise (13, 87, 101). Training using electrical stimulation resulted in a 60% reduction in muscle fatigue in people with SCI, but again, it is not clear if this is due to increased fatigue resistance or increased resistance to muscle damage (93).

Traditionally, oxidative capacity has been measured using the *in vitro* analysis of muscle biopsies (14, 27, 30, 46, 78, 97). Percutaneous biopsy technique is an important and acceptable technique in the study of conditions involving skeletal muscle (14). This is an invasive procedure where muscle samples are taken by a needle from the muscle of interest. These biopsies are analyzed for cross sectional area of a fiber type, fiber type percent, capillary density, and oxidative enzymes such as SDH. Not only are these biopsies invasive, but also they do not necessarily give an accurate representation of *in vivo* capacities.

Magnetic resonance spectroscopy (MRS) has been used as a noninvasive method for measuring muscle oxidative metabolism (71-76, 78, 80, 83). Phosphocreatine recovery kinetics is a way to quantify muscle metabolism. Muscle metabolism can be quantified as the maximal rate of adenosine triphosphate (ATP) production stimulated by the demands of the work placed on the muscle. To restore the supply of ATP during exercise, cytoplasmic adenosine diphosphate (ADP) increases and PCr is used. The creatine is then diffused to the mitochondria where it is phosphorylated to PCr by mitochondrial ATP. ATP concentrations remain steady due to the rapid rate of this process catalyzed by the enzyme creatine kinase. With the synthesis of PCr from creatine and phosphate, mitochondrial ATP from ADP is in equilibrium. Mitochondrial capacity can be represented by the time constant of PCr recovery. McCully et al (18) found that there was a significant correlation between *in vivo* and *in vitro* measurements of oxidative capacity in able bodied humans.

A potential complication of using PCr levels to indicate changes in oxidative metabolism is the influence of muscle pH on the creatine kinase equilibrium. As the intensity of exercise increases, there is a greater change in pH (109). A decrease in pH will result in the Pi peak shifting closer to PCr since the Pi peak position is pH dependent. A drop in muscle pH,

associated with accumulation of lactate or H^+ , can slow the process of PCr recovery, independent of changes in ATP synthesis after exercise. In turn, this may lower measured oxidative capacity (80).

MRS has been an effective way to measure PCr recovery. The ability to measure mitochondrial function in vivo provides an advantage in that along with the assessment of mitochondrial function, it can measure the energetic state of the cell. ATP, ADP and pH are all possible measurements to investigate (68). There is one published report that looked at muscle metabolism in vivo using the rate of phosphocreatine (PCr) recovery in persons with SCI (66). It was a very small sample ($n=3$) in which they observed PCr in individuals with SCI was slow to recover after exercise. PCr was depleted using a fatigue protocol that began with rest, 3 minutes of tetanic unilateral contractions, and a minimum of 40 minutes of recovery. These individuals with SCI reached their steady state after exercise between 35 and 40 minutes. A limitation to this study was that pH was not controlled, and end stimulation pH values reached ~ 6.2 , so it is not clear how much oxidative metabolism was reduced in this study. Because of this, better controlled and well powered studies are needed to assess mitochondrial function using ^{31}P MRS in people who have SCI. It would be advantageous to have a procedure, in vivo, that accurately measures mitochondrial function in patients with SCI using electrical stimulation.

Purpose:

The general aim of this study is to increase our understanding of how SCI influences muscle oxidative metabolism, in particular in individuals who are healthy, as well as in those who are severely deconditioned. This will be accomplished by measuring the rate of phosphocreatine resynthesis after exercise in individuals with SCI and able-bodied controls. This study will determine the extent of mitochondrial function after a spinal cord injury. Determining

the mitochondrial function of persons with SCI may help to recognize the need for exercise and contribute to the development of exercise programs to diminish the high prevalence rate of metabolic and cardiovascular diseases.

Specific Aims

1. Measure muscle metabolism after electrical stimulation in individuals with SCI and able-bodied controls
2. Compare PCr recovery rates after electrical stimulation and after voluntary exercise in able-bodied people

Hypotheses

1. Phosphocreatine recovery rates will be slower in individuals with SCI compared to the able-bodied controls indicating reduced mitochondrial function.
2. Phosphocreatine recovery rates from electrical stimulation would not be different from PCr recovery after voluntary contractions in able-bodied control subjects

Significance of the Study

The significance of the study is to contribute to the development of future studies which include the relationship between mitochondrial function and diabetes and heart disease. Also, the determination of how exercise programs can alter mitochondrial function in people with SCI.

CHAPTER II

REVIEW OF LITERATURE

SCI

Spinal Cord Injury is damage to the spinal cord that results in a loss of motor and/or sensory function. Approximately 250,000 people live with SCI in the United States. There are about 10,000 new SCI's every year; the majority of them, 80.9%, involve males and 19.1% females between the ages of 16 and 30(1). Catz et al (29) reported that the cause of injury is a road accident at 32.8%, work accident at 26.8%, fall from a height at 16.8%, suicide attempt at 13.6%, and others at 10.4%.

Throughout the past several decades, the life of individuals with SCI has improved but is still less than the able-bodied population (103). Morbidity and mortality that is associated with SCI is not necessarily due to the neurological deficit but to the complications it brings about (29). Persons with SCI have an increased prevalence of diabetes mellitus and cardiovascular disease. Cardiovascular disease now exceeds renal and pulmonary conditions for those with long-term SCI. They also have a greater risk for obesity, lipid disorders, metabolic syndrome, and diabetes to occur prematurely and at a higher prevalence (4, 85, 103, 104). Studies have reported the rate of diabetes in individuals with SCI to be from 13% to 22%, about three times ($RR=2.62$) that of the able-bodied population (9, 65). Garshick et al (37) reported that cardiovascular disease is almost four times more prevalent in persons with SCI than able-bodied ($RR=3.66$). Cardiovascular disease and diabetes are related to the individuals' physical activity level, the level of the spinal cord lesion, and time post injury (60).

Body Composition and SCI

Within the first 6 months post spinal cord injury, their body composition deteriorates markedly (3, 24-26, 103, 111). Leg lean tissue mass can be decreased by up to 15% and total body lean tissue mass can be decreased to about 9.5% (3, 103, 111). Due to their immobility and reduction of forces of gravity, individuals with SCI experience rapid bone loss (10, 36, 52, 100, 103). The most predominant finding is the large loss of bone during their first year due to disuse (5, 103). The bone mineral content is dependent on the level, completeness, and duration of SCI. It's been reported that the bone mineral content is decreased by 25 to 50% (60, 103).

According to Kocina et al (60), men and women with SCI who are physically active have above average fat mass, 16 to 24% for men and 24 to 32% for women, and sedentary SCI individuals have 'at risk' levels of body fat, above 25% for men and 32% for women.

Centrally located adipose tissue has been associated with heightened risk for CVD and type 2 diabetes. They have higher prevalence for abdominal obesity, which predisposes them to increased risks for diabetes and CVD compared with the general population (6, 9, 35, 60, 61, 88).

Skeletal muscle atrophy is associated with greater IMF accumulation in individuals with SCI. Intramuscular fat continues to increase over time (45). It has been reported that within the first 6 weeks of injury, thigh cross sectional area is at least 33% smaller in the SCI group compared to matched controls. Intramuscular fat was 126% greater in the SCI group when compared to the controls (16, 32). Although 3 months post injury thigh cross sectional area did not change significantly, intramuscular fat increased another 26% in the SCI individuals (16, 45).

Fatigue and SCI

There is a negative correlation between fatigue resistance and time since injury (40). Following a spinal cord injury, skeletal muscles become less resistant to fatigue. This decrease in

the resistance to fatigue leads to a reduction in torque and during repetitive activation the contractile speeds of muscle twitch and tetanus are significantly slower (22, 38, 39, 42, 92, 98, 99). Gerritis et al (22) reported that there is a force decline in paralyzed muscle of 60% and only 15% in the muscles of the control individuals. The most widely supported explanation for the high levels of fatigue observed in individuals with SCI has been attributed to the increased proportion of fast-twitch fibers. Fast twitch fibers have greater ATP turnover rates compared to slow twitch fibers. “Slow to fast” fiber conversion has been shown to occur after 1-2 years post-injury with increased expression of myosin heavy chain IIa and IIx (21, 41). Burnham et al (21) reported that a near complete conversion from ‘slow to fast’ muscle occurs over a period of approximately 70 months post-injury. Muscle biopsies from 2 to 11 years post-injury indicate significantly less slow-twitch fibers than controls (69% vs. 14%, respectively) and smaller mean fiber cross-sectional area (69).

Along with fiber type change, studies have demonstrated reduced oxidative enzyme activities (46, 69). Over time, the paralyzed skeletal muscle contains smaller fibers that have a greater energy demand (greater contractile speed) and a lower capacity to supply it, leading to greater muscle fatigue. These factors likely account for some of the difference in fatigability between SCI and able-bodied, with other factors possibly including altered calcium handling and/or muscle injury occurring during the onset of muscular contractions.

Biopsy and Muscle Metabolism

A common method to measure muscle metabolism is taking a muscle biopsy from the muscle of interest using the percutaneous biopsy technique (25, 28, 58). Biopsies are taken to determine fiber CSA, fiber type percent, aerobic and anaerobic enzyme activity, and capillarity (25). Aerobic and anaerobic enzyme activities are determined using Succinate dehydrogenase

(SDH) and α -glycerophosphate dehydrogenase (GPDH) respectively. Castro et al (26) reported that within the first six months of spinal cord injury there is no decline in SDH activity. SDH activity was maintained in relation to muscle fiber volume. The average muscle fiber cross sectional area for individuals with SCI at six months was about one-third that of able bodied controls. The average fiber SDH activity was about 10% lower. Longer periods of inactivation after SCI have shown a reduction in SDH. Martin et al (69) showed a 48-67% lower SDH activity per unit fiber volume. Bogdanis et al (19) reported that PCr was depleted by over 80% in the vastus lateralis after maximal cycling for 30 s by using muscle biopsies.

MRS and PCr Recovery Kinetics

³¹P-NMR uses a Radio Frequency (RF) coil that emits a radio pulse at a frequency that is absorbed by the corresponding phosphorus containing compounds within the muscle. These phosphorus atoms then transmit the radio frequency pulse back to the RF coil. This conveys a phosphorus spectrum based on the metabolic state of the muscle. NMR can only detect metabolites that are free in solution, not bound compounds. The area of each peak represents concentration of specific phosphorus atoms which can then be used to infer the concentration of the compounds that contain these atoms. In skeletal muscle there are five distinguishable peaks in the spectra. They are from right to left, three ATP peaks (gamma, alpha, beta), phosphocreatine (PCr), phosphodiester (44), and inorganic phosphate (Pi). Quantification assumes that skeletal muscle ATP concentration is 8.2 mmol/L of intracellular water (109). Calculation of ADP levels assume that total creatine is 42.5 mmol/L of intracellular water, PCr plus Pi equals 42.2 mmol/L of intracellular water, these components are equally distributed and there is no change in total creatine during exercise (107). As the quality of ³¹P MRS measurements improved, the ability to measure the kinetics of muscle metabolism in vivo also

improved (84). Many studies have used MRS as an in vivo method to evaluate PCr recovery kinetics (2, 70, 106, 107). Researchers began to use this method for different modes of exercise with different populations. Studies include isometric, eccentric, and concentric movements in subjects who are physically conditioned (62, 63, 86, 105) have disease (53, 55) or are sedentary (112).

Exercise and PCr

The control of mitochondrial function in skeletal muscle in vivo is obtained from the relationship between the rate of mitochondrial oxidation and the concentration of phosphorous metabolites. The creatine kinase reaction must be at equilibrium. PCr is used to phosphorylate ATP. The rate in which PCr is broken down is dependent on the intensity of the muscular contraction. There is a strong relationship between a decrease in force and a decrease in PCr during high intensity exercise (19, 20). Measuring metabolite levels during steady state exercise was the initial way studies of muscle metabolism using ^{31}P MRS were done. This was because of the low signal to noise values obtained from low field strength magnets required 1-5 minutes to obtain adequate signals. These studies did show relationships between the metabolic responses to exercise and disease status, training status, and age. The recovery kinetics of ADP and PCr imply that the rate of PCr resynthesis has a hyperbolic dependence on the concentration of ADP (54). The time constant (τ) or rate constant ($1/\tau$) of PCr recovery is used as a measure of oxidative capacity in skeletal muscle (33, 48, 57, 59, 77). The rate constant of phosphocreatine recovery is directly related to maximal citrate synthase activity (33, 78, 89). These findings show that mitochondrial content is directly related to the time course of PCr breakdown at the onset of submaximal exercise and PCr resynthesis following exercise (84). There have been many studies that have shown that endurance athletes have a faster PCr recovery kinetics than sprinters and

untrained individuals (31, 33, 64, 83, 105). Endurance training has been shown to reduce PCr recovery (81) and increase biochemical markers of oxidative capacity (43). The recovery time of PCr is also faster after short-term high-intensity interval training (33).

pH and PCr

The influence of muscle pH and glycolysis is a key confounding factor in the use of phosphorous metabolites to measure oxidative metabolism. During the resynthesis of PCr, hydrogen ions are released. This causes a change in muscle pH that will shift the creatine kinase equilibrium. This is the equation: $\text{PCr} + \text{MgADP}^- + \text{H}^+ \rightleftharpoons \text{MgATP}_2^- + \text{creatine}$. The high H^+ concentration seen with a decrease in pH affects PCr by reducing the apparent rate of resynthesis. This means that metabolite levels such as PCr measured during exercise will be sensitive to both the amount of mitochondria and the production of hydrogen ions due to glycolysis. Research utilizing muscle biopsies were among the first to quantify the relationship between pH and the CK reaction. Salhin et al (95) found that there was a significant correlation of $r=0.92$ ($p<0.01$, $n=34$) between pH in the muscle and the creatine kinase equilibrium. The oxygen availability affects the initial phase of PCr recovery and pH affects the later stages of recovery. With the development of MRS there have been many more studies that have quantified the effects of pH on PCr recovery. Changes in pH are represented by a shift in the Pi peak. Its position is dependent on the pH of the muscle. Pi is present in two forms, HPO_4^{-1} and H_2PO_4 . Since the Pi peak position is pH dependent, a decrease in pH will result in the Pi peak shifting closer to PCr on the MRS spectra. One of the first to examine this was Arnold et al (2) who found that PCr resynthesis was slower following heavy exercise. It was later found that the extent of acidosis at the end of exercise is what determines the rate of resynthesis (11). At the end of exercise glycolysis has stopped, yet pH continues to fall due to the release of protons

during the resynthesis of PCr. Numerous studies have found that pH is a factor in the biphasic recovery of PCr due to the influence of the CK equilibrium (2, 47, 94, 95, 106, 107).

Fiber Types and MRS

There have been a few studies that have shown PCr is resynthesized faster in slow twitch versus fast twitch muscle fibers (23, 51, 108). It has been thought that the faster recovery was due to an increase in capillaries, increased mitochondrial density, and higher oxidative enzyme activity. In the vastus lateralis, the fast twitch fibers are located closer to the surface, while slow twitch fibers are deeper in the muscle. In a study by McCully et al (78), during high intensity exercise, fast twitch glycolytic muscle fibers become acidotic after thirty seconds of exercise. The pH values reached as low as 6.3 after one minute. Slow twitch oxidative muscles have pH values of 6.9 at the end of a minute of exercise. The exchange of H^+ between muscle fibers is relatively slow. Within a muscle during maximal exercise, the different types of muscle fibers activated result in fibers having different pH values and Pi peaks with different frequencies. Subjects with mixed fiber populations will have multiple Pi peaks and the relative area of the different peaks will give an estimate to the proportion of fiber types (12, 90).

CHAPTER III

EVALUATION OF MUSCLE METABOLISM IN INDIVIDUALS WITH SPINAL CORD INJURY¹

¹Tara Mulcahy, Qun Zhao, Kevin McCully. To be submitted to *Spinal Cord*

Abstract

Individuals with a spinal cord injury (SCI) are at risk of cardiovascular disease and diabetes. Impaired muscle oxidative metabolism could contribute to disease risk. The purpose of this study was to evaluate oxidative muscle metabolism in the legs of people after spinal cord injury using the rate of phosphocreatine (PCr) resynthesis after exercise. A second purpose was to evaluate PCr recovery rate following voluntary exercise and electrical stimulation in able-bodied subjects. PCr resynthesis was measured after electrical stimulation or exercise using ^{31}P magnetic resonance spectroscopy with a 10 cm surface coil placed over the right vastus lateralis in a 3 Tesla magnetic resonance spectrometer. Subjects with SCI were electrically stimulated for 60 seconds at 4 Hz. The able-bodied (AB) subjects first performed a 39-second maximal voluntary isometric contraction (MVIC) and electrical stimulation at 4 Hz for 60-90 seconds. Each exercise bout was separated by ~8 min to allow for PCr recovery to be completed. After electrical stimulation, PCr recovery rate was $42.7 \pm (8.6)$, seconds mean (SD) and $82.4 \pm (30.6)$ seconds for AB and SCI subjects, respectfully. In AB subjects, PCr recovery rate was $25.9 \pm (4.7)$ seconds following voluntary exercise. The PCr recovery rate with electrical stimulation was significantly higher than MVIC ($p < 0.001$). Using electrical stimulation, the PCr recovery rates were slower in individuals with SCI compared to able-bodied individuals, $p < 0.001$. In conclusion, mitochondrial capacity in paralyzed muscle as measured by PCr recovery rates was about half of the able-bodied subjects. This decrease in mitochondrial capacity may play a role in the decline in insulin sensitivity in people with SCI.

Introduction

The number of individuals who have a spinal cord injury in the United States is approximately 259,000 persons (1, 4). Individuals with spinal cord injury have a higher risk for insulin resistance, diabetes, metabolic syndrome, and cardiovascular disease (4, 8, 49). Heart disease and diabetes are predictors of mortality in individuals with SCI (4, 37). Because of the restrictions associated with a sedentary lifestyle, individuals with SCI become deconditioned. After 1 year of injury, individuals with SCI have a higher prevalence for abdominal obesity, intramuscular fat accumulation, a decrease in muscle fiber cross-sectional area, a shift towards fast glycolytic type II fibers, reduced capillary density, and decreased mitochondrial function. Decreased mitochondrial function might play a role in the development of these diseases.

Magnetic resonance spectroscopy (MRS) has been developed as a noninvasive technique for measuring muscle oxidative capacity. The MRS approach to measuring oxidative capacity is the rate constant of PCr resynthesis after submaximal exercise. Phosphocreatine recovery kinetics have been measured using ^{31}P magnetic resonance spectroscopy (66) in various subject populations, and muscle types with differing exercise intensities (17, 48, 50, 56, 70, 80, 81, 83, 109). These studies often examine recovery kinetics in the calf muscles and use an in magnet ergometer to deplete phosphocreatine (PCr). Ergometers, although sufficient to deplete PCr, can be a major limiting factor in performed PCr recovery tests. Restrictions of the magnet place certain size and magnetic limitations on the ergometers, which often require that each ergometer be custom built. The ability to perform PCr recovery tests without the use of an ergometer was extremely beneficial.

There has only been one study to use in vivo ^{31}P MRS to measure PCr in paraplegics and there were only 3 subjects tested (66). It would be advantageous to have a procedure, in vivo,

that accurately measures mitochondrial function in patients with SCI using electrical stimulation.

The purpose of this study was to measure the rate of phosphocreatine resynthesis after exercise in individuals with SCI and able-bodied controls to determine the extent of mitochondrial function after a spinal cord injury. It was hypothesized that a maximal voluntary isometric contraction would produce the same recovery rate of electrical stimulation and that individuals with SCI would have the same recovery rate as sedentary control subject.

Methods

Subjects

Individuals with spinal cord injury were classified as ASIA-A. The control group consisted of healthy able-bodied individuals. SCI subjects were recruited as part of a larger study. The study was conducted with the approval of the Institutional Review Board at the University of Georgia and all subjects provided written informed consent.

Procedure

This is a cross sectional study on two groups of subjects. Each subject was only tested on one occasion.

MRS Measurements

Subjects were tested in a 3 Tesla whole body magnet (GE Healthcare, Waukesha, WI). A ^1H and ^{31}P dual tuned radio-frequency (RF) surface coil (Clinical MR Solutions, Brookfield, WI.) was placed over the vastus lateralis of the subject's right leg. Manual shimming on ^1H was applied to get a better signal-to-noise ratio (SNR) and less spectrum distortion, after an auto-shimming by a pre-scan sequence. A free induction decay (FID) chemical shift imaging (CSI) pulse sequence was applied to acquire the ^{31}P spectrum. Resting spectra were acquired every 3 seconds until 120 scans were taken. The resulting spectra were zero filled (from 2048 to 6144

points) phased and averaged in a custom analysis program (Winspa, Ronald Meyer, Michigan State University). The area under the curve for each peak (Pi, PDE, PCr, α ATP, β -ATP, and γ -ATP) was determined using integration.

Exercise Protocol

Four foil electrodes were placed apart on the vastus lateralis. The electrodes were tested on the subject and placed back in the magnet to be stimulated twice. The first minute was that of rest. The next 60 to 90 seconds were of electrically stimulated isometric contractions. The frequency was set at 4 Hz and the intensity was adjusted to each individual for proper depletion of PCr. Current was set to produce a ‘vigorous twitch’ that appeared to be maximal in the people with SCI, was set to maximal tolerable levels in able bodied people. The final six minutes were used to measure the rate of recovery.

Maximal voluntary isometric contraction (MVIC)

The able-bodied individuals will perform two maximal voluntary isometric contractions (MVIC) following each stimulation. This consists of a minute of rest followed by a 39 second duration MVIC, with vocal encouragement, to deplete PCr. The subjects remained as still as possible for six minutes after while recovery data was collected.

ATP Concentrations

Resting spectra were acquired every 3 seconds until 120 scans are taken. The resulting spectra were phased and averaged in a custom analysis program (Winspa, Ronald Meyer, Michigan State University). The area under the curve for each peak (Pi, PDE, PCr, α ATP, β ATP, and γ ATP) was determined using integration. pH was calculated using the following equation (82) :

$$\text{pH}=6.77+\log((\text{Pishift}-3.27)/(5.68-\text{Pishift}))$$

Phosphocreatine peaks were determined from the peak heights from individual spectra. PCr peak heights during recovery after exercise were fit to an exponential curve:

$$PCr = End - \Delta * \exp(-Time/T_c)$$

Statistical Analysis

A two-group unpaired t-test was conducted to compare the findings of the patients with SCI and the able-bodied individuals. A two-group paired t-test will be used to analyze the difference between the electrically stimulated able-bodied individuals and the MVIC performed by the able-bodied individuals. A p value of 0.05 was used for tests of significance.

Results

All subjects were able to complete the studies with no adverse events. All subjects showed sufficient muscle activation with electrical stimulation and had enough muscle mass to provide adequate signal quality for ^{31}P MRS. All able-bodied subjects were capable of performing multiple isometric contractions of the quadriceps muscle for thirty-nine seconds each. The physical characteristics of the subjects are shown in Table 3.1.

Resting

Representative resting spectra are shown in Figure 3.1. Resting metabolites are shown in Table 3.2. There were no differences found for the phosphorous metabolite ratios during rest for able-bodied individuals, except that the phosphodiester peak was significantly higher in individuals with SCI, $p = 0.03$. One individual with SCI did have very high Pi/PCr, 0.384, and low PCr/ATP ratios, 3.399.

Voluntary versus electrical stimulation exercise in able bodied

End exercise metabolic values are shown in table 3.3. There was statistical significance, $p < 0.001$, between a maximal voluntary contraction and electrical stimulation. The average T_c

recovery values after the MVIC and the Tc recovery values after 60-90 s of electrical stimulation of the vastus lateralis were significantly different from each other ($p < 0.001$). There was also a significant difference ($p < 0.001$) in Vmax values. Values were higher for an MVIC compared to electrical stimulation.

Measurements between SCI and able-bodied controls

End exercise Pi/PCr ratio and muscle pH was not significantly different between the two groups ($p = 0.12$) (Table 3.3). Representative PCr recovery curves for able-bodied subjects and individuals with SCI are shown in Figure 3.2. The average time constant after electrical stimulation in individuals with SCI was significantly higher ($p < 0.001$) than the able-bodied population (Figure 3.4). The Vmax values were also significantly reduced in the paralyzed individuals compared to the controls ($p < 0.001$).

Discussion

The primary finding of this study was that individuals with spinal cord injury have about half the mitochondrial function as found by phosphocreatine recovery kinetics. This is in general agreement with a previous study which measured PCr recovery rates in people with SCI (66). However, the study by Levy et al. (66) only had 3 subjects who were combined into one recovery curve. The PCr recovery rates may have been slowed due to the very low end exercise pH values reported in their study (end exercise pH was 6.2). In our study, the end-exercise value in paralyzed individuals was 6.95 ± 0.15 . We found MVIC Tc recovery values that were very similar to the values reported by others for a normal, healthy population. Haseler et al (48) reported a recovery time of 25.0 ± 2.7 s in six healthy men and Walter et al (109) reported a time constant of 32.1 ± 9 s for the calf. McCully et al (78) examined recovery kinetics in the calf to young (28.3 ± 6.8 years) and old (66.0 ± 6.0 years).

In this study, an able-bodied control group was used to be able to compare the results of individuals with spinal cord injury to the able-bodied population. The able-bodied individuals did not produce the same phosphocreatine recovery kinetics for a maximal voluntary contraction and electrical stimulation. If we were to use the Tc values from the MVIC to compare to the individuals with spinal cord injury they would seem more impaired than they actually may be. Therefore, to compare the phosphocreatine recovery kinetics of able-bodied individuals to those with spinal cord injury, it was important to use electrical stimulation. One reason for this significant difference is that we didn't deplete enough phosphocreatine making it hard to measure a recovery rate after voluntary exercise. Another reason could be the recruitment pattern of the two modes of exercise. Further studies may need to address the reasons for why PCr recovery rate differed in able-bodied subjects after voluntary and electrical stimulation exercise.

Our study did not find evidence that resting Pi/PCr or PCr/ATP levels were different between people with SCI and able-bodied subjects. Elevated Pi/PCr ratios have been seen after muscle injury, and people with SCI are more susceptible to muscle injury. However, our measurements were made prior to starting any leg exercise program, and so it is possible that under normal conditions, paralyzed muscle does not show much evidence of muscle injury. Normal values were found for the phosphorous metabolite ratios during rest in spite of the severe disuse and atrophy. According to Pathare et al (91), after 2 weeks of immobilization by casting, the Pi/PCr values increased significantly from 0.08 to 0.14. There is one outlier that has a much higher Pi/PCr ratio. This subject had a very high resting ratio (0.384) and a very low resting PCr (27.9 mM) when compared to the others in the group. There could be a few reasons for this. The individual may not have been completely at rest while collecting the resting spectra. It was also the only individual with SCI that had diabetes. We need to test more diabetic individuals to

determine if this high ratio is due to the disease itself or just the individual. When the subject is omitted from the analysis, the resting values between the controls and SCI are very similar numerically.

The PDE peak was significantly higher in the individuals with SCI compared to the able-bodied controls. Some studies report that this peak may increase with age (79, 96). It is unclear what this difference in peak height could be but a possibility is an indicator of accelerated age.

There are limitations to the current study. The control group was not exactly comparable to the group of individuals with SCI in terms of age. There were 3 subjects that are age-matched so we combined the control group to include a younger population. The age difference between our groups should make a small difference in the recovery rate of phosphocreatine (79). Our study also had relatively small sample sizes. While the study achieved statistical significance in its major findings, a larger sample size maybe more representative of the population of people with SCI.

In conclusion, ^{31}P MR spectroscopy provides an adequate noninvasive measurement for obtaining in vivo information of the changes in the oxidative metabolism of the vastus lateralis muscle. Electrical stimulation recovery measurements of able-bodied muscle appeared to be more appropriate for comparisons to electrical stimulation recovery measurements in people with SCI. In addition, people with SCI had significantly reduced muscle oxidative metabolism in their paralyzed muscles than able-bodied controls. More matched controls need to be tested to truly compare these results to the SCI population.

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Table 3.1. Descriptive characteristics of subjects

Variable	Group	
	SCI	Able Bodied
Age, yr	32 \pm 7	25 \pm 4 *
Sex (M/F)	4/2	7/5
Height, cm	178.2 \pm 5.2	174.5 \pm 9.9
Weight, kg	85.2 \pm 18.6	74.4 \pm 13.3

Values are mean \pm SD; n = 12 AB controls and n = 6 SCI patients.

* Significantly different ($p < 0.05$) than the able-bodied control group

Table 3.2. Resting Pi/PCr and pH values

Variable	Resting Pi/PCr	Resting PCr (mM)	Resting PDE peak (mM)	Resting pH
SCI	0.137 ± 0.123	40.96 ± 6.93	2.95 ± 1.2	7.05 ± 0.03
Able Bodied	0.084 ± 0.02	43.04 ± 4.03	1.6 ± 1.4 *	7.06 ± 0.02

Values are presented as mean ± SD. There were no differences ($p > 0.05$) in the average resting Pi/PCr or pH between the SCI group and the control group.

* Significantly different ($p < 0.05$) than SCI resting PDE peak values.

Table 3.3. End Exercise Pi/PCr and pH values

Variable	MVIC		Electrical Stim	
	End Exercise Pi/PCr	End Exercise pH	End Exercise Pi/PCr	End Exercise pH
SCI			0.59 ± 0.29	6.95 ± 0.15
Able Bodied	0.35 ± 0.2	7.08 ± 0.03	0.47 ± 0.09	7.04 ± 0.07

Values are presented as mean ± SD. There is no significant difference ($p > 0.05$) in Pi/PCr ratio or pH values between SCI and the able-bodied control group. There is no significant difference between end exercise Pi/PCr or values between MVIC and electrical Stimulation.

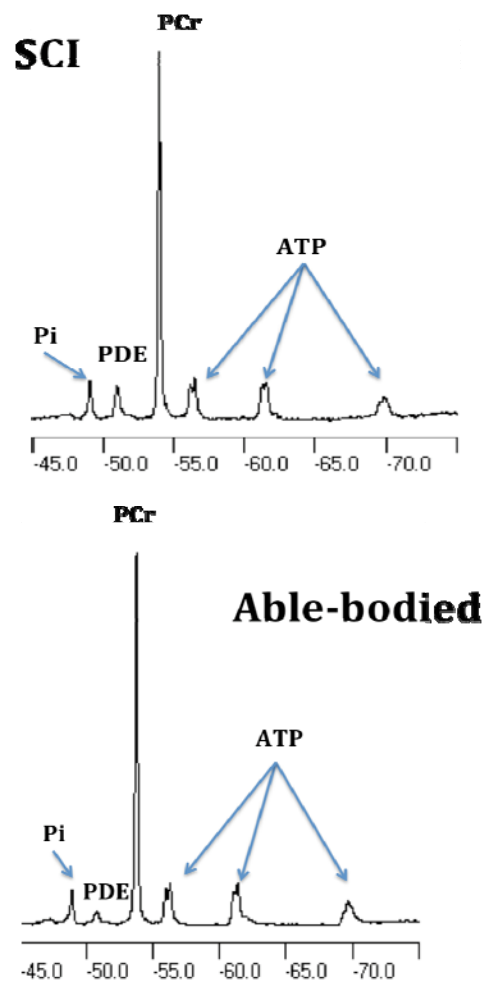


Figure 3.1

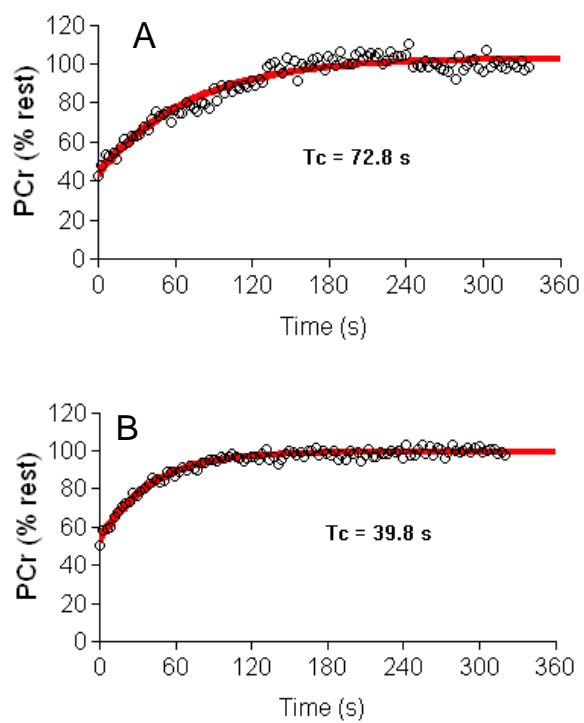


Figure 3.2

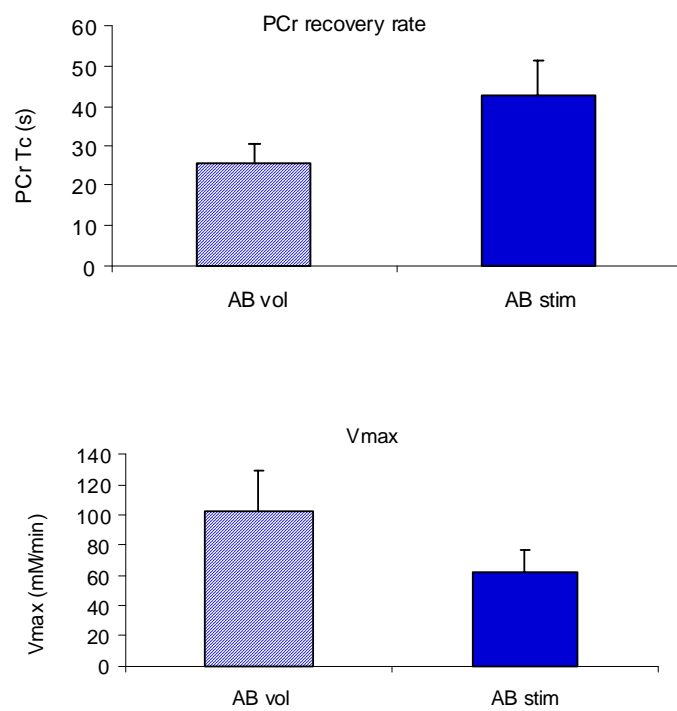


Figure 3.3

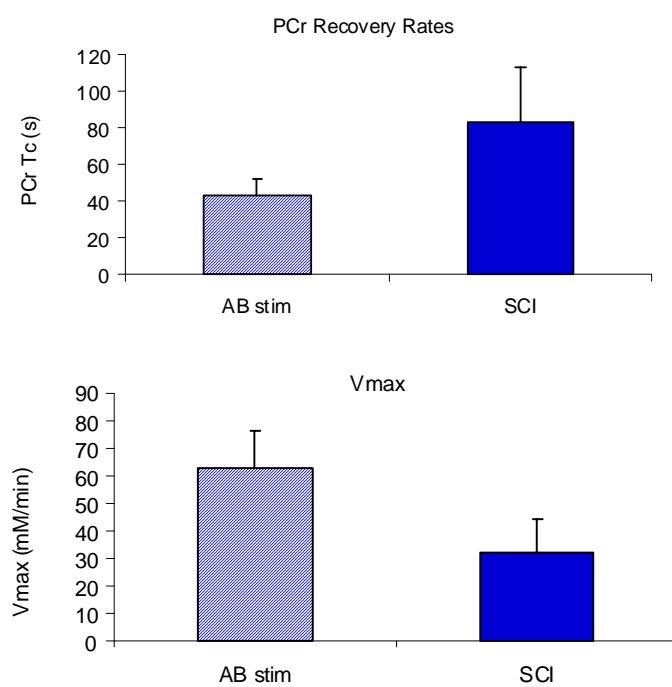


Figure 3.4

CHAPTER IV

SUMMARY AND CONCLUSION

The purpose of the study was to measure mitochondrial function in individuals with SCI. The study was successful in depleting phosphocreatine to a level sufficient enough to measure the recovery rate in not only healthy able-bodied controls but subjects with SCI as well. This study was successful in utilizing the unique *in vivo* measurement to evaluate functional oxidative capacity in the same muscle fibers as previous studies used *in vitro* measurements from muscle biopsies. It has been shown that there is a good correlation between *in vivo* and *in vitro* measurements of oxidative metabolism (78). MRS is an effective method of performing noninvasive measurements of oxidative capacity in a wide variety of subjects and is much easier than the alternative of a muscle biopsy.

Based on the results obtained in this study, individuals with SCI do seem to have impaired mitochondrial function. They are about half the oxidative capacity of the able-bodied population. According to the resting data, their phosphorous metabolites are no different than the controls. The only difference is in the amount of phosphodiester. However, it is possible that higher phosphodiester values indicate increased lipid with muscle, or an increased rate of aging of muscle. This is because previous studies have shown that age is associated with an increase in the phosphodiester peak (79, 96). More subjects need to be tested to confirm these results.

One of the major limitations to this study was the recruitment of participants. It was very difficult to recruit individuals with SCI to go into the MRI. In order for the individuals with SCI to go into the MRI they had to get medical clearance. This consisted of making sure the surgical equipment in their body could be tested in a 3-tesla magnet for safety reasons. There was only

one subject that was diabetic. More subjects with diabetes need to be tested. In the able-bodied population more age-matched, physically inactive individuals should be tested. This would make these two groups more comparable than the current subjects.

Impaired mitochondrial function is a contribution to the deteriorating health of individuals with SCI. It is now possible to evaluate whether exercise can improve muscle metabolism in complete SCI patients with electrical stimulation. Training programs can now be implemented to determine if participating in exercise can enhance their mitochondrial function and overall health. This study was also important in determining the differences between a maximal voluntary contraction and electrical stimulation.

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