THE EFFECTS OF STRENEOUS EXERCISE ON INTRAMUSCULAR MAGNESIUM CONCENTRATIONS AND MUSCLE METABOLISM

by

TIFFANY NICOLE TURNER

(Under the Direction of Kevin McCully)

ABSTRACT

^{31}P magnetic resonance spectroscopy (^{31}P MRS) has been used to measure muscle oxidative metabolism and intramuscular magnesium concentrations. The purpose of this study was to evaluate our ability to make these measurements in the quadriceps muscles of twelve males before and after strenuous walking. In vivo phosphocreatine (PCr) recovery kinetics was measured after 30-39 second bouts of isometric exercise. PCr recovery averaged 39 seconds and had an ICC of 0.819. The mean end exercise PCr was 47.6% and the mean end exercise pH was 6.97. Intramuscular magnesium concentrations averaged 0.388 and had a COV% between days of 7.91. Strenuous walking did not produce significant alterations in either PCr recovery kinetics or intracellular magnesium levels. In conclusion, despite excellent signal to noise values, PCr recovery after exercise has relatively low reproducibility and prior exercise is unlikely to alter measurements of resting metabolites or muscle metabolism.

INDEX WORDS: PCr recovery kinetics, Intramuscular magnesium, Exercise, Muscle metabolism, Reproducibility

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DEDICATION

To my family: Because I never had to ask for your love or support

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Hey-anyone who doesn't kick me out of the program when the smells from my lunch make others wonder if an animal has died in the lab is a true friend!

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CHAPTER ONE

Introduction

Phosphocreatine recovery is a means to quantify muscle metabolism. Oxidative, or muscle metabolism is essentially the rate of adenosine triphosphate (ATP) production needed to meet the demands of a prescribed workload. It is composed of four processes, β-oxidation, citric acid cycle, electron transfer chain and oxidative phosphorylation, which occurs in the mitochondria. *Chance et al.* illustrated a metabolic model whereby metabolic by products such as ADP, Pi, NADH, and oxygen regulate the production of ATP (15).

During exercise, cytoplasmic adenosine diphosphate (ADP) increases as adenosine triphosphate (ATP) decreases. To replenish the supply of cytoplasmic ATP, the phosphate from phosphocreatine (PCr) is used to renew the supply. The resulting creatine diffuses to the mitochondria and is phosphorylated into phosphocreatine by mitochondrial ATP. This process, coupled with the enzymatic activity of creatine kinase (CK) occurs at such a rapid rate that ATP concentrations essential remain steady. Due to the fact that this reaction occurs instantaneously, the production of phosphocreatine can be related to the production of mitochondrial ATP, and therefore related to oxidative metabolism. In the past, muscle biopsies were used to quantify these changes (11), but due to their invasive and painful nature subject recruitment and measurements per subject were limited. Advances in magnetic resonance spectroscopy has now allowed for a noninvasive means to measure oxidative metabolism.

There are two ways to evaluate muscle metabolism during exercise: Pi/PCr ratios to quantify ADP levels during exercise at different intensities (65, 73, 110), or measuring PCr recovery kinetics after exercise(6, 33, 39, 45, 65, 73, 74, 77, 110, 114, 121). While both ways are effective, PCr recovery does not require precise measurements of work intensity or muscle mass; it requires good time resolution and adequate depletion of PCr to generate an exponential recovery curve.

PCr depletion is dependent on exercise intensity. The more intense the exercise, the greater the change in pH. If pH drops below 6.9 a biphasic recovery curve will be seen with an initial slower component. If pH remains at rest, approximately 7.0-7.1 in the muscle, a nonoexpotential recovery curve is seen. Changes in pH have been related to the chemical shift of the inorganic phosphate peak on the MRS spectra. PCr's ionization constant, pK_a, at rest greatly differs from the pH at rest, therefore, it is unaffected. As exercise intensity increases, pH more closely resembles the pK_a of PCr. 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. The high H^+ concentration seen with a decrease in pH affects PCr by reducing the rate of resynthesis. *Roussell et al.* (94) and *Walter et al.* (115) both found that the initial phase of recovery is independent of pH changes and the secondary phase of recovery is what is effected. This discovery allows the slope of the initial stage to be used to calculate a recovery time constant that is independent of pH changes (115).

Mitochondrial function can be measured through phosphocreatine recovery kinetics due to the relationship between PCr resynthesis and ATP production. The creatine kinase reaction is fast enough such that PCr levels can be considered to be in equilibrium with ADP levels. Thus

mitochondrial production of ATP from ADP is in equilibrium with the synthesis of PCr from creatine and phosphate. Following exercise, the rate of ATP production based on oxidative metabolism should be initially fast, and should decrease exponentially as ADP levels fall to resting levels. Based upon this, rate constant of PCr recovery should represent the maximal rate of oxygen consumption (62, 79). The time constant of PCr recovery (inverse of the rate constant) should reflect mitochondrial capacity (14). *McCully et al* found a significant correlation between in vivo and in vitro measurements of oxidative metabolism (72).

While MRS has proven to be an effective tool to measure PCr recovery, the majority of the studies include measurement from the calf and employ the use of an in magnet ergometer. Few studies have examined PCr in the quadriceps (91). The use of an in magnet ergometer is a limiting step in performing PCr recovery tests due to the size and magnetic limitations. It would be advantageous to have a procedure that would adequately deplete PCr without the use of this tool.

Despite the use of PCr resynthesis as a measure of muscle metabolism since ~1990, very few studies have examined the reproducibility of these measurements. With the advent of new magnet spectroscopy systems and the use of different exercise modalities, there is a definite need to characterize the reproducibility of these important measurements.

Magnesium may also play a key role in muscle metabolism due to the fact that it is an essential cofactor in over 325 enzymatic reactions (83). Magnesium's primary cellular role is a cofactor in ATPase activity (96),(101). It serves to stabilize the structure of ATP in ATP-dependent enzymatic reactions by acting as a ligand for the phosphate groups. In glycolysis magnesium aids in the stability of the ATP by forming an active complex with the enzyme

before the substrate binds (96). This is seen in the rephosphorylation of phosphocreatine by mitochondrial ATPase activity. During exercise, the PCr supply depletes and the mean cytoplasmic ATP concentration decreases because it is converted into ADP-AMP-IMP. It has been shown that muscle magnesium levels significantly increase when exhaustion is reached (53). Free cytoplasmic magnesium increases because the break down products of ATP have a lower affinity, whereas resting ATP has a high affinity for magnesium. Since magnesium acts as an activating ion for phosphorylation and dephorylation reactions, it is possible that shifts in magnesium concentrations could lead to impaired muscle metabolism (95).

Numerous studies have examined the role of magnesium in the blood, sweat, and urine (12, 26, 41, 51, 88, 93) during exercise. Intramuscular magnesium concentrations have been studied to a lesser degree due to the invasive and complex nature of muscle biopsies. With the advances in technology, MRS has allowed noninvasive, in vivo measurements to become more readily available. Successful studies have suggested that MRS can be used to measure and track intracellular magnesium changes, which have been linked to changes in energy metabolism (30, 36, 60, 63, 75, 108). Several previous studies have suggested that patients with enhanced fatigue have altered intramuscular magnesium levels (75). Blood measurements of intracellular magnesium have been shown to be sensitive to inflammation, although little is know about how intramuscular magnesium might response to inflammation. It is plausible to speculate that alterations in magnesium will affect the energy metabolism in the muscle. However, intracellular magnesium measurements are rarely reported and it is not clear how reproducible they are or whether such measurements are sensitive to prior exercise.

Study aims

The aims of this study were to:

1. Make repeated measurements and determine the reproducibility of phosphocreatine recovery in the quadriceps muscles using isometric exercise in healthy human subjects.
2. Make repeated measurements and determine the reproducibility of intramuscular measurements of magnesium concentrations form the quadriceps with ^{31}P MRS.
3. Determine effects of prior strenuous exercise on PCr recovery kinetics and intramuscular magnesium concentrations in the quadriceps of health human subjects.

Hypotheses

It is hypothesized that:

1. We can obtain reproducible measures of the time constant of phosphocreatine recovery using ^{31}P MRS in healthy human quadriceps muscle.
2. We can obtain reproducible measures of intramuscular magnesium concentrations using ^{31}P MRS in healthy human quadriceps muscle.
3. Prior strenuous exercise will significantly change intramuscular magnesium and phosphocreatine recovery kinetics thirty to sixty minutes post exercise.
4. Decreases in either intracellular Mg^{2+} or resting ATP will result in decrease muscle metabolism measured as the time constant of PCr recovery.

Significance of the study

This is the first study performed by our laboratory to measure muscle metabolism as phosphocreatine recovery in the quadriceps muscle using the new 3 Tesla GE magnet located in the Biomedical Imaging Research Center. Thus, this study will serve as a pilot study for future studies such as those currently being conducted on the effects of quercitin on muscle metabolism. Future studies will examine muscle metabolism in patient populations such as people with spinal cord injuries. The determination of reproducibility of the phosphocreatine recovery measurements will allow better planning of sample sizes in these studies. A finding of low reproducibility may lead to changes in experimental methodology to improve the outcome of future studies. While intramuscular magnesium is a potentially measurable variable that may be linked to muscle fatigue and muscle inflammation, little is know about this measurement. This study will be the first to carefully determine the reliability of intramuscular magnesium measurements as well as to determine how stable these measurements are in response to prior exercise. Successful measurements of intramuscular magnesium will lead to future studies to examine magnesium in various patient populations.

CHAPTER TWO

Review of Related Literature

MRS and PCr recovery kinetics

^{31}P-NMR uses a surface coil that emits a radio pulse at a certain frequency that is absorbed by the corresponding compounds within the muscle. These compounds resend the radio frequency pulse back to the surface coil, which conveys a spectrum based on the metabolic state of the muscle. NMR can only detect metabolites that are free in solution, not bound compounds. It does not give absolute values, rather each value is representative relative to each other. Because of this, some assumptions are made. Skeletal muscle ATP concentration is 8.2mmol/L of intracellular water, total creatine is 42.5mmol/L of intracellular water, PCr plus Pi equals 42.2 mmol/L of intracellular water, these components are uniformly distributed, and there is no change in total creatine during exercise (104). With the advent of improved quality and time resolution of ^{31}P MRS measurements, the ability to measure the kinetics of muscle metabolism improved (79). Many studies have successfully used MRS to evaluate in vivo PCr recovery kinetics (1, 65, 106, 107). Studies include isometric, eccentric, and concentric movements in subjects who are physically conditioned (50, 57, 84, 103), have disease (46, 47, 120) or are sedentary (118). Recovery kinetics as a measure of muscle metabolism has several advantages over steady state measurements.

Exercise and PCr

At the onset of exercise, PCr is used to phosphorylate ATP and the rate of PCr breakdown is dependent on the intensity of the muscular contraction. Strong relationship between decreases in force and decreases in PCr during high intensity exercise has been found (8, 9, 80). *Sargeant* and *Dolan* (100) studied effects of submaximal exercise induced fatigue on short-term power output during isokinetic cycling. They reported the recovery of power had a half-time of 32 seconds, which is similar to the half time to recovery reported by *Cooke et al.* and others following exhaustive exercise (20). Initial studies of muscle metabolism using ^{31}P MRS measured metabolite levels during steady state exercise. This was due because of the low signal to noise values obtained from low field strength magnets and 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.

pH and PCr

A key confounding factor in the use of phosphorous metabolites to measure oxidative metabolism is the influence of muscle pH and glycolysis. Because hydrogen ions are released during the resynthesis of PCr, changes in muscle pH will shift the creatine kinase equilibrium. This is illustrated in the equation: $PCr + MgADP^{-} + H^{+} \Leftrightarrow MgATP^{2-}$ + creatine. The high H^{+} concentration seen with a decrease in pH affects PCr by shifting the equilibrium to the right (in the equation above. This means that levels of 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. *Harris et al.* (32) and *Sahlin et al.* (98, 99) both hypothesized that pH was

the limiting factor in the CK equilibrium. *Salhin et al.* (99) 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. They further concluded that oxygen availability affects the initial phase of PCr recovery and pH affects the later stages of recovery. Many more studies have quantified the effects of pH on PCr recovery with the development of MRS. Shifts in pH are represented by shifts in the Pi peak because its position is pH dependent. Pi is present is two forms, HPO_4^{-1} and H_2PO^4. During rest, the pKa is near 6.8 and the ratio of the two forms is approximately 60/40. Changes in pH cause the ratio to shift toward one extreme due to the fact that the pH is dependent on the concentration of the forms. 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. *Arnold et al.* (1) was one of the first to examine this and found that PCr resynthesis was slower following heavy exercise. *Behdahan et al.* (4) found that the extent of acidosis at the end of exercise determined the rate of resynthesis. 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 (1, 32, 98, 99, 104, 105).

Fiber Types and MRS

Studies have shown that PCr is resynthesised faster in slow twitch or type 1 fibers versus fast twitch of type 2 fibers (11, 40, 109). It has hypothesized that the faster recovery was due to increased capillaries, increased mitochondrial density, and higher oxidative enzyme activity. The vastus lateralis is similar to the calf in that the fast twitch fibers are closer to the surface, while slow twitch fibers are deeper in the muscle. Calf muscles from elite sprinters and

endurance runners were examined using ^{31}P MRS (67). During high intensity exercise, fast twitch glycolytic muscle fibers become acidotic after thirty seconds and reach pH values of 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, the presence of different types of muscle fibers within a single muscle during maximal exercise results in fibers having different pH values and Pi peaks with different frequencies. Double or split Pi peaks can be seen if data is collected from differing fiber types, slow vs. fast. 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. *Bernus et al.* (5) and *Park et al.* (86) have seen up to three Pi peaks which they concluded as fast twitch glycolytic, fast twitch oxidative, and slow twitch oxidative fibers.

Magnesium

It is well known that Mg is an essential cofactor in over 325 enzymatic reactions (83) and because of this, changes in magnesium may show a great effect (10). Magnesium's primary cellular role is a cofactor in ATPase activity (101). It serves to stabilize the structure of ATP in ATP-dependent enzymatic reactions by acting as a ligand for the phosphate groups. In glycolysis magnesium aids in the stability of the ATP complex or is required as part of the metalloenzyme. Magnesium-ATP is needed to catalyze the steps involving hexokinase and phosphofructokinase and Magnesium-ADP is needed for phosphoglycerate and pyruvate kinase. Magnesium binds to these enzymes by carboxylate groups on the protein and form an active complex with the enzyme before the substrate binds (96). Since magnesium acts as an activating

ion for phosphorylation and dephorylation reactions, it is possible that shifts in magnesium or ATP concentrations could lead to fatigue (95).

Muscle magnesium levels significantly increase when exhaustion is reached and it has been suggested that fatigue during endurance exercise is due to the increased magnesium levels inhibiting the release of calcium from the sarcoplasmic reticulum (53). Once fatigue is attained, force production falls due to contractile properties and reduction of calcium release from the sarcoplasmic reticulum. PCr supply depletes and the mean cytoplasmic ATP concentration decreases because it is converted into ADP-AMP-IMP. Free cytoplasmic magnesium increases because the break down products of ATP have a lower affinity, whereas resting ATP has a high affinity for magnesium. The rise in free magnesium concentration seems to be a factor in reducing calcium release, because an increase in free magnesium has been shown to cause a block of depolarization-induced calcium release (53, 54).

Magnesium and exercise

Numerous studies, such as marathon running, long distance cross-country skiing, cycle ergometry, swim training, and tennis have examined the effects of exercise on intracellular blood, urine, sweat and muscle magnesium levels (12, 26, 41, 51, 88, 93). Overall, studies have found that submaximal exercise leads to hypomagnesium: a transient decrease in plasma Mg concentrations. Magnesium deficiencies reduce physical performance and the magnesium state may have an effect on exercise capacity (18, 44, 52, 61, 87). Cellular levels of ATP and creatine phosphate appear to become rapidly depleted with magnesium deficiency (87).

Magnesium in blood

Erythrocyte magnesium studies have produced conflicting evidence, yet this is the most common method of collection. Some studies indicate that a decrease in plasma magnesium concentration is due to the shift of magnesium into the red blood cells (64, 85, 89, 102, 119) while other studies have shown a decrease in cellular magnesium rather than an increase in erythrocyte magnesium. Although the means to depletion is debated, numerous investigators have verified a reduction in serum or plasma magnesium concentrations with exercise (12, 23, 26, 51).

Magnesium in sweat and urine

Strenuous exercise can also cause increased magnesium excretion through sweat and urine. It is difficult to obtain accurate measurements through excretion due to the fact that these processes are homeostatically regulated. Studies have found ssignificant decreases in urinary excretion of Mg in prolonged submaximal exercise where the excretion increases during the recovery period to reach levels higher than those measured before exercise (31, 58).

Magnesium and soft tissue

Approximately half of the total body magnesium is found in the soft tissue. Seven and nine mmol of magnesium per kilogram of wet tissue is found in skeletal muscle and liver (112, 113) while free magnesium ranges from 0.3 and 3.0 mmol/L (21). *Corkey et al*. (21) found that small changes in the total cell magnesium may affect larger changes in the free magnesium. Decreases in magnesium during exercise have been linked to possible shifts of magnesium from the extra cellular fluid to skeletal muscle. Exercising muscles appear to slowly increase in

magnesium content, which is paralleled by a decline in plasma magnesium concentration. This suggests that serum magnesium reduction could be explained by the redistribution into the muscle during heightened metabolic need (23).

Magnesium and MRS

In previous years, needle biopsies were the most common method to examine muscle metabolism, yet magnetic resonance spectroscopy is quickly becoming the preferred method due to its non-invasive nature (24, 25). Numerous studies have suggested that MRS can be used to measure and track intracellular magnesium changes, which have been linked to changes in energy metabolism (30, 36, 60, 63, 75, 108). According to previous research, the resting magnesium concentration in healthy individuals is expected to be 0.36mM ± 0.5mM (75). *Resnick et al*. (90) found a decrease in erythrocyte free magnesium in untreated subjects with hypertension. Approximately ten percent of the environmental magnesium is in the form ^{25}Mg and can be determined with NMR (92).

Near-infrared spectroscopy (NIRS)

NIRS is a noninvasive method used to quantify changes in tissue oxygen levels. Difficult to quantify NIRS, therefore, the units are expressed in arbitrary unit called optical density. Various wavelengths of light are absorbed by the oxygenated and deoxygenated forms of the heme groups. Due to the fact that NIR varies among individuals, it is commonly calibrated by the range of muscle oxygenation caused by arterial occlusion after reactive hyperemia (13). It is assumed that a minimum of 5 minutes of ischemia will flush out HbO_2 and that reactive

hyperemia will eliminate HHb. Numerous studies have evaluated skeletal muscle using this approach (29, 43, 49, 81, 82, 34).

Electromyography (EMG)

Measures the muscles electrical activity by detecting small electrical potentials from the muscle. Surface EMG is noninvasive and gives information on overall muscle function. Many studies have examined exercise and EMG activity (80, 111).

CHAPTER THREE

THE EFFECTS OF STRENOUS EXERCISE ON INTRAMUSCULAR MAGNESIUM CONCENTRATIONS AND MUSCLE METABOLISM

T N Turner, Q. Zhao, and K K McCully, to be submitted to NMR in Biomedicine

Abstract

^{31}P magnetic resonance spectroscopy (^{31}P MRS) has been used to measure phosphorous containing compounds in skeletal muscle. Perhaps the most established use has been to measure muscle oxidative metabolism using the rate of recovery of phosphocreatine after exercise, although intramuscular magnesium concentrations have been measured using the frequency shift of the beta-ATP peak. The purpose of this study was to evaluate our ability to measure muscle metabolism and intramuscular magnesium in the quadriceps muscles twice before and twice after two hours of moderate intensity walking. Twelve normal, healthy male volunteers were tested in a 3 Tesla magnet. In vivo phosphocreatine (PCr) recovery kinetics was measured after 30-39 second bouts of isometric exercise. Out of 12 subjects, the data from one subject was not used. The mean end exercise PCr was 47.6% and the mean end exercise pH was 6.97. PCr recovery averaged 39 seconds ($p = 0.892$) and had an interclass correlation coefficient between days (ICC) of 0.819. Intramuscular magnesium concentrations averaged 0.388 mM and had a COV% between days of 7.91%. Prior strenuous exercise did not produce significant alterations in either PCr recovery kinetics or intracellular magnesium levels ($p = 0.440$). In conclusion, despite excellent signal to noise values, PCr recovery after exercise has modest reproducibility. In addition, prior exercise is unlikely to alter measurements of resting metabolites or muscle metabolism.

Introduction

Phosphocreatine recovery kinetics have been measured using ^{31}P magnetic resonance spectroscopy (MRS) in various magnet strengths, subject populations, and muscle types with differing exercise intensities (6, 33, 39, 45, 65, 73, 74, 77, 110, 114, 121). While PCr has been studied extensively, the reproducibility of these measurements inbetween or within days has only been statistically quantified in a few papers. With the advent of new magnet spectroscopy systems and the use of different exercise modalities, there is a definite need to characterize the reproducibility of these important measurements. Also, the majority of these studies examine recovery kinetics in the calf muscles and use an in magnet ergometer to deplete phosphocreatine (PCr). While in magnet ergometers are a sufficient means to aid in the depletion of PCr, they can be a major limiting step 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 would be a definite advantage.

It is not clear what factors may effect mitochondrial function. Studies have examined age, varying work intensities, and patient populations (69, 72, 76, 114). While the effects of training have been examined (68), it is unclear what effects vigorous activity will have on muscle oxidative capacity in a healthy population

Resting metabolism has been measured using MRS and it is hypothesized that changes in intramuscular magnesium concentrations may be a potential marker for abnormal mitochondria function (75). Magnesium is a cofactor in ATP dependent reactions and provides stability to ATP by binding and aiding in the transfer of phosphate groups. Due to this characteristic, shifts

in either magnesium or ATP concentrations may lead to impaired energy metabolism. Several previous studies have suggested that patients with enhanced fatigue have altered intramuscular magnesium levels (70). Blood measurements of intracellular magnesium have been shown to be sensitive to inflammation, although little is know about how intramuscular magnesium might response to inflammation. It is plausible to speculate that alterations in magnesium will affect the energy metabolism in the muscle. However, intracellular magnesium measurements are rarely reported and it is not clear how reproducible they are or whether such measurements are sensitive to prior physical activity.

The purpose of this study was to: 1) determine the reproducibility of measurements of the time constant of PCr recovery and intramuscular magnesium concentrations, and 2) to evaluate how muscle magnesium and mitochondrial function through PCr kinetics are affected by prior long duration exercise. It was hypothesized that reliability of the measurements would be within commonly accepted standards and that prior exercise would alter intracellular magnesium and the time constant of PCr recovery.

Methods

Subjects

A total of 12 healthy, college aged males (mean age= 22 ± 1.9 yrs) were recruited because of their athletic ability and low subcutaneous fat. The physical characteristics of the subjects are shown in Table 3.1. All subjects participated in physical activity at least twice a week and were capable of performing multiple isometric contractions of the quadriceps muscle for forty seconds each. The study was conducted with the approval of the Institutional Review Board at the University of Georgia and all subjects provided written informed consent.

Experimental design

A one group with repeated measures design was used in this study. Each subject had a total of five test days which included one familiarization testing session followed by 4 test sessions separated by 1-30 days. Initially, a familiarization trial was preformed in the lab where maximal voluntary isometric contractions and oxygen saturation in the muscle were measured. The next four testing sessions were preformed in the 3 Tesla large bore magnet at the Biomedical Imaging Research Center in the Coverdale building where MRS was preformed to collect intramuscular magnesium, ATP concentrations and PCr recovery kinetics. Testing session three involved a strenuous exercise protocol where the subjects preformed vigorous incline treadmill walking for two hours in the lab. During this exercise, heart rate, perceived exertion and EMG activity of the quadriceps were collected. Thirty to sixty minutes post exercise, MRS was used to evaluate intramuscular magnesium, ATP concentrations and PCr recovery kinetics. Testing session four was preformed one to three days post exercise and was identical to testing sessions one and two. Each subject reported no physical activity twenty-four hours prior to testing other than the exercise performed in this study.

Maximal voluntary isometric contraction (MVIC)

Muscle EMG measurements were made to acclimatize the subjects to the isometric exercise protocol. MVIC was quantified using EMG activity of the right quadriceps during various durations. Two electrodes were placed approximately three and five inches above the knee along the vastus lateralis. A grounding electrode was placed on the knee cap. Signals were sent into a data acquisition system (Biopac) set to collect at 2000 samples per second. The subject lay supine and was instructed to maximally contract the quadriceps muscle with and

without visual feedback of the EMG activation. Between each subsequent MVIC exercise the subject was allowed to rest for approximately five minutes. The subject would perform two 20 second warm up contraction and one 40 second contraction without visual EMG feedback. One 40 second contraction while watching the EMG activity of the vastus lateralis was then collected, which was followed by two 40 second contractions without visual feedback. The root mean squared signal during each contraction was calculated and analyzed.

Muscle oxygen saturation

Muscle oxygen saturation was measured during isometric exercise to estimate muscle activation prior to making MRS measurements. Muscle oxygen saturation was quantified using continuous wavelength near infrared spectroscopy (NIM, Inc Philadelphia PA). Eight channels with a separation distance of 3 cm were collected at 3 Hz. The subject remained in the supine position while a sensor was placed on the vastus lateralis of the right quadriceps and wrapped with an athletic bandage. A blood pressure cuff was placed as proximal on the upper leg as possible, see Figure 3.1. The subject performed a 40 second MVIC with vocal encouragement and after two minutes of recovery the muscle was made ischemic for 10 minutes using a blood pressure cuff inflated to 220mmHg. Recovery data was collected for three to five minutes after the cuff was released. Oxygen saturation signals during exercise were normalized to the minimum value during ischemia and the maximum value during reactive hyperemia.

Intramuscular magnesium and ATP concentrations

Resting measurements of magnesium and ATP were taken using MRS in a 3 Tesla whole body magnet (GE Medical). A pair of coils (^{1}H and ^{31}P) were placed on the vastus lateralis of

the subject's right quadriceps. Resting spectra were acquired every 3 seconds until 120 scans were 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. Magnesium and pH were calculated using the following equations:

$$pMg=4.24-\log((18.58-\beta ATPshift)^{0.42}/(-15.74+\beta APTshift)^{0.84}$$

$$Mg=10^{(-PMg)}*100$$

$$pH=6.77+\log((Pi_{shift}-3.27)/(5.68-Pi_{shift}))$$

Phosphocreatine recovery kinetics

Once resting measurements of magnesium and ATP were taken, the subject performed a 30-39 second duration MVIC to deplete PCr. The subject was then instructed to remain as still as possible while recovery data was collected for approximately four minutes. This procedure was repeated two or three times. Phosphocreatine peaks were determined from peak heights from individual spectra using a MatLab routine. PCr peak heights during recovery after exercise were fit to an exponential curve:

$$PCr=End-Delta*Exp(-Time/Tc)$$

Exercise protocol

Incline treadmill walking was performed for two hours where the subjects reached a target level that was between forty and sixty percent of their heart rate reserve. During this time, speed/grade variations, EMG activity, heart rate and perceived exertion were collected. The subject remained seated for approximately thirty minutes to establish a resting heart rate. During this time, two EMG electrodes were placed on the vastus lateralis of the right quadriceps

approximately three and five inches above the knee and one electrode on the knee cap. All outputted into Biopac hardware in conjunction with Acknowledge Software set to collect at 2000 samples per second. The area was shaved and or sprayed with adhesive prior to electrode placement. Athletic tape and bandages were then wrapped around the knee and quadriceps. To measure EMG activity, the subject was instructed to remain seated with the right leg elevated perpendicular to the torso and perform two 3 second MVICs. The subject immediately got on the treadmill where 120 seconds on EMG walking activity was collected. This procedure was repeated every 30 minutes. A percentage of activation of the vastus lateralis was calculated using the 120 seconds of walking compared to the maximal EMG activity calculated for the three second MVICs. Polar heart rate monitors were used to ensure the heart rate reserve remained between forty and sixty percent and Borg scale measurements were used to quantify perceived exertion. Both measurements were collected every 10 minutes and speed/grade was adjusted between 3-5mph and 5-8% respectively to ensure the testing remained submaximal. To ensure exercise intensity remained consistent between subjects, heart rate reserve was calculated using the Karvonen Formula:

220 - Age = Maximum Heart Rate

Max Heart Rate - Rest. Heart Rate x Intensity + Rest. Heart Rate = Heart rate reserve

Subjects were allowed to drink 32 ounces of water or a sports beverage.

Statistical analysis

All values are reported as means ± standard deviation. Repeated measures ANOVA and Reliability measures (SPSS) were conducted to evaluate the variation between and within days. Independent sample t-test (SPSS) was conducted to compare mean EMG differences pre and post visual feedback. A linear regression was used to evaluate whether oxygen saturation as a

percentage of exercise could predict a person's end exercise PCr. Analysis were conducted with statistical significance accepted at $\alpha = 0.05$.

Results

Reproducibility

Based on exclusion criteria for PCr recovery kinetics, eleven of the twelve subjects were successful. At least a 20% depletion of PCr and an r^2 value above 0.6 was set to evaluate the success of each subject. Although adequate depletion was attained during post test 2, one subject was rejected because adequate depletion was not attained during the initial pre tests. Three of the remaining one hundred and thirty two tests were excluded because the r^2 value was below 0.6 and two other tests were incomplete. A total of eleven subjects and one hundred and twenty seven tests were analyzed. Based on exclusion criteria for Mg and ATP concentration, all twelve subjects were analyzed. A signal to noise ratio of 7:1 was set as exclusion criteria. The mean signal to noise ratio for all subjects was 47:1. Tables 3.2-3.4 show the reproducibility measurements for PCr recovery kinetics, various compounds and $[Mg^{+2}]$. PCr_{Tc} had a COV% between days of 18.4% and a COV% within days of 8.43%. ICC between and within days for PCr was calculated as 0.819 and 0.928 respectively. End exercise PCr had an ICC between days of 0.87 and a COV% of 11.1%. Intramuscular magnesium had an ICC between days of 0.352 and a COV% between days of 7.91%.

NIRS and EMG

Figure 3.3 demonstrates a mean desaturation of 47% after exercise (sd=20.0, n=12) and a mean of 83% during rest (sd=4.62, n=12) using NIRS. EMG showed a mean level activation of

76mV (sd=43.8mV) during the pre forty second MVIC. The forty second MVIC in which visual feedback of the EMG signal was given had a mean level of activation of 92mV (sd=48.5mV). Two post visual forty second MVICs had a mean activation of 89mV and 90mV (sd=50.6 and 44.7) respectively.

Muscle metabolism measure as PCr recovery kinetics

Representative resting spectra are shown in figure 3.5. Signal to noise had a ratio of approximately 1:47 . Figure 3.5 also demonstrates PCr values at the end of exercise. Mean values for end exercise PCr were 46.0% for pretest 1, 52.0% for pre test 2, 48.9% thirty to sixty minutes post exercise, and 43.5% one to three days post exercise. See Table 3.2. pH and Pi/PCr at the end of exercise and rest are also show in Table 3.2. NIRS percent exercise was a poor predictor of PCr end exercise. (F=0.132, p=0.724). See figure 3.6.

PCr recovery time constants were 39.9 seconds, 39.0 seconds and 38.7 seconds on average for pre test one, pre test two, and one to three days post exercise (sd=12.7 seconds, 16.9 seconds, and 12.4 seconds). Mean recovery time constant thirty to sixty minutes post exercise was 37.5 seconds (sd=14 seconds). Figure 3.7 represents the mean exponential recovery curve for PCr. Mauchly's W was not significant (0.567, p=0.424) so sphericity was not violated. It was statistically significant that there was no change in the PCr recovery kinetics (F=0.206, p=0.892, df=3) between the four test days. See Figure 3.8.

Intramuscular magnesium concentrations

Magnesium levels averaged 0.414 and 0.376 mmol (sd=0.151 and 0.034) over the initial pretests. A significant difference thirty to sixty minutes post exercise (p = 0.386, sd=0.035) and

one to three days post exercise (p = 0.375, sd=0.037) was not found. See table 3.3. Figure3.9 demonstrates that there was no difference in intracellular magnesium concentrations with exercise. Mauchly's W was significant (W=0.030, p <0.001); therefore, Greenhouse-Geisser was used to show that there was no statistically significant evidence that the mean intramuscular magnesium concentration differed between the four test days (p=0.440).

Discussion

This study was successful in measuring PCr recovery in the quadriceps of eleven of the twelve subjects. We found T_c recovery values that were higher than the values reported by others in the calf and quadriceps for a normal, healthy population. *Haseler et al (33)* reported a recovery time of 25.0 ± 2.7 s in the calf of six healthy men and *Walter et al* (114) reported a time constant of 32.1 ± 9 s for the calf. *McCully et al* (72) examined recovery kinetics in the calf of young (28.3 ± 6.8 years) and old (66.0 ± 6.0 years) subjects. *McCully et al* (72) reported that four young subjects had a recovery time of 31.0 ± 5.3 s and six older subjects had a recovery time of 56.7 ± 21.6s. Values reported for the quadriceps were also different than our reported values. *Barker et al.* (3) reported a time constant to recovery using the quadriceps of eight male subjects of 23 ± 9 s. *McKeough et al.* (78) found a half time to recovery for the quadriceps in five male subjects of 35 ± 3 s. In an abstract submitted to ACSM, *Williams et al.* (117) found a time constant to recovery that was more closely related to our results. They found that the quadriceps of seven male subjects had a recovery time constant of 38 ± 4 s, where our subjects had a mean recovery time constant of 39.9 ± 12.7 s during pre test one. While our subjects had a mean age of twenty two, they appear to have a recovery time constant of an older population (72)

or a comprisomized population (110). It is unclear what factors may have led to this difference. Our data had good signal to noise of approximately forty seven to one and a reasonable curve fit that was greater than r^2=0.6. Since the data does not appear to be a contributing factor to the variation from other reported scores, it is possible that an analysis which is independent of pH should be used. *Toussaint et. al.* (110) found that eight healthy males had a recovery time in the calf of 31.8 ± 7.6 s before correction for changes in pH. After the pH corrections in three of the eight subjects, recovery values decreased to 27.5 ± 8.0 s. *Walter et. al* (114) examined the difference between using k_{max} ($1/T_c$) versus V_{max} as a means to evaluate PCr recovery kinetics in the calf. V_{max} is independent of pH changes because it estimates a time constant from the slope of the first initial points of the PCr recovery curve. There was not a mean pH change within our subjects, yet differences within each test were noted. These slight changes may account for the variation, therefore a V_{max} calculation may be beneficial in determining an accurate time constant to recovery.

Our successful measurement of PCr recovery differed from previous studies in that we did not use an in magnet ergometer. We found that a MVIC of the quadriceps was sufficient to deplete PCr and generate a nonoexpotential recovery curve. This agrees with the results found by *Karlsson et al. (42)*. They found a significant difference between resting phosphocreatine values compared to end values at thirty, fifty, and eighty percent MVIC. The use of a MVIC is advantageous because it excludes the size and magnetic limitations of inmagnet ergometers. It serves to facilitate a pathway for more PCr recovery studies due to the ease and reduced cost of measurement. Previous studies also were biased toward measurements in the calf, which can be explained by the ease of contraction using an ergometer. Success using voluntary isometric contractions will allow for a wider range of muscles to be examined. It is possible to speculate

that the successful MVIC may be contributed to the EMG practice each subject preformed prior to the initial pretest. It cannot be stated with certainty that this was the driving force behind the success due to the fact that all subjects practiced the MVIC with EMG feedback. Future studies are needed to compare subject performance and subsequent results with and without prior practice.

The reproducibility of our PCr recovery data was also verified. We have been able to find three studies which statistically document the validity of such measurements (55, 71, 114). Similar to our PCr recovery data, the variability between and within days for our measurements were higher than those previously reported. *Walter et al* (114) tested four people on two different days and found a k_{pcr} COV% of 8, 9 and 10% for varying workloads and durations in the calf muscle. This is similar to a study by *McCully et. al* (71) who also examined the COV% in the calf for varying work intensities within the same day. They found a within subject variation for PCr recovery time of 13% between different work loads. *Larson-Meyer et al* (55) found a COV% of 5.0 ± 2.9s after testing the calf of eight female subjects one month apart. Our study tested eleven male subject's quadriceps and found a COV% of 8.43% and 18.4% for within and between days. While the reported data on reliability appears to disagree, we are unsure as to the discrepancies in the large variation between our data and the reported. To our knowledge, our study has quantified reproducibility with the largest sample size. It is possible that the increased variation was due to the different testing/retesting times of each subject, which was between 1-30 days. Yet, based on the reproducibility results of *Larson-Meyer et al.* (55), it can be argued that one month test/retest resulted in extremely reproducible data. Also our subjects stated that their physical activity did not change from the start to completion of the study

and it is unlikely that mitochondrial adaptations would occur in thirty days to account for such variability. More testing is necessary to validate these measurements.

This study also found that we could make resting measurements of intramuscular magnesium concentrations in the quadriceps. Magnesium has successfully been studied in the brain and other tissues using ^{31}P MRS (2, 16, 38, 59, 60, 66). Previous studies have used AMARES to analyze each spectra, but we chose WinSpa due to the inability of AMARES to precisely phase each spectra. During this study, we found that slight alterations in the phasing of the β-ATP peak led to large changes in the reported magnesium concentrations. If the phase diverged from the baseline, thereby altering the detectable area under the curve by 0.01 degrees, the resulting magnesium concentration could vary between approximately seven and nineteen percent. This large range highlights the sensitivity of these measurements to proper phasing; therefore we chose to integrate the area under the curve using WinSpa. Although we chose different methods of analysis that ensured the most accurate phase, our results were higher than what we would have predicted based on the literature. In a study by *Iotti et al*. (37) the magnesium concentration in the calf was found to be 0.32 mmol and *Malucelli et al.* (63) found a similar concentration in the calf of 0.31mmol. We reported values that were higher in the quadriceps during the two pre testing sessions; they averaged 0.41 and 0.38 respectively and were more closely related to the values reported in the calf by *Ryschon et al.* (97). We are unsure as to the discrepancy between the literature reported values and ours.

The reproducibility between days for intramuscular magnesium was higher than the predicted literature values. *Ryschon et al.* (97) looked at the COV% when the coil was repositioned on the calf in five subjects between each of the five acquisitions. They found a COV% that was less than 6.1% for each subject. *Wray et al.* (116) calculated the variability

between days and found a variation of 4.1% when evaluating magnesium levels in the calf. We found a significantly higher variability of 7.91% between days in the vastus lateralis. We are unsure why our variability is higher due to the fact that we had good signal to noise. We expected to find relatively reliable results between days due to the fact that our signal to noise was forty seven percent better with two hertz line broadening and that we collected and averaged a total of one hundred and twenty scans. The higher sample size aided our improved signal to noise ration due to the fact that the ratio increases with the square root of the sample size. This variation between days may not be entirely due the ability of our 3T magnet to make such measurements, but may represent inconsistencies in the analysis, such as phasing.

We did not find any effect of exercise on oxidative metabolism or intramuscular magnesium concentrations. It was hypothesized that since magnesium is involved in numerous enzymatic reactions, changes in its concentration would alter metabolism. Numerous studies have reported sweat, urinary, and blood magnesium changes with exercise. *Bohl et al.* (10) stated that exercise is a stressor that may upset body magnesium homeostasis because it is associated with increased magnesium excretion through sweat and urine. *Consolazio et al*. (19) estimated that during endurance exercise, the sweat loss of magnesium was about one percent of the total body content. Excretion of magnesium has also been evaluated from urinary losses. *Lijnen et al.* (58) examined urinary magnesium losses in healthy males prior, immediately post, and twelve hours after a marathon. They found a significant increase in urinary magnesium excretion twelve hours post as compared to premarathon levels. *Rose et al.* (93) was the first to report a serum magnesium decrease after a marathon. They studied eighty highly trained males and found a significant mean decrease in serum magnesium levels post-marathon compared with pre-marathon values. Since this study, researchers have hypothesized that decreases in serum or

plasma magnesium levels may be due to shifts of magnesium into erythrocytes (12, 88), adipose (26, 51), or shifts of magnesium from the extracellular fluid into skeletal muscle . It was hypothesized by *Costill et al.* (22) that the reduction in serum magnesium is due to the redistribution of magnesium into the skeletal muscles during exercise because of the increased metabolic need. While changes in sweat, urinary, and blood have been extensively evaluated, changes in muscle magnesium have been researched to a lesser degree. *Lamb et al.* (53) found that muscle magnesium levels increase significantly during exhaustive exercise. They believe that this increase in magnesium would inhibit the release of calcium from the sarcoplasmic reticulum. *Blasev and Lamb* (7) examined the effect of increased free magnesium levels, low ATP concentration, and increased AMP and IMP concentrations on calcium release in skinned rat skeletal muscle fibers. They found that calcium release is controlled by concentrations of ATP, AMP, IMP and magnesium. They hypothesized that the reduction in calcium may be due to higher free magnesium levels and lower ATP concentrations near the calcium release channels. The purpose of our study was to test the effect of long duration exercise on intramuscular magnesium levels. We found that two hours of vigorous walking did not alter magnesium concentrations in the muscle when measured pre/post exercise. This may be due to the fact that the vigorous walking did not adequately activate the quadriceps muscles. EMG showed that only two percent of the quadriceps were active during the exercise. Since the quadriceps did not reach exhaustion, it is unlikely that magnesium changed significantly. Due to the decreased activation, a very small effect size (0.06) was detected. The precision of our measurements would allow us to detect a ten percent change, but the overall effect in our study was too small. From this study, we can state that two hours of vigorous walking does not alter magnesium in the quadriceps. We are not sure of the effect of prior vigorous exercise on the calf

or the effects of a more strenuous prior exercise protocol that targets the quadriceps. Further research with an exercise protocol that adequately fatigues the quadriceps in necessary to reevaluate changes in exercise induced magnesium concentrations.

Numerous studies have examined the effects exercise using varying work intensities to deplete phosphocreatine concentrations. *Arnold et al.* (1) found that the rate of PCr resynthesis was slower following heavy exercise where subjects squeezed a rubber bulb of a sphygmomanometer for 150 seconds at 500mmHg when compared to light exercise of a 270 second contraction at 100mmHg. *McCann et al.* (65) examined PCr recovery in the forearm of four adult males during moderate and heavy exercise. PCr recovery time constants for moderate work were found to be 35 ± 13.2s while heavy work showed an increased time constant to recovery of 50 ± 22.9s. Other studies have evaluated the metabolic effects of training. In a study by *Kent-Braun et al.* (48) seven subjects preformed wrist curls five days a week for eight weeks and they concluded that the training led to an increased capacity of oxidative metabolism.. Other studies have shown increases in mitochondria, oxidative enzymes (28, 35) and capillary density (56) as a result of training. Other studies have looked at the effects of prior exercise, such as a warm-up, on PCr recovery kinetics. *McCann et al.* (65) found a difference between the initial work bout and subsequent work bouts in the forearm of four male subjects. To correct for these differences, each subject preformed a warm-up contraction that was approximately half the length of a single moderate intensity contraction was preformed. *Walter et al.* (114) also measured PCr recovery kinetics in the gastrocnemius of eight male subjects, measured as V_{max}, with and without a warm-up and did not find a significant difference in the times to recovery. They concluded that unlike whole body oxygen consumption, which requires several minutes to achieve maximal values, large muscle ATP synthesis rates can be achieved without prior warm-

up. Our results agreed with *Walter et al.* (114) in that no difference between initial and subsequent contraction were seen. It has been hypothesized that an intense warm-up will enhance muscle oxygen delivery (17, 27), therefore, PCr recovery may be altered due to oxygen availability (33). It is also possible that changes in magnesium concentration may effect the rate of PCr recovery because decreases in bound magnesium may limit these phosphorylation reactions. Since magnesium in a primary stabilizing cofactor in ATP dependent reactions (10, 101), changes in bound magnesium levels may alter the phosphorylation and dephosphorylation of the mitochondrial ATP (95). The phosphate from the mitochondrial ATP is used to rephosphorylate PCr; therefore, changes in this oxidative metabolism may be reflected through magnesium concentrations and/or phosphocreatine recovery kinetics (95). Our prior exercise differed from *Walter et al.* (114) and *McCann et al.* (65) in that vigorous incline walking was preformed for two hours in place of a short duration exercise protocol. No difference in PCr recovery kinetics were seen as a result of the long duration exercise. Since no changes in magnesium were detected post exercise, PCr remained consistent and we concluded that oxidative metabolism measurements in the quadriceps are not effected by prior vigorous activity. These results have significant implications on future muscle metabolism studies. In the past, subjects restrained from vigorous physical activity twenty-four hours prior to testing, but these results have shown that the measurements are more robust. The potential limitations to this conclusion is the intensity of the prior exercise and the target muscle to be studied. Since only two percent of the vastus lateralis was activated during the long duration exercise protocol, it is plausible that a change in either magnesium or phosphocreatine was not evident due to the inactive muscle fibers. The effect of walking on the calf muscle were not evaluated; therefore only the effects of exercise on muscle metabolism can be evaluated. To our knowledge, this is

the first study to evaluate PCr recovery using ^{31}P MRS after long duration exercise and further tests are necessary to establish the reliability of these measures

CHAPTER FOUR

Summary and Conclusions

While we were unable to identify changes in PCr recovery kinetics or magnesium concentrations after two hours of vigorous exercise, this study did discover valuable information that will benefit future research in this area.

The majority of studies that examine muscle metabolism through PCr recovery use in magnet ergometers as a means to deplete PCr. The problem with this is that in magnet ergometers are expensive and time consuming to create. To our knowledge this is one of the first studies to examine PCr recovery using a maximal voluntary isometric contraction of the quadriceps. This is valuable because it demonstrates that ergometers are not necessary to successfully evaluate recovery kinetics in healthy humans. This study shows that if a subject is capable of performing isometric contraction, adequate depletion of PCr will be achieved and the mitochondrial function quantified.

Valuable information was also found concerning prior exercise and metabolism in the quadriceps. In the past, subjects were required to not perform any vigorous physical activity twenty four hours prior to testing. It is now known that these measurements are more robust. This study shows that magnesium concentrations and muscle metabolism in the quadriceps are not effected to a detectable difference after long duration submaximal exercise. This increases the availability of potential subjects to be tested by extending the population to athletes who normally perform daily physical activity part of their training regime. In the past, recruitment of this type of population was more difficult because the subject was forced to deviate from their

prescribed exercise training. This information also benefits the researcher because it is now known that the results will not be altered if a subject jogs to the magnet. Magnet time is expensive, and if the researcher expects that the results may be compromised, the test will be canceled. This not only wastes the researcher and subject's time, but slows the progression of the study. It was shown that if a subject is late and rushes to the magnet, the researcher can be confident that this slight change in physical activity will not alter metabolism results in the quadriceps.

Parameters for muscle metabolism in healthy individuals have been established and can now be compared to various compromised populations. This is beneficial because training programs to improve muscle metabolism can now be related not only to pre/post improvements within subjects, but also provide a baseline for comparison between healthy and compromised individuals. One example is PCr recovery in SCI subjects. Impaired muscle metabolism is a contributing factor to their deteriorating health. Current policy states that there is no physical benefit of exercise for complete SCI patients and only provides temporary mental benefits. It is now possible to evaluate whether exercise can improve muscle metabolism in complete SCI patients. If the above is true, exercise protocols will now be assigned to all complete and incomplete patients as a means to improve mental and physical health.

This study also had an impact on future studies on magnesium. It was learned that the spectra phase is critical to achieve reliable results. Small shifts in the β-ATP correspond to large magnesium concentration changes.

CHAPTER FIVE

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Figure legend

Figure 3.1: NIRS and cuff placement.

Figure 3.2: Placement of NIRS probe on the quadriceps muscle for determination of oxygen saturation during isometric exercise.

Figure 3.3: Representative results for oxygen saturation during rest and exercise. Oxygen saturation from one channel representing the average response to exercise is shown.

Figure 3.4: EMG activity of the vastus lateralis. Root mean squared EMG values during the six practice trials are show (means and SD). There was a significant enhancement of the EMG signal after the subject was allowed to see their emg signals.

Figure 3.5: Representative resting and end exercise spectra from one subject.

Figure 3.6: Linear regression of NIRS depletion and PCr depletion.

Figure 3.7: Representative PCr recovery curve after isometric exercise.

Figure 3.8: PCr Tc to recovery pre/post exercise. Values are presented as means $\pm$ SD.

Figure 3.9: Intramuscular [Mg^{+2}] pre/post exercise. Values are presented as means $\pm$ SD.

Table 3.1 Subject characteristics

	Subject characteristics			Physical activity		
	age	height	weight		min/week	
	yrs	cm	kg	light	moderate	heavy
mean	22	179	82	131	76	30
sd	1.9	3.7	33.1	78.5	42.3	31.8

n = 12

Table 3.2: Changes in PCr Tc to recovery pre/post exercise

	Pre 1	Pre 2	30-60 min post ex	1-3d post ex
mean	39.9	39.1	37.5	38.7
sd	12.7	16.9	14	12.4
ICC $_{\text{b/t pre 1 \& 2}}$	0.819			
COV% $_{\text{b/t pre 1 \& 2}}$	18.4			
ICC $_{\text{within pre 1}}$	0.928			
COV% $_{\text{within pre 1}}$	8.43			

Table 3.3 Changes in muscle metabolites pre/post exercise

		Rest			End ex	
		Pi/PCr	PCr/ATP	pH	PCr %	pH
Pre 1	mean	0.089	4.24	7.07	46.1	6.94
	sd	0.029	0.664	0.031	11.6	0.07
Pre 2	mean	0.092	4.68	7.06	52	6.97
	sd	0.012	0.893	0.029	14.1	0.105
30-60min post ex	mean	0.080	5.14	7.06	48.9	6.98
	sd	0.017	0.981	0.022	14	0.103
1-3d post ex	mean	0.092	5.12	7.05	43.5	6.98
	sd	0.023	1.08	0.019	7.8	0.078
$COV\%_{between\ pre\ 1\ and\ pre\ 2}$		15.1	8.60	0.21	11.1	0.79
$ICC_{between\ pre\ 1\ and\ pre\ 2}$		0.13	0.79	0.45	0.87	0.64

Table 3.4 [Mg^{+2}] changes pre/post exercise

	Pre 1	Pre 2	30-60 min post ex	1-3d post ex
mean	0.414	0.376	0.386	0.375
sd	0.151	0.034	0.035	0.037
ICC $_{\text{b/t pre 1 \& 2}}$	0.352			
COV% $_{\text{b/t pre 1 \& 2}}$	7.91			

Figure 3.1 NIRS and Cuff placement

Figure 3.2 Oxygen saturation measured with NIRS

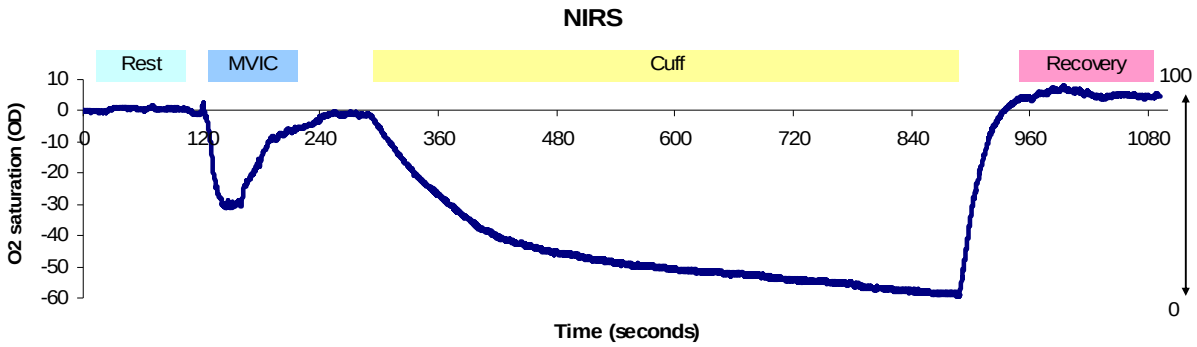

Figure 3.3 Oxygen saturation during rest and exercise

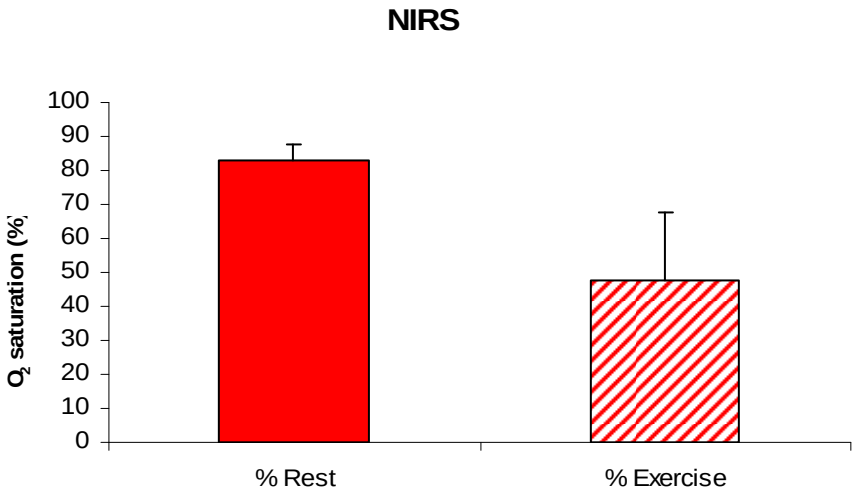

Figure 3.4 EMG activation of the vastus lateralis

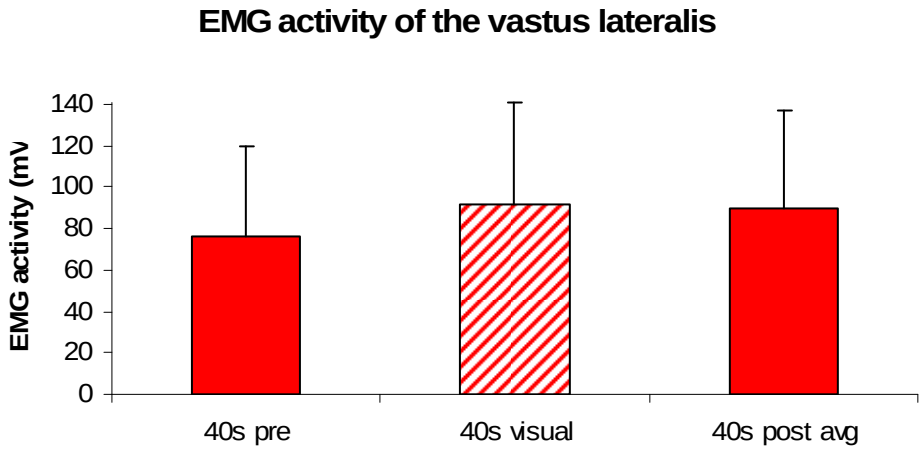

Figure 3.5: Resting and end exercise spectra

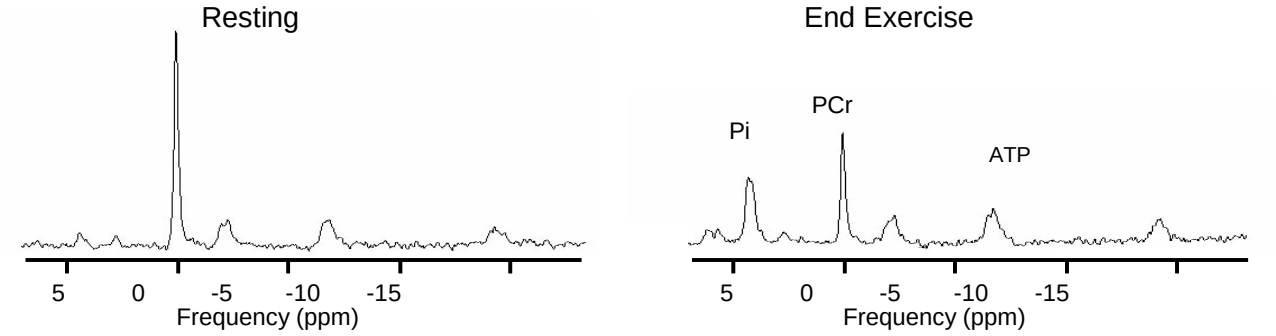

Figure 3.6: Linear regression of NIRS depletion and PCr depletion

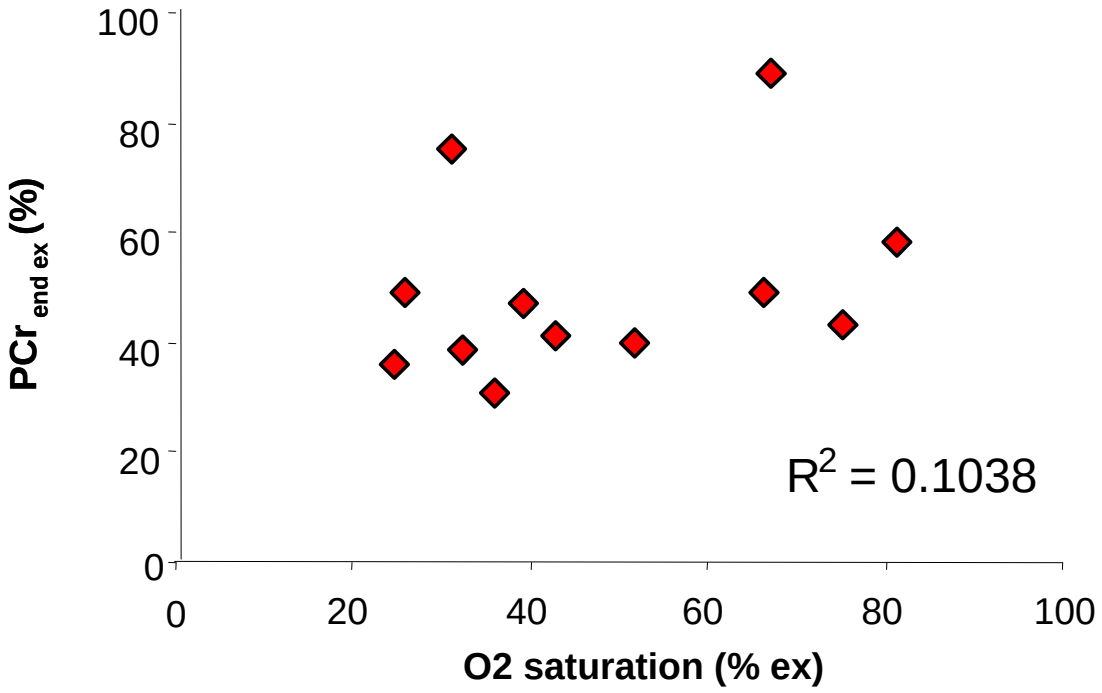

Figure 3.7: PCr recovery curve

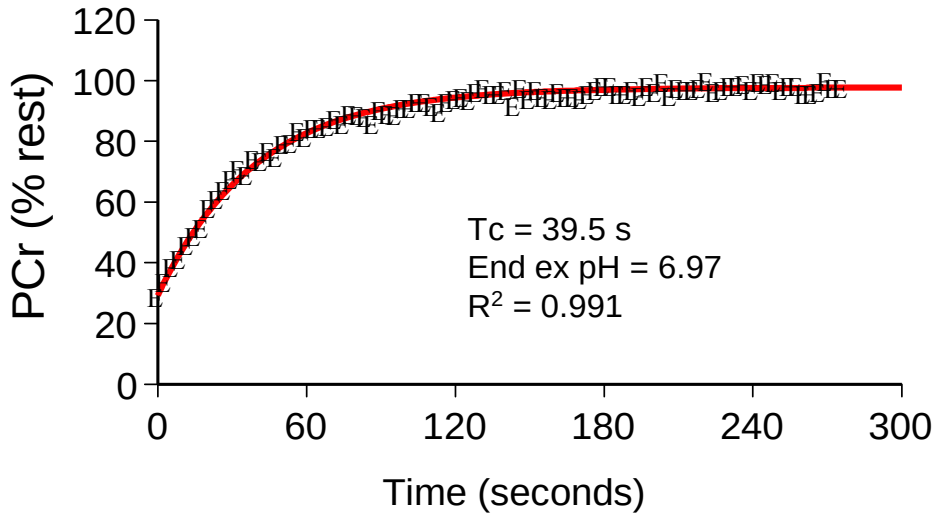

Figure 3.8: PCr Tc to recovery pre/post exercise

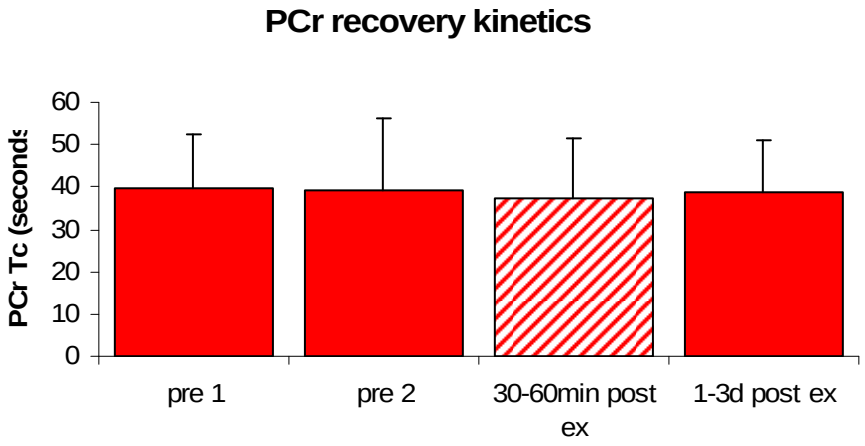

Figure 3.9: Intramuscular [Mg^{+2}] pre/post exercise