7-3-2012

Effect of Creatine Supplementation on Wrist Flexion Work and Power after Immobilization

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EFFECT OF CREATINE SUPPLEMENTATION ON WRIST FLEXION WORK AND POWER AFTER IMMOBILIZATION

BY

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DISSERTATION

Submitted in Partial Fulfillment of the
Requirements for the Degree of

Doctor of Philosophy
Physical Education, Sports and Exercise Sciences

The University of New Mexico
Albuquerque, New Mexico

May 2012

ACKNOWLEDGMENTS

I would like to thank Dr. Suzanne Schneider, Dr. Len Kravitz, Dr. Robert Robergs, Dr. Christine Mermier, Dr. Carole Conn, and Dr. Ann Gibson for your guidance and support throughout this dissertation process.

Dr. Suzanne Schneider, thank you for your assistance all the way through my doctoral experience. Your expertise, honesty, and openness to opposing views enabled me to make this research idea become a reality. Thank you for teaching me the importance of acquiring the necessary background and current knowledge when exploring potential research opportunities.

Dr. Rob Robergs, thank you for teaching me the importance of critical thinking, not accepting the status quo, but to challenge conventional thinking when new data supports an updated view, and to strive for professionalism in exercise physiology.

Dr. Len Kravitz, thank you for your sound advice and enthusiasm over the years and for teaching me the essential components to the science and art of teaching. Thank you for inspiring everyone to excel in both written and verbal presentations.

Thank you, Steve, Dean, and the rest of the group at New Mexico Resonance for your time, generosity, and patience during data collection. I would also like to thank Genevieve Birren for assistance in the completion of this manuscript. To my friends and family, thank you for believing in me.

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ABSTRACT

The purpose of this dissertation was to identify the effect of creatine (Cr) supplementation during immobilization on muscle performance. Twenty-five healthy, active male (n = 14; age 28 ± 5 years) and female (n = 11; age 22 ± 4 years) subjects performed wrist flexion exercise before (PRE) and after (POST) one week of wrist/forearm cast immobilization. During the immobilization period, subjects consumed Cr (20g) or placebo PL (4% CHO flavored solution) interspersed throughout the day. On the first day of immobilization subjects consumed two doses of Cr (10 g) or PL. Subjects consumed 5 g of Cr or PL on four occasions from day 2 to day 7 for a total of 20 $g \cdot d^{-1}$. On day 8, subjects were instructed to consume a total of 5 g CR or PL, and the cast was removed prior to POST test. Exercise was performed on a custom developed rotational ergometer device attached by a cord to suspended weights across a wall pulley. Wrist flexion exercise commenced with an incremental protocol to fatigue followed by a 4.8 min rest. Then constant-load exercise (CL1) was performed with the peak weight

achieved in the incremental protocol for 2.4 min, followed by another 4.8 min rest. The second constant-load exercise bout (CL2) used the same weight as CL1 for a final 2.4 min endurance exercise bout. Total work and average power was quantified by the kg load, the distance the weight was lifted, and the time to conduct the work. Immobilization caused a significant decrement in forearm total work (-3.17 ± 2.27% PL; -2.61 ± 1.89% CR) and average power (-3.43 ± 2.35% PL; -2.61 ± 1.89% CR) during the incremental protocol, regardless of Cr supplementation. During the first CL bout, both total work and average power decreased in PL after immobilization (-28.9 ± 9.63%; $p < 0.05$), but not in CR. However, during CL2, both total work and average power decreased in CR (work -14.39 ± 4.54%; power -10.52 ± 4.6% and PL (work -21.98 ± 8.28%; power -21.3 ± 8.25%) groups after immobilization. The significance of these findings are; 1) 7 days of wrist immobilization will significantly decrease wrist flexion work and power, 2) this decrement can be slowed with Cr supplementation for an initial constant load exercise bout performance, and 3) however, this beneficial effect may be lost with repeated bouts of constant load exercise. Future research should help elucidate the underlying mechanisms of fatigue after immobilization to explain possible differences in incremental and constant load endurance exercise performance.

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SYMBOLS / ABBREVIATIONS

≥: greater than or equal

≤: less than or equal

>: greater than

<: less than

±: plus-minus sign

ADP: adenosine diphosphate

ANOVA: analysis of variance

ATP: adenosine triphosphate

BM: body mass

bpm: beats per minute

BW: body weight

°C: degrees Celcius

CK: creatine kinase

CL1: constant load exercise bout 1

CL2: constant load exercise bout 2

cm: centimeter

CP: creatine phosphate

Cr: creatine

CR: creatine monohydrate

CSA: cross-sectional area

df: degrees of freedom

$d \cdot wk^{-1}$: days per week

fCr: free creatine

$g \cdot d^{-1}$: grams per day

$g \cdot kg^{-1}$: grams per kilogram

h: hour

$hrs \cdot wk^{-1}$: hours per week

J: Joule

kcal: kilocalorie

kg: kilogram

kgm: kilogram meter

$kgm \cdot min^{-1}$: kilogram meters per minute

km: kilometer

L: liter

$L \cdot min^{-1}$: liters per minute

lb: pound

LBM: lean body mass

MHC: myosin heavy chain

mg: milligram

min: minute

ml: milliliters

$mmol \cdot L^{-1}$: millimoles per liter

$ml \cdot min^{-1}$: milliliters per minute

MRF4: myogenic regulatory factor 4 protein

n: subject number

PFK: phosphofructokinase

P: power

^{31}P-MRS: phosphorus-magnetic resonance spectroscopy

Pi: inorganic phosphate

PL: placebo group

PV: plasma volume

s: second

SE: standard error

std: standard deviation

TBW: total body water

tCr: total creatine

W: watt

CHAPTER 1

Introduction

Creatine (Cr), which comes from the Greek word *kreas*, meaning flesh, is a naturally occurring protein compound found in meat and fish. It is also endogenously synthesized from the amino acids arginine, glycine, and methionine in the human liver and pancreas (Wyss & Kaddurah-Daouk, 2000). In an average 70 kg adult, the total Cr pool amounts to ~120 g, of which most (~ 95%) is contained in skeletal muscle with ~ 65% in phosphorylated form of phosphocreatine (PCr) (Casey & Greenhaff, 2000). During high-intensity exercise, PCr hydrolysis plays a crucial role in skeletal muscle metabolism by maintaining a high rate of adenosine triphosphate (ATP) production. Cr acts through several mechanisms, including: 1) aids in rapid rephosphorylation of adenosine diphosphate (ADP) back to ATP via the creatine kinase reaction (Hespel et al., 2001; Yquel, Arsac, Thiaudiere, Canioni, & Manier, 2002); 2) enhances myosin head cross-bridge recycling and tension maintenance (Wyss & Kaddurah-Daouk, 2000); 3) buffers acidosis by consuming H^+ during the creatine kinase reaction, thereby improving cellular homeostasis (Mesa, Ruiz, Gonzalez-Gross, Sainz, & Garzon, 2002); 4) increases the rate of glycolysis through the phosphofructokinase reaction and thus the rapid production of ATP (Demant & Rhodes, 1999); 5) enhances oxidative phosphoylation and decreases protein and/or nucleotide degradation via increased oxygen uptake coupled to a reduction in ammonia accumulation (Rico-Sanz & Mendez Marco, 2002); and, 6) shortens muscle relaxation time during intermittent maximal isometric contractions by means of facilitated calcium uptake by the sarcoplasmic reticulum (Van-Leemputte, Vandenberghe, & Hespel, 1999).

Oral Cr supplementation at a dose of 20 g·day^{-1} for 3-5 days (loading phase) increases total muscle creatine (TCr) content, free creatine (fCr), and PCr (Casey, Constantin-Teodosiu, Howell, Hultman, & Greenhaff, 1996). Early work using muscle biopsy showed that Cr ingestion favorably improved muscle metabolism by increasing PCr availability in type II muscle fibers (Greenhaff, Bodin, Soderlund, & Hultman, 1994). Cr ingestion was also shown to enhance muscle PCr resynthesis during recovery from intense muscle contractions resulting in enhanced fatigue resistance during short duration high-intensity exercise (Balsom, Soderlund, Sjodin, & Ekblom, 1995). Subsequent non-invasive research on human skeletal muscle *in vivo* using phosphorus magnetic resonance spectroscopy (^{31}P MRS) has shown similar muscle metabolic improvements during intermittent maximal exercise. Yiquel et al. (2002) examined the effect of Cr ingestion (20g/day for 6 days) on muscle power, PCr resynthesis, inorganic phosphate (Pi), and pH during 8 repeated brief bouts of maximal dynamic plantar flexion exercise. Following Cr supplementation, resting muscle PCr increased by 15%, along with an increased rate of muscle PCr resynthesis during recovery, a lower Pi concentration, higher muscle pH, and improved maintenance of muscle power output. In another study using ^{31}P MRS by Rico-Sanz (2000), Cr supplementation (5 g·d^{-1} x 11d) reduced net muscle PCr utilization, Pi accumulation, and acidosis during repeated bouts of low-intensity isometric exercise (32% of MVC) of the plantar flexors to exhaustion.

There is evidence that suggests Cr supplementation at rest and during resistance training may be an effective intervention to reverse or maintain lower-body muscle mass during and after limb immobilization (Hespel et al., 2001). Most recently, Johnston and co-workers (2009) examined the effects of cast-induced immobilization of the upper limb

between Cr and placebo interventions. After baseline measures of lean tissue mass (dual-energy X-ray absorptiometry), strength (1-repetition maximum [RM] isometric single arm elbow flexion/extension), and muscle endurance (maximum number of single-arm isokinetic elbow flexion/extension repetitions at 60% 1 RM), subjects had their dominant or non-dominant upper limb immobilized at 90˚ elbow flexion for 7 days. Compared to placebo, Cr supplementation better maintained muscle mass, elbow flexor strength, elbow flexor endurance, elbow extensor strength, and elbow extensor endurance during immobilization. These results indicate that short-term Cr supplementation attenuates the loss in muscle mass and strength during upper-arm immobilization.

Limb immobilization, bed rest, weightlessness, and aging are all associated with a decline in muscle strength and endurance capacity with concomitant reductions in muscle size, type II muscle fiber diameter, mitochondrial enzyme activity, and high-energy phosphate metabolism. There is evidence to suggest that reduced levels of resting muscle PCr in the elderly may be responsible for the age-related decline in muscle strength and endurance (McCully et al., 1991). In addition, PCr resynthesis rates following exercise decrease by ~8% every decade after 30 yr of age (McCully & Posner, 1992). Smith et al. (1998) studied young (30 ± 5 yr) and middle-aged (58 ± 4 yr) men and women using ^{31}P MRS during single-leg knee-extension exercise. Resting muscle PCr was lower and initial PCr resynthesis rate was slower in the middle-aged group. Following 5 days of Cr ingestion at 0.3 g·kg^{-1}·day, resting PCr increased 15% in the young group and 30% in the middle aged group. The middle-aged group also had greater improvements in PCr hydrolysis during exercise and initial PCr resynthesis rate after exercise compared with the young group. These results suggest that Cr would be an effective nutritional

supplement for improving muscle metabolism and performance for men and women of increasing age, as well as if forced to endure prolonged inactivity (e.g. spaceflight) or joint/limb immobilization such as during recovery from musculoskeletal injury.

Problem Statement

Cr supplementation has been shown to improve muscular strength and endurance in a variety of studies and from a diverse number of research laboratories. However, application of Cr supplementation to mitigate the atrophy and detraining from limb immobilization in human subjects has only been researched by one prior investigation (Johnston, Burke, MacNeil, & Candow, 2009). Though the results of Johnston et al. (2009) were positive to the benefits of Cr supplementation to preservation of muscle mass and strength following 7 days of upper-limb immobilization, this study did not directly assess muscle work and peak power during forearm immobilization. Given that there seems to be a role for Cr in maintenance of muscle integrity and function during immobilization, which has application to athletes who are injured or the elderly, this topic warrants further investigation to assess muscle function during and following high intensity exercise. We expect that Cr supplementation will mitigate the decrement in forearm work and power during one week of writs/forearm immobilization.

Purpose of the Study

This study was conducted to compare the effects of short term immobilization of the forearm between Cr and placebo interventions. Forearm wrist flexion exercise during

incremental exercise, and repeated bouts of intense exercise were analyzed for total muscle work and average muscle power.

Hypotheses

The following research hypotheses were tested in this study.

Hypothesis I.

There will be a significant ($p < 0.05$) decrease in the PL group, but not the Cr group following immobilization for work and power during an incremental, dynamic wrist flexion exercise to exhaustion.

Rationale. Cr supplementation has demonstrated effectiveness to improve exercise performance. Cr has also shown to attenuate muscle atrophy, strength, and endurance of the upper limbs following cast-induced immobilization (Johnston, Burke, MacNeil, & Candow, 2009).

Hypothesis II.

There will be a significant ($p < 0.05$) decrease in the PL group, but not the Cr group following immobilization for work and power during constant load dynamic wrist flexion exercise 4.8 minutes following the incremental exercise bout to exhaustion.

Rationale. While the results from the Johnston et al. (2009) study measured isometric strength and endurance, it is reasonable to expect Cr supplementation will maintain dynamic wrist flexion exercise to fatigue following immobilization.

Hypothesis III.

There will be a significant ($p < 0.05$) decrease in the PL group, but not the Cr group following immobilization for work and power during constant load dynamic wrist flexion exercise 4.8 minutes following the first steady-state exercise bout to exhaustion.

Rationale. Cr supplementation has been demonstrated to improve intermittent exercise performance through several physiologic mechanisms, most notably rephosphorylation of ATP via the creatine kinase reaction. Therefore, it is reasonable to expect Cr will improve forearm flexion work and power during the final steady-state exercise bout.

Scope of the Study

Twenty-five healthy, resistance-trained male (n = 14) and female (n = 11) subjects were randomly assigned to either a treatment group or placebo group. Approximately one week prior to forearm immobilization, both groups performed a familiarization protocol consisting of incremental wrist flexion exercise to exhaustion determine the peak load for the incremental protocol. Immediately after the incremental protocol, subjects rested for 4.8 min, and then perform two additional bouts of wrist flexion exercise involving 2.4 min of exercise with a load equal to the load attained during the last stage of the incremental protocol, interspersed with 4.8 min of recovery. Subjects completed pre-testing on day 1, and then ingested Cr or placebo starting with the evening meal on day 1. On day 2, subjects were fitted with a hand/forearm cast, and the cast remained on through the morning of day 8. The cast was then removed immediately prior to post-immobilization exercise testing.

Assumptions

The following assumptions were made in this study:

1. The subjects ingested Cr as instructed.
2. All subjects had not used Cr supplements for at least 2 months prior to testing.
3. The subjects remained in a cast during the treatment period.
4. The subjects exerted maximal effort for the exercise test protocol.

Limitations

The following limitations were identified for this study:

1. The study group consisted of healthy, trained male and female subjects in the age range of 18 to 33 years old; therefore, the results can only be generalized to a healthy, trained population of similar age.
2. This study measured the muscles of the forearm. Caution must be employed when generalizing the findings to muscles or muscle groups that may contain different fiber types and/or metabolic properties.
3. This study used immobilization to study the effects of muscle atrophy. Care should be used when extrapolating the results to other conditions that exacerbate muscle atrophy such as aging, muscle wasting diseases, spaceflight, or bed rest.

Significance of the Study

This study identified the effectiveness of Cr supplementation to maintain muscle function during short-term immobilization. No prior study has used forearm/wrist immobilization combined with Cr supplementation. Additionally, muscle power output and time to fatigue were assessed. The results of this study may provide insight into the muscle performance changes that occur during immobilization as well as the effectiveness Cr supplementation to offset muscle dysfunction. If demonstrated effective, Cr supplementation would be a cost-effective therapeutic intervention to slow the changes in muscle during immobilization. Future research could explore the effectiveness of Cr supplementation during other muscle disuse scenarios such as bed rest and spaceflight.

Definition of Terms

The terms in this study have been operationally defined as follows:

Acidosis. A decrease in pH (increase in free hydrogen ion concentration).

Adenosine diphosphate (ADP). A nucleotide that is the product of the ATPase reaction.

Adenosine triphosphate (ATP). A multifunctional nucleotide that transfers chemical energy within cells for metabolism.

Amino acids. Molecules that contain a carboxylic acid group, an amine group, and a variety of side chains.

Ammonia. A bi-product of the AMP deaminase reaction that forms IMP.

Anabolism. The metabolic process that requires energy to build molecules from smaller units.

Anaerobic capacity. The capacity of skeletal muscle to regenerate ATP from non-mitochondrial respiration pathways.

Anaerobic metabolism. Reactions of metabolism that do not require the presence of oxygen.

Anaerobic threshold. The exercise intensity when there is an abrupt increase in creatine phosphate hydrolysis and glycolysis, resulting in increased lactate production and the decrease in muscle creatine phosphate.

Atrophy. Decrease in size.

Buffering capacity. The capacity to remove free hydrogen ions from solution.

Catabolism. The metabolic process involving the breakdown of relatively complex molecules with the subsequent release of energy.

Concentric muscle action. Muscle actions involving muscle shortening.

Creatine. A nitrogenous compound that is a product of the creatine kinase reaction.

Creatine kinase. An enzyme that facilitates the regeneration of ATP via creatine phosphate hydrolysis and ADP.

Creatine monohydrate. The supplemental, powdered form of creatine widely used a dietary supplement.

Creatine phosphate. See phosphocreatine.

Dietary supplement. Substances that are not considered drugs or normal foods or food additives.

Eccentric muscle action. Muscle actions involving muscle lengthening.

Endogenous. Substances that are produced within an organism or cell.

Ergogenic. That which improves physiologic variables associated with exercise performance such as drugs or dietary supplements.

Ergometry. A science concerning the measurement of work.

Exogenous. Substances that are produced outside an organism or cell.

Extracellular. Outside the cell.

Free creatine. Creatine in the skeletal muscle cells that is in the non-phosphorylated form.

Hypertrophy. Increase in size.

Inorganic phosphate. Phosphorus found as a free phosphate ion in solution.

Intracellular. Inside the cell.

Muscle disuse. General term used to describe situations and consequences of reduced muscle actions such as during immobilization, bed rest, or spaceflight.

Palmaris longus. An anterior arm muscle that acts as a wrist flexor. It originates on the medial epicondyle of the humerus and inserts on the palmar surface of the hand.

Phosphagen system. Energy system used to generate ATP quickly during high-intensity exercise through the ATPase reaction, creatine kinase reaction, adenylate kinase reaction, and AMP deaminase reaction.

Phosphocreatine. Creatine in the phosphorylated form that serves as an energy store in skeletal muscle.

Phosphorus Magnetic Resonance Spectroscopy (P-MRS). A non-invasive method of measuring muscle biochemistry with a magnetic field that excites phosphorus nuclei to produce a detectable signal used to quantify high energy phosphate metabolites.

Plasma Volume. The non-cellular portion of the blood.

Power. Work performed in a given time period.

Protein. Compounds consisting of one or more polypeptides, or peptide-bound chains of amino acids.

Total Body Water. The total amount of water in the body.

Work. Force applied against gravity for a given distance.

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CHAPTER 2

This chapter presents a review manuscript, entitled “Exploring the Potential of Creatine to Maintain Muscle Function During Immobilization” that will be submitted to the International Journal of Sports Nutrition and Exercise Metabolism. It is authored by Jeremy C. Fransen and Suzanne Schneider. The manuscript follows the formatting and style guidelines of the journal. The references cited are provided at the end of the manuscript.

Exploring the Potential of Creatine Ingestion to Maintain Muscle Function During Immobilization

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ABSTRACT

Limb immobilization (casting) results in a loss of muscle mass along with decrements in muscle strength and endurance. In addition, immobilization has been shown to impair high energy phosphate catabolism in skeletal muscle. Creatine ingestion in conjunction with resistance training can significantly increase muscle force production, augment muscle size increases, and improve energy-rich phosphate catabolism in skeletal muscle. Surprisingly, there are few research studies investigating the potential of oral creatine supplementation to mitigate the muscle changes during immobilization. Early investigations found that creatine supplementation can maintain muscle size, strength, and endurance during short-term (1-2 wk) immobilization. Future research should focus on whether creatine supplementation can also prevent muscle metabolic consequences during immobilization. Creatine ingestion during short-term immobilization may help to identify the underlying mechanisms that contribute to the reported ergogenic effects.

Key Words: Casting, Muscle performance, Creatine phosphate

INTRODUCTION

The effects of muscle disuse include decreases in muscle cross-sectional area (CSA) (Hather, Adams, Tesch, & Dudley, 1992), reduced muscle specific force (Yue, Bilodeau, Hardy, & Enoka, 1997), alterations in muscle contractile properties (Seki, Taniguchi, & Narusawa, 2001), increased muscle protein degradation (Berg, Dudley, Haggmark, Ohlsen, & Tesch, 1991), change in muscle fiber type distribution from type I to type IIb fibers (Desplanches, Mayet, Sempore, & Flandrois, 1987), neuromuscular adjustments (Duchateau & Hainaut, 1987), and a shift in muscle metabolic properties including loss of oxidative properties (Neeti Pathare et al., 2005). The aforementioned muscle changes occur as a result of inactivity, ageing, bed rest, spaceflight, limb suspension, or joint immobilization. Joint immobilization has been particularly useful as a model to investigate changes in skeletal muscle from disuse (Berg et al., 1991; Miles et al., 1994). Recent evidence suggests that creatine (Cr) supplementation may slow the rate of muscle loss and dysfunction during cast-induced immobilization (Johnston, Burke, MacNeil, & Candow, 2009). The goal of this article is to review the role of Cr in muscle energy metabolism and exercise performance, muscle function, the consequences of muscle disuse during immobilization, and to argue for the potential use of oral Cr supplementation to minimize the changes in muscle size and function during immobilization.

Biochemistry of creatine

Cr is a nitrogenous amino acid compound with the chemical name α-methylguanidinoacetic acid. Cr has a positive charge with a molecular weight of 131 Da

(Mesa, Ruiz, Gonzalez-Gross, Sainz, & Garzon, 2002). Cr was discovered in 1835 by the French scientist Chevreul, with the first supplementation studies beginning in the early 1900s (Cathcart, 1909; Chanutin, 1926; Mendel & Rose, 1911). The role of Cr in energy metabolism was clarified with the discovery of phosphocreatine (PCr) in 1927, followed by the creatine kinase (CK) reaction in 1934 (Wyss & Kaddurah-Daouk, 2000). CK catalyzes the reversible transfer of the γ-phosphate group of ATP to the guanidine group of Cr to yield ADP, PCr and H^+ (Fig. 1). Thus, Cr is the substrate of CK to form PCr (Mesa et al., 2002). The high-energy phosphoryl group of PCr is transferred to ADP during exercise to maintain ATP concentrations in the muscle. Cr can then be recycled or transformed to creatinine (Crn) to be excreted in the urine (Mesa et al., 2002). During recovery from intense exercise, the large pool of PCr in fast-twitch skeletal muscle allows immediate regeneration of ATP (Fig. 1). Due to the high cytosolic content of CK in skeletal muscle, the CK reaction remains at near equilibrium maintaining an almost constant ATP concentration, and thus buffers the cytosolic phosphorylation potential that is crucial for optimal cellular ATPase function (Wyss & Kaddurah-Daouk, 2000).

The demand for Cr approximates 2 $g \cdot d^{-1}$ to replace the catabolized Cr that is excreted from the kidneys as Crn (Harris, 1992). The daily requirement for Cr can be met through a combination of *de novo* biosynthesis (~ 1 $g \cdot d^{-1}$) and intestinal absorption of dietary Cr (~ 1 $g \cdot d^{-1}$) (Mesa et al., 2002). The amino acids glycine, arginine, and methionine are involved in the endogenous synthesis of Cr in the liver, kidney, and pancreas. The first two steps of Cr biosynthesis involve the transfer of the amidino group of arginine to glycine, which is catalyzed by L-arginine:glycine amidinotransferase (AGAT), to yield L-ornithine and guanidinoacetate. Guanidinoacetate is then methylated

at the amidino group via the action of S-adenosyl-L-methionine:N-guanidinoacetate methyltrasferase (GAMT) to yield S-adenosyl-L-homocysteine and Cr (Wyss & Kaddurah-Daouk, 2000). The formation of guanidinoacetate by AGAT is the rate-limiting step in Cr synthesis (Walker, 1979). Endogenous Cr biosynthesis is reduced following increased dietary Cr ingestion (Walker, 1979), but returns to normal once dietary intake is reduced (Persky & Brazeau, 2001).

An omnivorous diet provides approximately 1 $g \cdot d^{-1}$ of Cr with normal plasma Cr levels ranging from 50-100 $\mu mol \cdot L^{-1}$ (Harris, 1992; Williams, 1998). Typical dietary sources of Cr include meat, fish, and other animal products. Following ingestion, Cr is absorbed by the gut and enters the bloodstream where it accumulates in CK-containing tissues, predominately skeletal muscle. Greater than 90% of Cr enters the skeletal muscle through the binding of specific transporter proteins located in the muscle fiber membranes (Mesa et al., 2002). The main Cr transporter expressed in skeletal muscle is creatine transporter 1 (CRT-1) (Sora et al., 1994). CRT-1 belongs to a super-family of neurotransmitter transporters and is mediated by Na^+-K^+ ATPase mechanisms which are dependent on extracellular concentrations of Na^+ and Cl^- (Dai, Vinnakota, Qian, Kunze, & Sarkar, 1999). The CRT-1 transport mechanism demonstrates a Michaelis-Menten constant (K_m) for Cr in the micromolar range of 15–30 $\mu mol \cdot L^{-1}$, which allows for sufficient Cr transport into skeletal muscle (Sora et al., 1994).

An average 70 kg male maintains approximately 120 g of Cr, of which 95% is stored in the skeletal muscle. The total creatine (TCr) pool in the muscle exists as both free creatine (FCr) and PCr, with approximately 60% of the TCr as PCr, and the remainder as FCr (Williams, 1998). As explained earlier, Cr is an important source of

energy for muscle contraction because of its rapid phosphorylation to form PCr, along with the reversible rephosphorylation of ADP to form ATP via the CK reaction. Due to the limited availability of ATP and PCr in muscle, Cr supplementation may increase TCr concentrations, facilitate intramuscular PCr and subsequent ATP formation, thus prolonging high-intensity exercise performance (P. D. Balsom, Soderlund, K., Sjodin, B., & Ekblom, B., 1995). Cr supplementation has also been shown to enhance myosin head cross-bridge recycling (Wyss & Kaddurah-Daouk, 2000), buffer acidosis by consuming H^+ during the CK reaction (Mesa et al., 2002), increase the rate of glycolysis through the phosphofructokinase (PFK) reaction (Demant & Rhodes, 1999), and shorten muscle relaxation time during intermittent maximal isometric contractions (Vandenberghe, Van Hecke, Van Leemputte, Vanstapel, & Hespel, 1999).

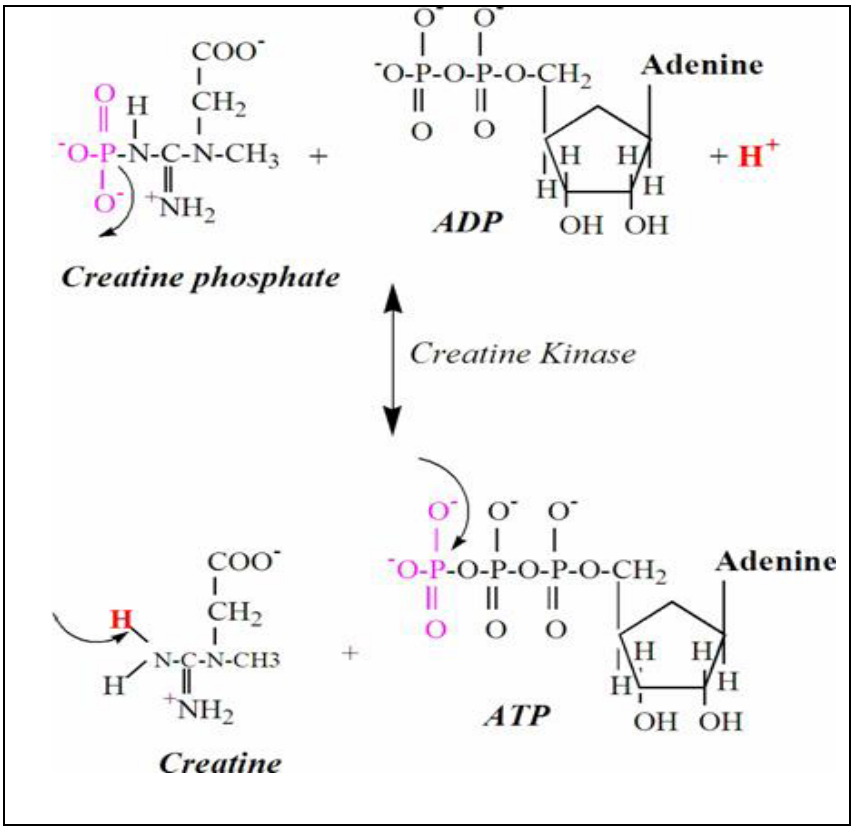

Figure 1: The creatine kinase reaction

Muscle changes during immobilization

There are substantial immediate and longer-term physiologic changes in human skeletal muscle as a consequence of disuse. Muscle fiber atrophy is one of the most studied responses to muscle disuse in humans (Sargeant, Davies, Edwards, Maunder, & Young, 1977a). Early research on the effects of muscle atrophy was conducted on patients that were plaster cast immobilized following limb trauma or surgery (Gibson et al., 1987; Haggmark, Jansson, & Ericksson, 1981; Sargeant et al., 1977a; Young, Hughes, Round, & Edwards, 1982). It should be noted that trauma, surgery, and pain may accelerate the atrophic response in the immobilized limb (Wolfe, Jahoor, & Hartl, 1989). Subsequent research has used immobilization as a model for muscle disuse atrophy in healthy volunteers (Miles et al., 1994; Yasuda, Glover, Phillips, Isfort, & Tarnopolsky, 2005). Additional models of disuse atrophy include limb suspension (Dudley et al., 1992) and bed rest (LeBlanc et al., 1988), which are often used to investigate muscle changes incurred during spaceflight. Although it is tempting to assign a value to the rate of muscle change or loss during disuse, research clearly shows that the type of disuse protocol and the muscle group affected determines the rate of decrement in size and function of the muscle (Brian C Clark, 2009).

Muscle atrophy following immobilization has been a topic of inquiry for over a century. Clinical observations of human skeletal muscle atrophy following mobilization corroborated earlier research with monkeys (Chor & Dolkart, 1936; Willis, Caiozzo, Yasukawa, Prietto, & McMaster, 1982). Anthropometrically measured leg volume has shown a 12% reduction following a mean of 131 days of leg immobilization (Sargeant, Davies, Edwards, Maunder, & Young, 1977b). Compared to disuse models of bed rest

and lower limb suspension, immobilization resulted in greater atrophy (20% decrement versus 8%) of the quadriceps femoris muscle (Convertino, Doerr, Mathes, Stein, & Buchanan, 1989). Veldhuizen et al. (1993) investigated the effect of thigh muscle size and function following four weeks of knee immobilization. Quadriceps cross-sectional area (CSA) was calculated with computed tomography (CT) and showed a decrease of 21% ± 7% ($p < 0.05$). In addition, muscle biopsy from the vastus lateralis revealed a 16% decrease ($p < 0.05$) in fiber diameter. There is less data and equivocal results concerning the atrophy of the upper limbs. For example, 21 days of wrist immobilization has resulted in no significant atrophy of the forearm muscles (Kitahara et al., 2003), while 9 days of wrist immobilization resulted in significant atrophy (4.1% decrease in CSA) of the forearm muscles (Miles et al., 1994). Although there was no significant change in forearm muscle size following 21 days of wrist immobilization, there was a significant decrease in grip strength and endurance and a prolonged post-exercise PCr recovery period.

There are also significant alterations in neuromuscular properties that appear within the first few days of muscle disuse. Research suggests that neural factors, primarily central activation, can explain approximately 50% of the strength loss during four weeks of muscle unweighting (B.C. Clark, Manini, Bolanowski, & Ploutz-Snyder, 2006). Immobilization also has been shown to alter the functional properties of motor units (Seki et al., 2001). For example, Clark et al. (2008) evaluated the neuromuscular properties during and following 3 weeks of hand/forearm cast immobilization. Immobilization was shown to impair central activation of skeletal muscle, corticospinal excitability, and muscle contractile properties. These changes are surprisingly rapid and

can significantly alter motorneural activity in as little as one week (Lundbye-Jensen & Nielsen, 2008).

Deficits in strength following immobilization cannot entirely be accounted for by decreases in muscle CSA and neural alterations. Metabolic factors, including changes in inorganic phosphate (Pi), have been shown to contribute to altered muscle contractile function (Cook & Pate, 1985). Pathare et al. (2005) studied the effects of 7 weeks of immobilization of the ankle joint on skeletal muscle using ^{31}P MRS and muscle biopsy. Following immobilization, there was a significant decrease in plantar flexor torque and a significant increase in the Pi concentration ($p < 0.001$). Single fiber measurements demonstrated an inverse relationship between Pi concentration and relative force production. The alterations in resting Pi concentration may contribute to strength deficits during immobilization independent of decreases in CSA and neuromuscular impairments (Pathare et al., 2006). These results suggest that Cr supplementation may be able to play a role in maintaining muscle function during immobilization by maintaining a lower Pi/PCr ratio and thus mitigating the decrease in muscle force production.

Creatine supplementation and skeletal muscle metabolism

Oral Cr supplementation at a dose of 20 $g \cdot d^{-1}$ for 3-5 days (loading phase) increases total muscle creatine (TCr) content, free creatine (fCr), and PCr. Early work using muscle biopsies showed that Cr ingestion favorably improved muscle metabolism by increasing PCr availability in type II muscle fibers as well as enhancing muscle PCr resynthesis during recovery from intense muscle contractions resulting in fatigue resistance during short duration high-intensity exercise (Greenhaff, 1994; Balsom et al.,

1995; Casey, Constantin-Teodosiu, Howell, Hultman, & Greenhaff, 1996). Subsequent non-invasive research on human skeletal muscle *in vivo* using ^{31}P MRS has shown similar muscle metabolic improvements during intermittent maximal exercise.

Yquel et al. (2002) examined the effect of creatine ingestion at 20 g·d^{-1} for 6 days on muscle power, PCr resynthesis, inorganic phosphate (Pi), and pH during 8 repeated brief bouts of maximal dynamic plantar flexion exercise. ^{31}P MRS of the medial gastrocnemius muscle was recorded on a superconducting magnet operating at 4.7 Tesla. Following Cr supplementation, resting muscle PCr increased by 15%, along with an increased rate of muscle PCr resynthesis, a lower Pi concentration, higher muscle pH, and improved maintenance of muscle power output. In another study using ^{31}P MRS by Rico-Sanz (2000), Cr supplementation (5 g ·d^{-1} x 11days) reduced net muscle PCr utilization, Pi accumulation, and decreases in pH during repeated bouts of low-intensity isometric exercise (32% of MVC) of the plantar flexors to exhaustion. These results suggest that Cr supplementation improves muscle metabolism during intermittent intense exercise due to a combination of enhanced muscle PCr stores, lowered Pi, higher pH, and improved PCr recovery kinetics.

The changes in muscle metabolism following Cr supplementation may be influenced by age, the muscle or muscle groups being tested, the type of muscular contraction, or the method used to assess PCr recovery kinetics. Smith et al. (1998) compared the effects of Cr supplementation on young and middle-aged men and women during dynamic knee-extension exercise in a whole body magnet resonance system and concluded that Cr ingestion improved PCr availability and resynthesis rate in middle-aged versus younger individuals. Other research have shown no effect of Cr

supplementation on PCr resynthesis rate of the gastrocnemius muscle during intermittent plantar flexor isometric contractions (Vandenberghe et al., 1999). Subsequent research have even shown a slowing of the PCr kinetics during exercise and recovery following Cr supplementation (Jones, Wilkerson, & Fulford, 2009). It should be noted, however, that both of the aforementioned studies finding no improvement or impaired PCr recovery kinetics reported an increase in resting PCr concentrations and subsequent improvements in muscle performance following Cr supplementation. While the exact mechanisms of muscle metabolic improvements may be debatable, the improvements in muscle strength and endurance following Cr supplementation remain consistent throughout the literature.

Creatine supplementation and short-duration high intensity exercise

Cr supplementation (~ 20 $g \cdot d^{-1}$) can increase isokinetic peak muscle torque (Greenhaff et al., 1994; Rossouw et al., 2000), peak power (Green et al., 2001), as well as improve 1 RM strength performance (Chrusch et al., 2001). Cr supplementation has been shown to increase muscle power during repeated, short bouts of high-intensity exercise in young healthy females and men 30-60 years of age (Bemben & Lamont, 2005; Lemon, 2002). For example, Cr supplementation (5×6 $g \cdot d^{-1}$) maintained exercise power by mitigating the decline in work output from baseline over 10 6-s bouts of intermittent high-intensity cycling exercise (P. D. Balsom, Ekblom, B., Soderlund, K., Sjodin, B., & Hultman, E., 1993). Cr supplementation (5×5 $g \cdot d^{-1}$) increased peak power by 20.1% (from 1061 ± 124 to 1325 ± 69 Watts) and mean power by 14.4% (from 582 ± 58.6 to 699 ± 26.1 Watts) during three Anaerobic Wingate Tests (AWT) with six minutes of active recovery between bouts (Havenetidis, 2003). Ziegenfuss et al. (2002) studied the

effects of 3 days of Cr supplementation (0.35 $g \cdot kg^{-1}$ of fat-free mass) on 10 male and 10 female power athletes during six maximal 10 s cycle sprints interspersed with 60 s of recovery. Cr supplementation resulted in statistically significant increases in body mass (0.9 +/- 0.1 kg, $p < 0.03$), total work during the first sprint ($p < 0.04$), and peak power during sprints 2 to 6 ($p < 0.10$).

The improvements in exercise performance from Cr supplementation may be due to increased skeletal muscle PCr which may serve as a temporal energy buffer as well as a modulator of glycolysis resulting in delayed neuromuscular fatigue. Stout et al. (2000) found improvements in neuromuscular fatigue after 5 days of Cr loading (4×5 $g \cdot d^{-1}$) in trained female athletes, with a significant ($p < 0.05$) increase in physical working capacity at the fatigue threshold. Most recently, Smith et al. (2007) examined the effects of 5 days of Cr loading (4×5 $g \cdot d^{-1}$) versus placebo (PL– 10 g of flavored dextrose powder) on the electromyographic fatigue threshold (EMG_{FT}) in college-aged women. Prior to and following supplementation, each subject performed a discontinuous incremental cycle ergometer test to determine their EMG_{FT} value from the vastus lateralis muscle. Cr supplementation increased EMG_{FT} from pre- to post-supplementation by 14.5% ± 3.5% ($p = 0.009$). There was no change for the PL group (-2.2 ± 5.8%; $p = 0.732$). These results suggest that Cr may delay the onset of neuromuscular fatigue during intermittent high intensity exercise.

Creatine supplementation and moderate to long-duration exercise

In addition to the improvements during high-intensity exercise, there is evidence that Cr supplementation can improve moderate to long-duration exercise performance.

Preen et al. (2001) examined the effect of Cr supplementation (20 $g \cdot d^{-1}$ for 5 days) on long-term (~80 min) repeated-sprint cycle exercise (10 sets of 5 or 6 x 6 s maximal bike sprints). Total work done increased 6% (from 251.7 ± 18.4 kJ to 266.9 ± 19.3 kJ; $p < 0.05$) following Cr ingestion whereas there was no change in the placebo group (254.0 ± 10.4kJ to 252.3 ± 9.3 kJ; NS). Cr supplementation has been shown to improve VO_2 kinetics during exercise below the ventilatory threshold (VT)] (Jones, Carter, Pringle, & Campbell, 2002). Nelson and co-workers (2000) investigated the effects of 7 days of Cr supplementation (4×5 $g \cdot d^{-1}$) on cardiorespiratory responses to a graded exercise cycle test to exhaustion. Cr significantly increased ($p < 0.05$) total test time vs. placebo. In addition, VO_2 and heart rate were significantly lower at the end of each of the first five exercise stages following Cr supplementation, but were unchanged after placebo. Moreover, Cr supplementation increased the VT (pre Cr = 2.2 ± 0.4 $l \cdot min^{-1}$ or 66% of VO_{2peak} vs. post Cr = 2.6 ± 0.5 $l \cdot min^{-1}$ or 78% of VO_{2peak}). In another study by Rico-Sanz and Mendez Marco (2000), Cr supplementation (20 $g \cdot d^{-1}$ x 5d) improved time to exhaustion during cycling from 29.9 ± 3.8 min pre-Cr to 36.5 ± 5.7 min post-Cr ($p < 0.05$) versus no change in the placebo group [38.1 ± 5.6 and 40.8 ± 5.7 min pre-treatment and post-treatment, respectively (NS)].

Molecular mechanisms of creatine supplementation for muscle hypertrophy

More recently, research has focused on the effect of Cr supplementation on molecular pathways in skeletal muscle synthesis and degradation. Olson et al. (2006) investigated the influence of Cr and protein supplementation on satellite cell and mononuclei number in human skeletal muscle during 16 weeks of resistance training.

Using a double-blind design, 32 healthy male subjects were assigned to strength training (3 days per week) with either Cr (6-24 g; n = 9), protein (20g; n = 8), or placebo (n = 8). Muscle biopsies were obtained at week 0, 4, 8, and 16 of the resistance training program. Significantly greater ($p < 0.05$) enhancements in the proportion of satellite cells were observed in the Cr supplementation group at weeks 4 and week 8. Furthermore, Cr supplementation resulted in an increase of 14-17% in muscle mean fiber area (MFA) as well as an increased number of mononuclei per fiber at week 4, 8 and 16, versus the strength training plus protein group that increased MFA by 8% in week 16 only. The results from this study demonstrate that Cr supplementation in conjunction with resistance training augments the training-induced increases in satellite cells and mononuclei in human skeletal muscle, resulting in enhanced muscle fiber growth

Cr can change the intracellular osmotic pressure resulting in movement of water into the cell, which can be a stimulus for protein synthesis or slowing protein breakdown (Ziegenfuss et al., 2002; Keller et al., 2003). The accumulation of Cr in skeletal muscle appears to stimulate transcriptional factors that regulate contractile protein synthesis, possibly as a result of increased myosin heavy chain synthesis (Willoughby, 2001). The increases in lean body mass following Cr supplementation could also be mediated via anabolic signaling pathways involving increases in insulin-like growth factor I (IGF-1), mRNA expression and eukaryotic initiation factor-4e binding protein-1 (4E-BPI) (Deldicque, 2005). Cr supplementation has been shown to upregulate mRNA content of genes and protein content of kinases involved in osmosensing and signal transduction, cytoskeleton remodeling, protein and glycogen synthesis regulation, satellite cell

proliferation and differentiation, and regulation of DNA replication and repair (Safdar, Yardley, Snow, Melov, & Tarnopolsky, 2008).

Creatine supplementation and immobilization

Hespel et al. (2001) studied the effects of Cr supplementation on muscle volume, function, and myogenic transcription factor expression during 2 weeks of leg immobilization and rehabilitation in human subjects (n = 22; 13 males and 9 females). Using a double-blind design, half the subjects consumed either Cr (20 g $\cdot d^{-1}$ down to 5 g $\cdot d^{-1}$; n = 11) or placebo (maltodextrin; n = 11) for a total of 12 weeks (2 weeks immobilization, 10 weeks rehabilitation). Cross sectional area (CSA) of the quadriceps muscle was assessed by NMR imaging. Muscle dynamic power and isometric force of the knee-extensor muscles were assessed on an isokinetic dynamometer. In addition, muscle biopsy samples from the vastus lateralis were taken to evaluate muscle myogenic transcription factors. Measurements were taken before and after immobilization, and after weeks 3 and 10 of rehabilitation training. During rehabilitation, dynamic power and CSA recovered at a faster rate in the Cr supplementation group versus the placebo ($p < 0.05$). Following rehabilitation, only the Cr group increased myogenic regulatory factor 4 (MRF4) protein expression ($p < 0.05$), which was found to be correlated with the change in mean muscle fiber diameter ($r = 0.73$). These results suggest that Cr can enhance muscle strength and hypertrophy during rehabilitation following disuse atrophy.

Research using the rat model has demonstrated the effectiveness of Cr to attenuate muscle wasting during immobilization (Aoki, Lima, Miyabara, Gouveia, & Moriscot, 2004). Cr loading for 14 days (7 days prior to immobilization and together with

immobilization) increased muscle Cr content in the lower leg muscles of the soleus by 25% and the gastrocnemius by 18%. This increase in intramuscular Cr content is hypothesized to play a role in the mitigation of muscle loss induced by immobilization. Additionally, the researchers found an additive effect on myosin heavy chain (MHC) shift to the fast phenotype with the combination of Cr supplementation and immobilization.

Cr supplementation has been used during arm immobilization to investigate its effect to alter the changes in muscle mass, strength, and endurance (Johnston et al., 2009). Using a single-blind, placebo-controlled, crossover design for 29 days, Cr was supplemented (20 $g \cdot d^{-1}$) to Cr naïve men (n = 7; 18-25 years) during 7 days of upper-limb immobilization. During immobilization, compared to placebo, Cr supplementation better maintained muscle lean tissue mass (Cr + 0.9% vs. PL –3.7%) of the upper arm as measured with dual-energy X-ray absorptiometry (DEXA). In addition, Cr supplementation significantly ($p < 0.05$) maintained elbow flexor strength (Cr –4.1% vs. PL –21.5%) and endurance (Cr –9.6% vs. PL –43%), and elbow extensor strength (Cr –3.8% vs. PL –18%) and endurance (Cr –6.5% vs. PL –35%) over placebo. These results show that short-term Cr supplementation can slow down the muscle atrophy and strength and endurance losses during periods of immobilization.

There are other mechanisms by which Cr supplementation may attenuate muscle dysfunction during periods of immobilization. For example, Cr supplementation has been shown to improve glucoregulation in skeletal muscle (Ceddia & Sweeney, 2004). Investigation into the effect of oral Cr supplementation on glucose transporter type 4 (GLUT4) protein content along with muscle glycogen and TCr content during

immobilization and subsequent training was conducted (Eijnde, Urso, Richter, Greenhaff, & Hespel, 2001). The right leg of each participant was immobilized for two weeks after which all subjects participated in a resistance exercise program for 10 weeks. Immobilization decreased GLUT4 in the placebo group by 20% ($p < 0.05$) with no significant change in the Cr group (+ 9%, NS). During rehabilitation, Cr intake increased GLUT4 by ~40% ($P < 0.05$), whereas the placebo group normalized pre-immobilization levels. Moreover, muscle glycogen and TCr were higher with Cr supplementation following 3 weeks of retraining. These results suggest that oral Cr ingestion may improve glucoregulation during immobilization and during subsequent rehabilitation training.

Safety of creatine supplementation

In the scientific literature, Cr has been shown to be a safe and effective supplement. In fact, weight gain is the only documented side effect of Cr ingestion (Dalbo, Roberts, Stout, & Kerksick, 2008). Despite the impressive safety record, concerns have been raised by the popular media and scientific community regarding possible adverse effects of Cr supplementation. There have been anecdotal reports in the popular literature that Cr may cause gastrointestinal upset, cause diarrhea, and promote muscle cramping and dehydration (Terjung et al., 2000). Schilling et al. (2001) examined the long-term (0.8 – 4 yr) safety of Cr supplementation in 26 competitive athletes (18 male and 8 females, 24.7 ± 9.2 yr) and found no evidence of increased incidence of muscle injury, cramps, or any other side effects. Some researchers speculated that Cr supplementation could result in an intracellular fluid shift that could reduce the extracellular fluid compartment and impair thermoregulation. In a recent meta-analysis

(Lopez et al., 2009), the authors concluded that there is no evidence that supports the concept that Cr supplementation either hinders the body's ability to dissipate heat or negatively affects body fluid balance. Controlled experimental trials of athletes exercising in the heat resulted in no adverse effects from Cr supplementation at recommended dosages of 5-20 $g \cdot day^{-1}$.

There are claims that Cr supplementation may promote liver damage and increase renal stress and/or impair renal function (Juhn, 1998). The concerns of Cr supplementation on renal physiology can be traced to four case studies of possible renal dysfunction in individuals believed to have been supplementing Cr (Kuehl et al., 1998; Pritchard & Kalra, 1998; Koshy et al., 1999; Loud et al., 2001). Conclusions regarding these case studies have been criticized because two of these individuals had pre-existing kidney disease, one may have been misdiagnosed, and the other was apparently taking only 25 mg of Cr per serving, which makes the connection of Cr ingestion to renal dysfunction dubious. In each of these cases, elevations in serum creatinine (Crn) were initially used to diagnose renal stress. However, Cr is naturally degraded to Crn with the increased serum Crn levels most likely due to the initial Cr loading phase. Studies measuring urinary Crn clearance and/or iohexol infusion techniques to assess glomular filtration found no renal dysfunction in individuals ingesting Cr from 21 months to longer than 5 years (Poortmans et al., 1997; Poortmans & Francaux, 1999; Rasmussen et al., 1999; Robinson et al., 2000). In summary, there is no scientific evidence that Cr supplementation at recommended doses in healthy individuals can induce impairment in renal function (Mesa et al., 2002). Likewise, there is no scientific evidence to suggest that Cr has any impact on liver function following short- or long-term supplementation

(Bemben & Lamont, 2005). Robinson et al. (2000) reported no adverse effects on hepatic function during short-duration (20 g $\cdot d^{-1}$ for 5 days) or long-term (3 g $\cdot d^{-1}$ for 63 days) Cr supplementation.

In an effort to examine the long-term health effects of Cr supplementation, Kreider et al. (2003) studied 98 college football players over a 21-month period. Cr was administered at 15.75 g$\cdot d^{-1}$ for 5 days and then averaged 5 g$\cdot d^{-1}$ for the remainder of the study. Fasting blood and 24-hr urine samples were collected at 0, 1, 1.5, 4, 6, 10, 12, 17, and 21 months of training. Short or long-term Cr supplementation had no significant effect on a 54-item panel of quantitative blood and urine markers or on a 15-item panel of qualitative urine markers. Cr supplementation did not cause any clinically significant changes in serum metabolic markers, muscle and liver enzyme efflux, serum electrolytes, blood lipid profiles, red and white whole blood cell hematology, or quantitative and qualitative urinary markers of renal function.

While the hypothetical side effects of Cr have been hotly debated in the public media, less attention has been directed to several possible health benefits of Cr supplementation. For example, research suggests that Cr may improve blood lipid profiles. In a study conducted by Earnest et al. (1996), Cr supplementation (20 g$\cdot d^{-1}$ for 5 days, followed by 10 g$\cdot d^{-1}$ for 51 days) in mildly hypertriglyceridemic and hypercholesterolemic subjects was shown to reduce plasma concentrations of total cholesterol, triacylglycerols, and very-low-density lipoprotein-C by 5-26%, while having no effect on low-density lipoprotein-C, high-density lipoprotein-C, and Crn concentrations. There is also evidence that Cr supplementation may provide neuroprotection against mitochondrial dysfunction by improving neuronal cell energy

metabolism thus delaying apoptosis that occurs during neurodegenerative disorders such as Alzheimer's Disease (Brewer & Wallimann, 2000), Huntington's Disease (Hersch et al., 2006), and Parkinson's Disease (Matthews, 1999).

Conclusions

Joint immobilization has multifaceted physiologic implications including changes in musculoskeletal morphology, neuromuscular function, muscle contractile properties, and metabolic processes. Cr supplementation in ambulatory subjects has been shown to enhance muscle glycolytic metabolism and improve local muscle and total body exercise performance. Moreover, Cr supplementation potentiates skeletal muscle anabolism when combined with resistance exercise training, and when used alone, mitigates muscle catabolism during periods of muscle disuse. Future research should focus on muscle metabolic consequences of immobilization combined with Cr supplementation. Potential benefits of Cr ingestion during short-term immobilization should be explored to identify the underlying mechanisms that contribute to the reported protective effects. Longer periods of immobilization and other disuse models such as bed rest and spaceflight could then be investigated to determine if Cr provides similar protection. In conclusion, Cr supplementation appears to be safe, effective, and possibly a useful countermeasure against the deleterious effects of muscle changes during joint immobilization.

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CHAPTER 3

This chapter presents a complete manuscript that describes the study in traditional journal article form including an abstract, introduction, experimental procedures, results, conclusions, and reference section. The manuscript, entitled “Effect of Creatine Supplementation on Wrist Flexion Exercise Performance after Immobilization” will be submitted to the Journal of Strength and Conditioning Research. It is authored by Jeremy C. Fransen, Robert Robergs, Micah Zuhl, and Suzanne Schneider. The manuscript follows the formatting and style guidelines of the journal. The references cited are provided at the end of the manuscript.

Effect of Creatine Supplementation on Wrist Flexion Work and Power after Immobilization

By

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Running Head: Creatine and immobilization

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ABSTRACT

The purpose of this study was to investigate the effect of creatine (CR) supplementation during immobilization on skeletal muscle total work and average power during repeated maximal intensity wrist dynamic contractions. Twenty-five healthy, active male (age 28 ± 5 years) and female (age 22 ± 4 years) subjects performed wrist flexion exercise before (PRE) and after (POST) one week of wrist/forearm cast immobilization. The wrist flexion exercise consisted of an incremental protocol to fatigue followed by two constant load (CL1 and CL2) exercise bouts at the maximal load obtained during the incremental bout. During the immobilization period, in a double-blind study design, CR subjects (9 men and 4 women) consumed CR (20 $g \cdot day^{-1}$) while placebo (PL) subjects (5 men and 7 women) consumed a 4% carbohydrate solution. Immobilization caused a decrement in forearm total work (-3.17% PL; -2.61% CR) and average power (-3.43% PL; -2.61% CR) during the incremental protocol, regardless of CR supplementation. During the first CL bout, both total work and average power decreased in PL after immobilization (-28.9 ± 9.6%; $p < 0.05$), but not in CR. However, during the second CL bout, both total work and average power decreased in CR (work -14.39 ± 4.54%; power -10.52 ± 4.6% and PL (work -21.98 ± 8.28; power -21.3 ±8.25%) groups after immobilization. Although the peak power for wrist flexion did not decrease significantly after 1 week of immobilization, arm endurance was significantly reduced. This impairment in arm endurance can be prevented by the ingestion of CR, suggesting the loss in short term endurance is due to either a depletion or altered kinetics of the phosphocreatine system.

Key Words: Casting, Creatine Phosphate, Muscle endurance

Introduction

Numerous studies have documented the loss of muscle size and function after spaceflight, bed rest, or joint immobilization (1, 3, 4). Immobilization has been used as a ground-based model to investigate the effects of microgravity on skeletal muscle function (4, 10, 14, 25). Muscle deconditioning occurs most rapidly during cast-induced immobilization, followed by unloading with limb movement (i.e., unloaded limb models, bed rest, or spaceflight) and, lastly, similar changes in muscle function occur with aging (6, 11, 20). The loss of muscle function after disuse has been associated with a loss of muscle contractile proteins (4), increased fatigability (36), neural adaptations (21), changes in muscle fiber phenotype (32), impaired contractility (27), and a glycolytic shift in metabolic properties (26).

Creatine monohydrate (CR) supplementation has been shown to potentiate increases in muscle mass and strength when combined with resistance training in ambulatory subjects (5, 34). CR supplementation also has been found to accelerate lower-body muscle mass recovery following 2 weeks of knee flexor immobilization when combined with a resistance training program (15). However, there is also evidence to suggest that CR supplementation may slow the rate of muscle loss and dysfunction during immobilization, independent of a resistance training stimulus. For example, CR supplementation was found to mitigate muscle loss in rat planar flexors during 14 days of hind limb suspension (1). In humans, Johnston et al. (17) reported CR supplementation better maintained muscle mass, isometric strength, and dynamic endurance of the upper arm flexors and extensors during isokinetic exercise after nine days of arm casting.

However, there has been no research demonstrating the effectiveness of CR supplementation on dynamic incremental wrist flexion exercise performance to fatigue following immobilization. Moreover, research by Johnston et al. (17) did not perform multiple endurance bouts, which may better reveal the ergogenic potential of CR as research has shown improved performance during intermittent exercise bouts (36).

The purpose of this study was to investigate the effect of dietary CR supplementation to preserve muscle endurance during repeated dynamic forearm flexion exercises following immobilization. We hypothesized that dietary CR supplementation will maintain muscle endurance after seven days of wrist casting, as evidenced by a maintenance of total work and average power during repeated endurance bouts of high intensity, constant load wrist flexion exercise.

Methods

Setting

All forearm ergometry data collection was conducted at New Mexico Resonance (NMR) in Albuquerque, New Mexico. Casting of the forearm/hand was conducted at the Exercise Physiology Laboratories at the University of New Mexico. Both of these facilities are located at an altitude of 1600 m (5,400 ft) with an approximate barometric pressure of 630 mmHg.

Subjects

Twenty-five active individuals (14 men, mean age 28 ± 5 years; 11 women, mean age 22 ± 4 years) participated in this study. On their initial visit each subject gave informed consent and completed a standard health history questionnaire to exclude subjects with metal implants, a history of liver dysfunction, diabetes, neurological disorders, or renal disease. The protocol was approved by the Institutional Review Board of the University of New Mexico. Each participant performed aerobic and/or resistive exercise 3-6 $d \cdot wk^{-1}$. They were asked to abstain from medications and were instructed to avoid changes in their diet and refrain from physical activity for 24 hours immediately before the exercise bouts in the study. Participants were excluded from the study if they had consumed supplemental CR up to two months prior to the study.

Table 1: Group Characteristics

Group	**Gender** *M or F*	**Height** *Cm*	**Weight** *Kg*	**Age** *yrs*
Placebo	5 M, 7 F			
mean		171	65.8	23
std		13	13.4	3
Creatine	9 M, 4 F			
mean		173	76.5	25
std		9	22.2	4

Experimental Protocol

The twenty-five subjects were randomly assigned to either a treatment group (CR, n = 13; 9 men and 4 women) or placebo group (PL, n = 12, 5 men and 7 women). The CR and PL groups were balanced in terms of age, height, and weight. This study was a double-blind protocol, where neither the subjects nor the researchers were aware of the treatment (PL or CR) during immobilization. Approximately one week before the immobilization of the

non-dominant forearm/hand, each subject performed a familiarization protocol, which consisted of a maximal incremental exercise test, followed by two constant load (CL1 and CL2) endurance tests. The maximal incremental test consisted of a continuous incremental protocol with 0.8 min stages. The weight started at 1 kg or 2 kg and was increased every 0.8 min by 0.5 kg or 1 kg, respectively depending on the baseline strength of the subject. Forearm wrist flexion exercise continued until volitional exhaustion (5.2 ± 0.69 min) using a custom-made arm ergometer (see below). Immediately after the incremental exercise protocol, the subjects rested for 4.8 min, and then performed two constant load wrist flexion exercise bouts of up to 2.4 min, against a weight which equaled the maximal weight moved during the incremental protocol. These two constant load endurance tests were separated by 4.8 min of recovery. The same protocol (the incremental test and two endurance exercises) was repeated approximately one week later before immobilization (pre-tests), and after 7 days of CR or PL ingestion immediately after the cast was removed (post-tests).

Creatine Ingestion

Subjects were fitted with a forearm/wrist cast within one week of the familiarization test (Figure 1). On the day of casting (day 1), each subject ingested either CR (5 g Cr + 10 grams sucrose) or placebo PL (10 g sucrose) starting with the afternoon meal and another 5 g of CR or PL with the evening meal, for a total of 10 g on day 1. The subjects were instructed to ingest CR (20 $g \cdot d^{-1}$) or PL (10 $g \cdot d^{-1}$of a 4% CHO flavored solution) throughout the rest of the immobilization period from days 2 to 7. The CR dose of 20 $g \cdot d^{-1}$ is based on prior research demonstrating sufficient loading in the muscles after 5 days

(13, 34, 35). They were instructed to consume 5 g of CR or the PL dissolved in 250 ml of warm water on four occasions, at equally spaced intervals on day 2 to day 7. The subjects were instructed to consume the supplement with food and to avoid simultaneous intake of caffeine to ensure CR absorption. On day 8, the subjects were instructed to consume 5 g CR or PL after breakfast. The cast was removed in the afternoon immediately prior to repeating the exercise testing sequence.

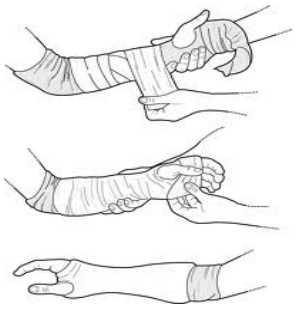

Figure 1. Forearm cast

Ergometry

Dynamic exercise was performed using a custom-made forearm flexion ergometer, so that the exercise could be performed with minimal movement and with the forearm positioned within the coil of a 1.9 Tesla superconducting magnet. The pronated hand rested around the rotational ergometer device, which was attached by a non-elastic cord to weights which were raised and lowered across a wall pulley (Figure 2). Maximal forearm wrist flexion exercise consisted of a downward wrist movement of 60° resulting in a 10-cm vertical displacement of the weight. The subject then lowered the weight under control back to the starting position. Repetitions were counted only if the subject could perform a full range of motion as indicated by the sound of the ergometer clicking and weight load hitting the end-point in the range of motion.

During each exercise, the subjects sat with their non-dominant arm extended laterally from their body, and with the forearm supported on the peripheral coil inside the magnet (Figure 2). The subject was instructed to smoothly press down on the handle during the concentric action (lifting the weight on the pulley) and to smoothly return the handle during the eccentric component of the movement cycle. The subjects were instructed not to strongly grip the handle during the contraction with the intent to isolate the wrist flexor muscles while minimizing involvement of finger flexor muscles. All exercise was done at a rate of one concentric and eccentric action cycle every 4 s and the rate was reinforced by a clicking sound that corresponded to the concentric and eccentric actions. Concentric and eccentric actions took 1.25 s each, with 1.5 s between contractions. During the incremental protocol, the loads were increased in .5 kg or 1 kg increments, depending on results during the familiarization session, until volitional fatigue, as indicated by the inability to maintain repetition cadence and/or complete the full range of motion. The subjects then rested for 4.8 min with their arm extended in the magnet. The subjects were then asked to perform a 2.4 min bout of wrist flexion exercise with a load equal to the load attained during the last stage of the incremental protocol. The subjects then rested another 4.8 min with their arm in the magnet to collect recovery data. Following the rest period, the subjects were again asked to perform one final 2.4 min exercise bout with the same load as the previous 2.4 min stage. The two constant load exercise bouts were terminated earlier if the subjects could not perform a complete exercise movement in the prescribed time interval. The subject then rested for a final 20 min with their arm in the magnet to obtain complete recovery data.

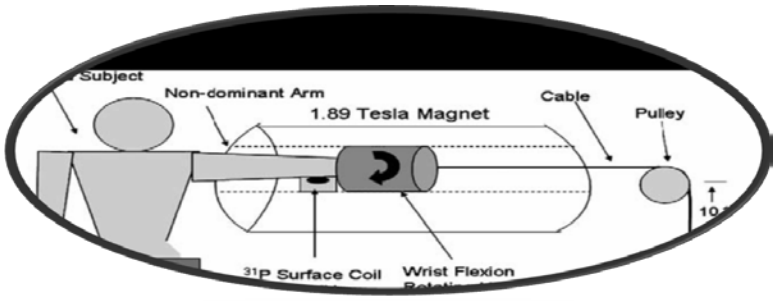

Figure 2: Schematic of exercise apparatus

Calculations

Percent change (%Δ) in total work and average power were determined as follows:

$$\%\Delta = \text{post-test} - \text{pre-test} / \text{pre-test} \times 100$$

Work (W) and Power (P) were determined as follows:

$$W\ (kg \cdot m) = \text{load (kg)} \times \text{distance (0.1 m)} \times \text{repetitions}$$

$$W\ (\text{Joules}) = kg \cdot m \times 9.80665$$

$$P\ (kg \cdot m \cdot min^{-1}) = W / \text{Time (min)}$$

$$P\ (\text{Watts}) = kg \cdot m \cdot min^{-1} / 6.118$$

Statistical Analysis

Independent t-tests confirmed there were no significant differences between the CR and PL groups for anthropometric characteristics or for work or power exercise results before immobilization.

Using data from before and after immobilization, the total work and average power during the exercise bouts were analyzed using a 2-factor (group; creatine vs. placebo and time; pre-cast, post-cast) ANOVA, with repeated measures on the time factor.

For the graphical representations, non-paired t-tests were performed to compare the percent changes in total work and average power between the pre- and post-immobilization tests. As CR was expected to have only positive effects on exercise performance after immobilization, a one-tailed t-test was used to determine differences between the group's pre-and post-immobilization.

Except for anthropometric data and completed repetitions (± *SD*), all values are expressed as mean ± *SE*. Statistical significance was set at $p \leq 0.05$. Data were analyzed using commercial statistical software (Statistica, v10, Statsoft Inc., Tulsa, OK). Sample size estimates were computed using commercial software (GPower 3.1, University of Kiel, Germany) and prior research which measured percent changes in isokinetic dynamic endurance following a similar period of arm immobilization (17).

Results

Where data for both groups were combined (the ANOVA time effect), there were significant ($p < 0.05$) decreases in forearm muscle work and average power after immobilization during all three exercise bouts: the incremental protocol, CL1 and CL2.

Incremental ANOVA test results:

Incremental work decreased from 466.05 ± 12.98 J to 457.98 ± 47.29 J for CR and from 409.89 ± 51.93 J to 395.73 ± 50.82 J (Figure 3).

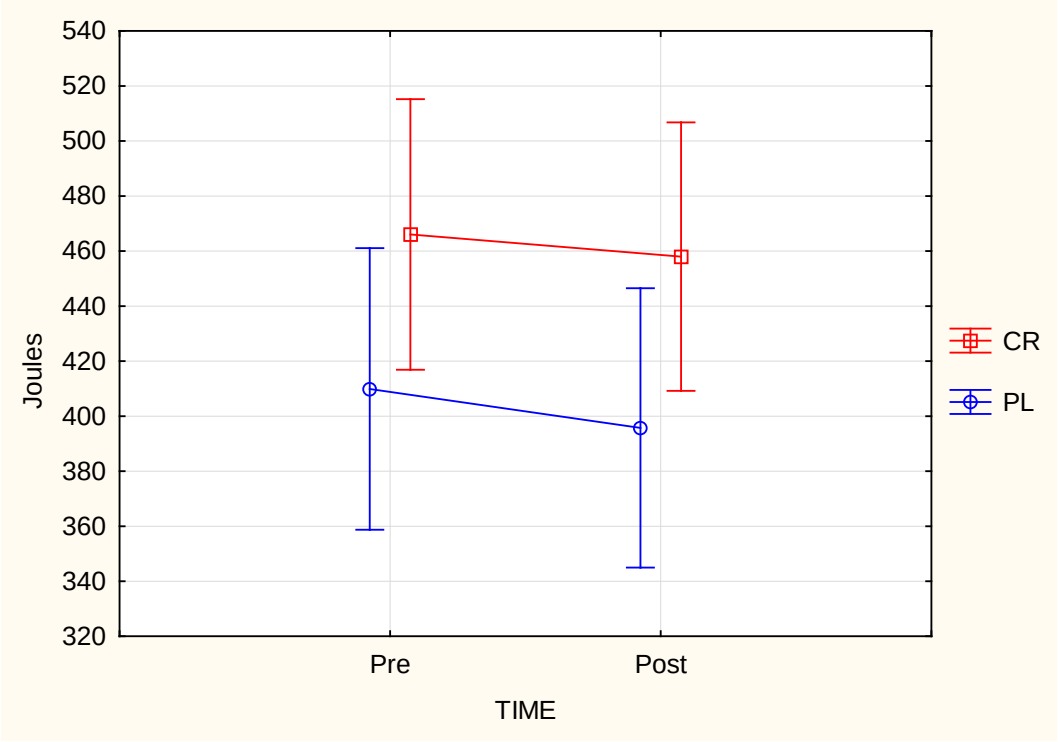

Figure 3. Incremental work (J) before (Pre) and after (Post) immobilization for creatine (CR) and placebo (PL) groups.

Incremental average power decreased from 6.21 ± 0.62 W to 6.11 ± 0.63 W for CR and from 5.55 ± 0.69 W to 5.38 ± 0.68 W for PL (Figure 4). Table 2 quantifies the total completed repetitions for CR and PL groups pre- and post-immobilization (± *SD*).

Table 2: Completed Repetitions for Incremental Protocol

	Pre Immobilization	Post Immobilization
PL	78 ± 11	76 ± 12
CR	78 ± 11	77 ± 12

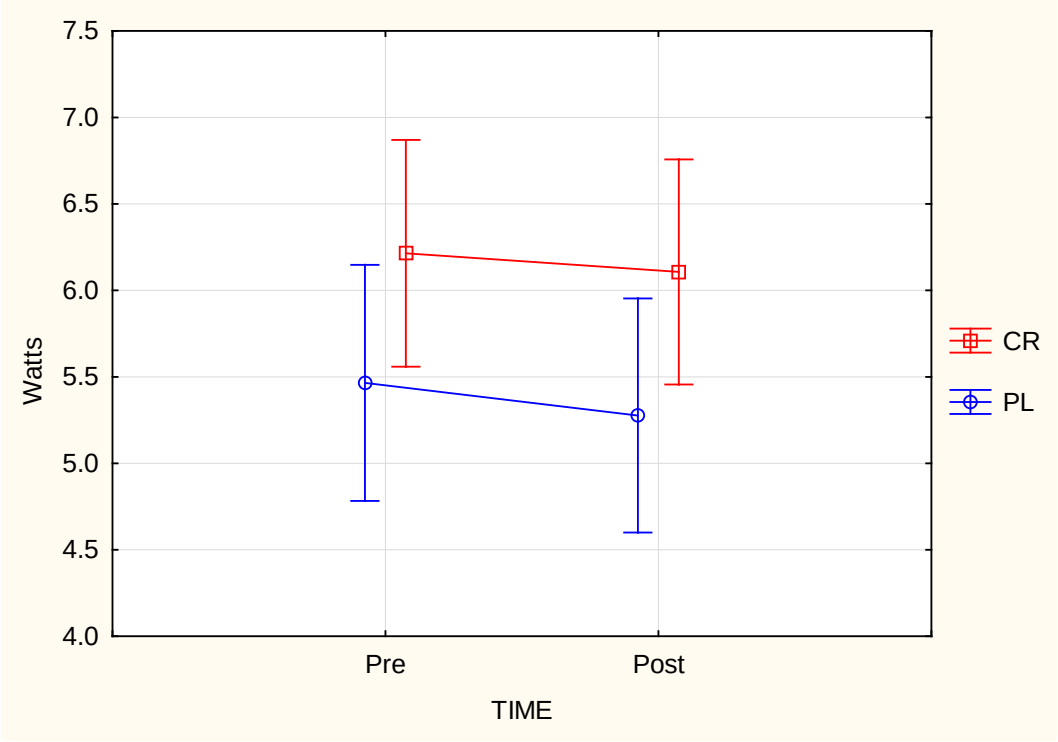

Figure 4. Incremental power (W) before (Pre) and after (Post) immobilization for creatine (CR) and placebo (PL) groups. Values are mean ± *SE*.

Incremental Percent Change Results:

From the non-paired t-tests of the percent changes, there were no significant differences between groups in the percent change in total work (CR -2.61 ± 1.89% vs. PL -3.17 ± 2.27%; Figure 5) or average power (CR -2.61 ± 1.89% vs. PL -3.43 ± 2.35%; Figure 6) during the incremental protocol pre- to post-immobilization. The peak load (kg) attained during the incremental protocol was the same for all subject's pre- and post-immobilization, with an average of 5.8 ± 2 kg (3.7 ± 0.5 kg for women and 7.3 ±1.2 kg for men).

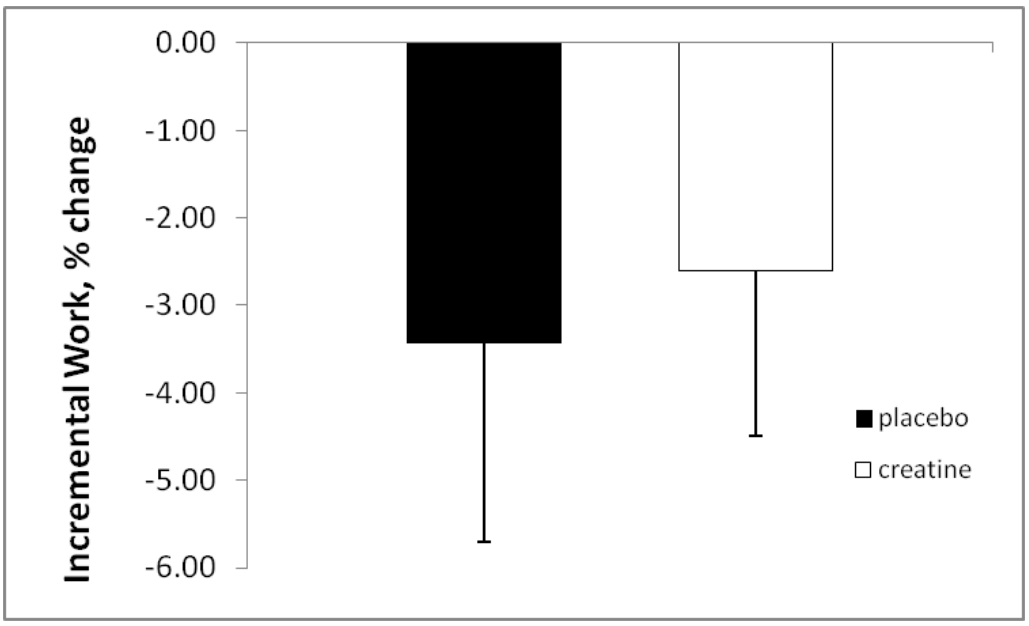

Figure 5. Percentage change in incremental work (J) after immobilization from creatine and placebo. Values are mean ± *SE*.

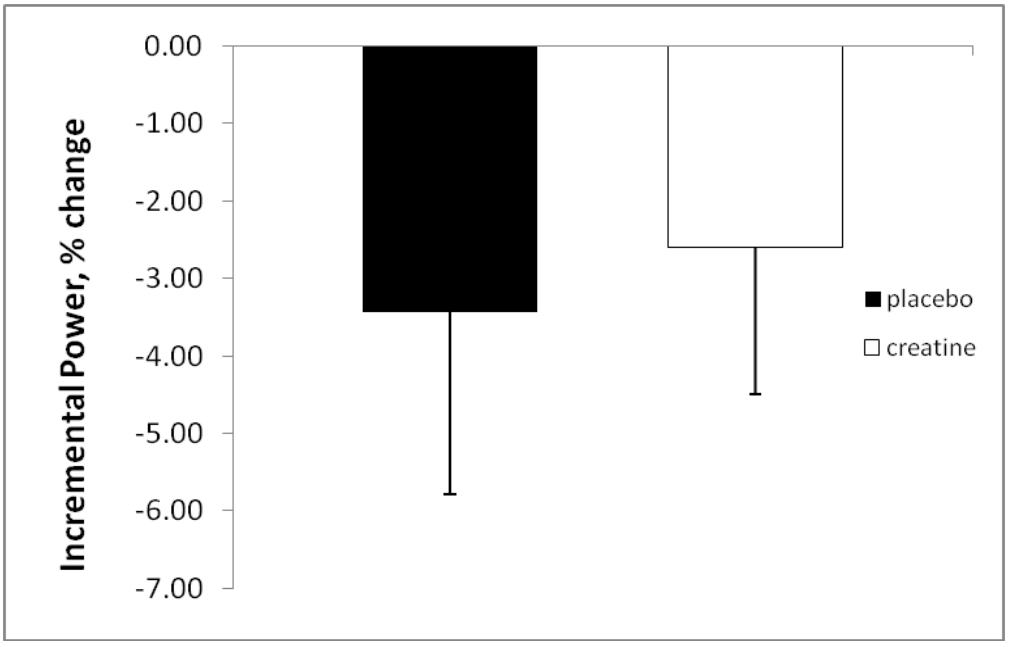

Figure 6. Percentage change in incremental power (W) after immobilization from creatine and placebo. Values are mean ± *SE*.

CL1 ANOVA results:

Total work decreased in CL1 from 214.54 ± 70.18 J to 196.89 ± 61.17 J for CR and from 187.23 ± 71.2 J to 143.31 ± 92.47 J for PL (Figure 7). Power decreased in CL1 from 1.49 ± 0.40 W to 1.32 ± 0.4 W for Cr and from 1.3 ± 0.49 W to 1.0 ± 0.64 W for PL (Figure 8). Table 3 quantifies the total completed repetitions for CR and PL groups pre- and post-immobilization (± *SD*).

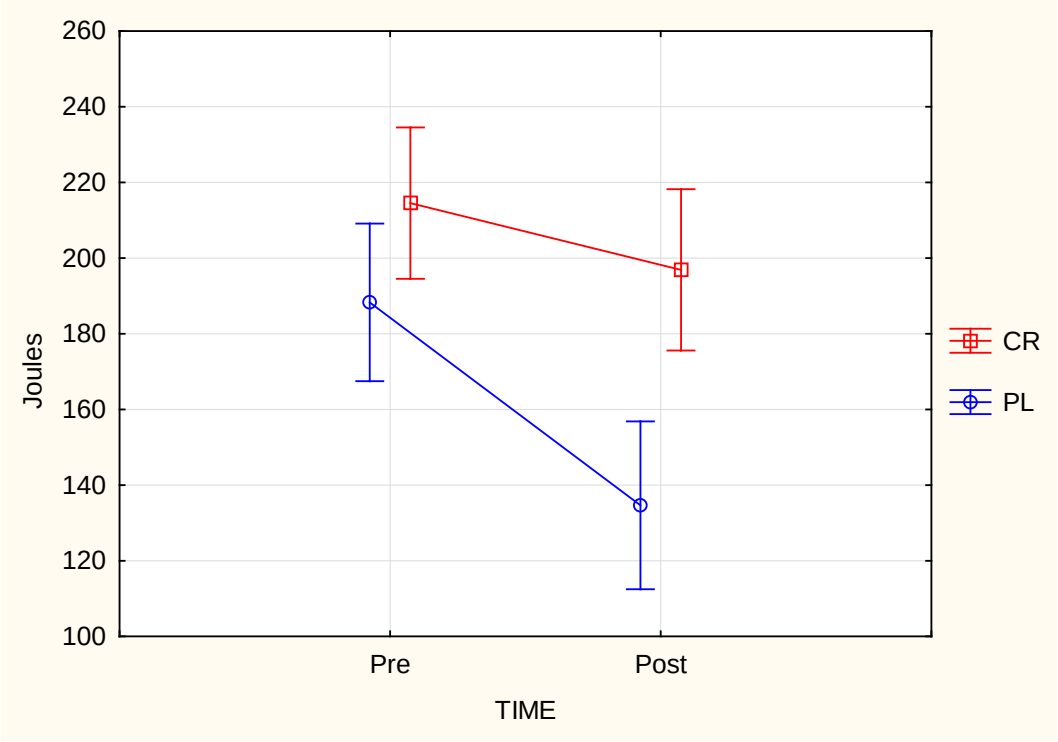

Figure 7. Constant load bout 1 work (J) before (Pre) and after (Post) immobilization for creatine (CR) and placebo (PL) groups. Values are mean ± *SE*.

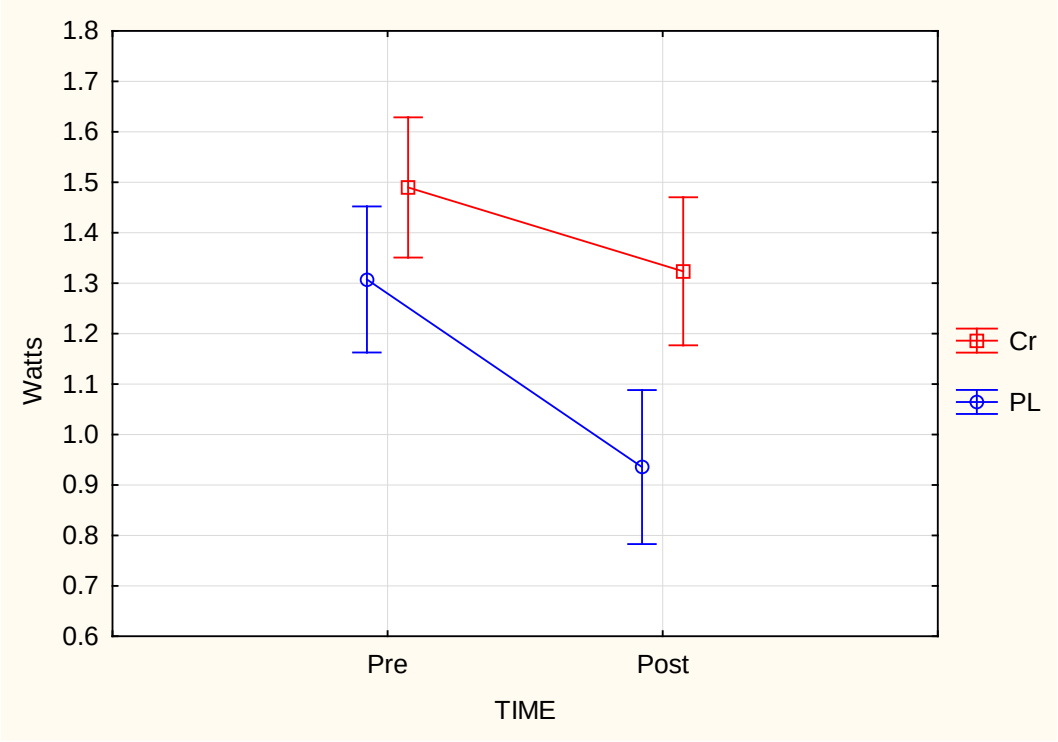

Figure 8. Constant load bout 1 power (W) before (Pre) and after (Post) immobilization for creatine (CR) and placebo (PL) groups. Values are mean ± *SE*.

Table 3: Completed Repetitions in CL1

	Pre Immobilization	Post Immobilization
PL	36 ± 1	29 ± 10
CR	36 ± 0	33 ± 2

CL1 Percent Change Results:

Compared to placebo, creatine ingestion attenuated the decrease in wrist flexion total work (CR -7.48 ± 1.62% vs. PL -28.96 ± 9.62%, $p < 0.05$; Figure 9) and power (CR -10.01 ± 2.38% vs. PL - 28.9 ±9.63%, $p < 0.05$; Figure 10).

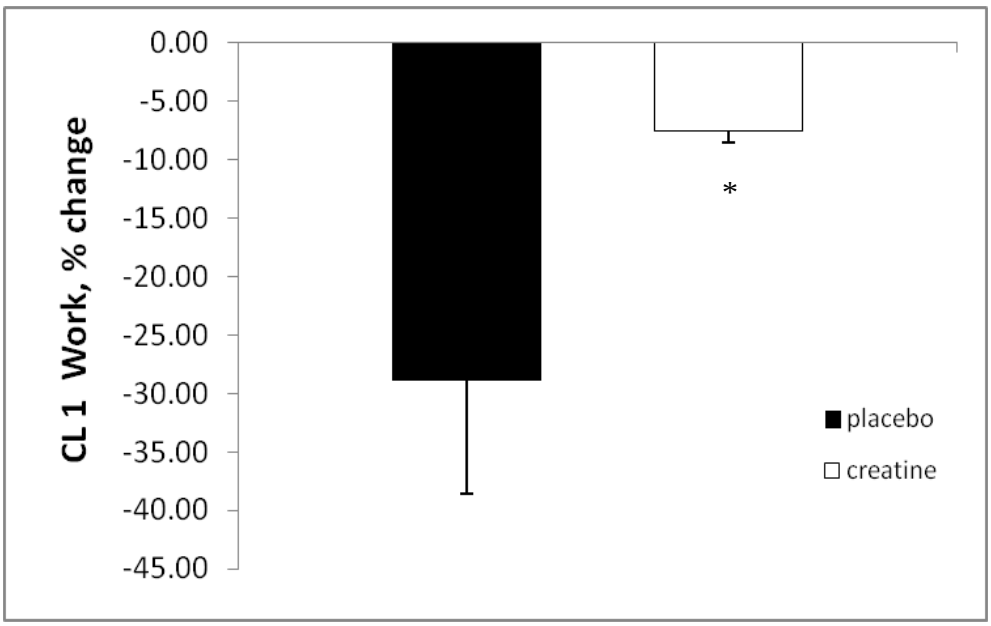

Figure 9. Percentage change in constant load bout 1 work (J) after immobilization from creatine and placebo. Values are mean ± *SE*. *Significant group time interaction, with creatine better maintaining strength over placebo ($p < 0.05$).

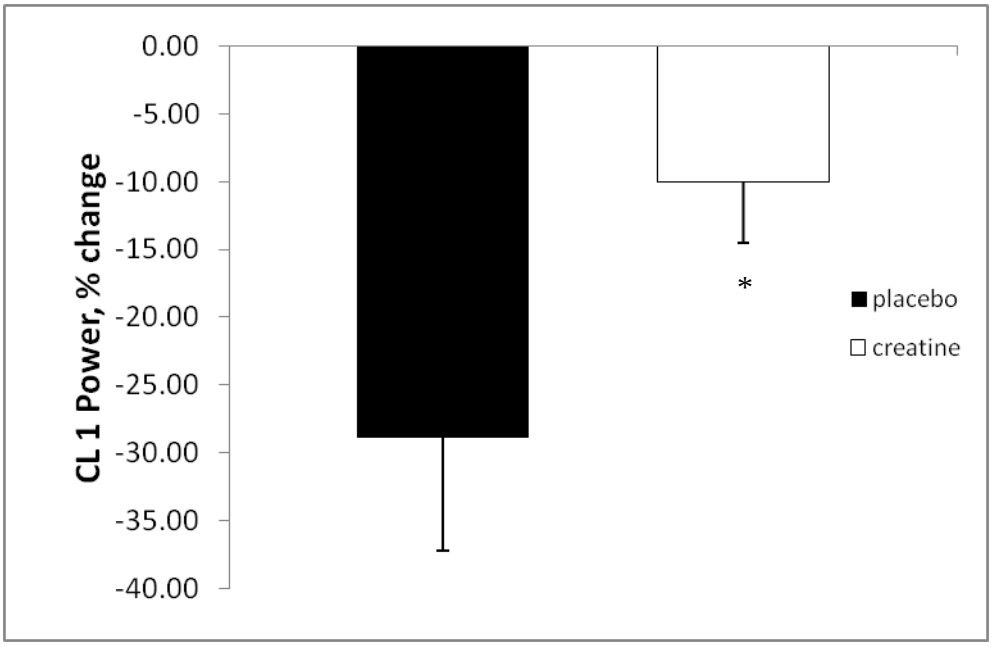

Figure 10. Percentage change in constant load bout 1 power (W) after immobilization from creatine and placebo. Values are mean ± *SE*. *Significant group time interaction, with creatine better maintaining strength over placebo ($p < 0.05$).

CL2 ANOVA Results:

Total work decreased in CL2 from 214.09 ± 19.61 J to 188.21 ± 18.58 J for CR and from 185.80 ± 20.72 J to 155.32 ± 22.62 J in PL (Figure 11). Power decreased in CL2 from 1.49 ± 0.14 W to 1.25 ± 0.12 W in CR and from 1.29 ± 0.14 W to 1.07 ± 0.16 W in PL (Figure 12). Table 4 quantifies the total completed repetitions for CR and PL groups pre- and post-immobilization (± *SD*).

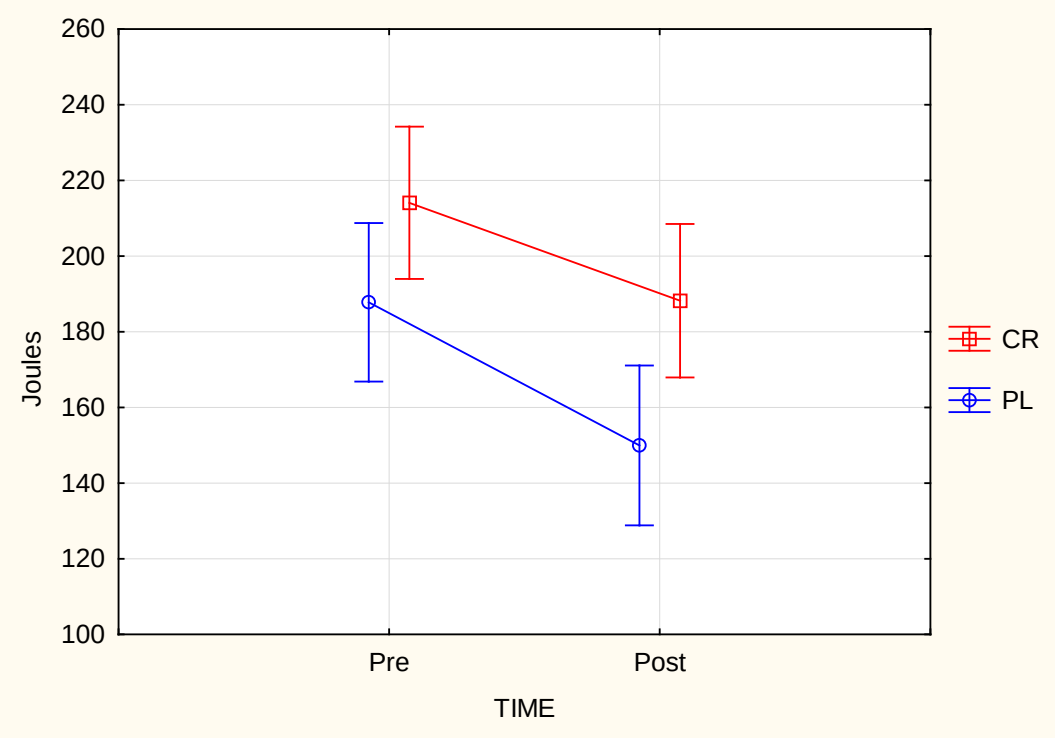

Figure 11. Constant load bout 2 work (J) before (Pre) and after (Post) immobilization for creatine (CR) and placebo (PL) groups. Values are mean ± *SE*.

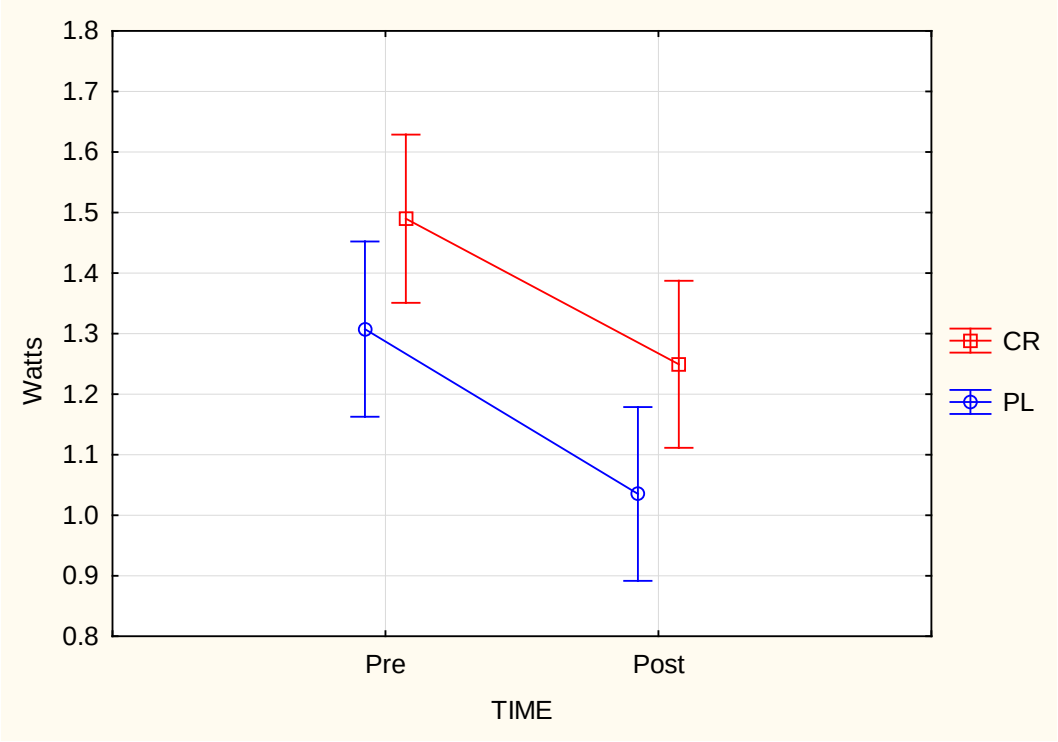

Figure 12. Constant load bout 2 power (W) before (Pre) and after (Post) immobilization for creatine (CR) and placebo (PL) groups. Values are mean ± *SE*.

Table 4: Completed Repetitions in CL2

	Pre Immobilization	Post Immobilization
PL	36 ± 2	30 ± 10
CR	36 ± 1	32 ± 6

CL2 Percent Change Results

There were no differences in the percent decrements in total work (-10.52 ± 10.62% vs. PL -21.3 ± 8.25%; Figure 13) and average power (-14.39 ± 4.54% vs. -21.98 ± 8.28; Figure 14) between CR and PL after immobilization during the CL2 exercise bout.

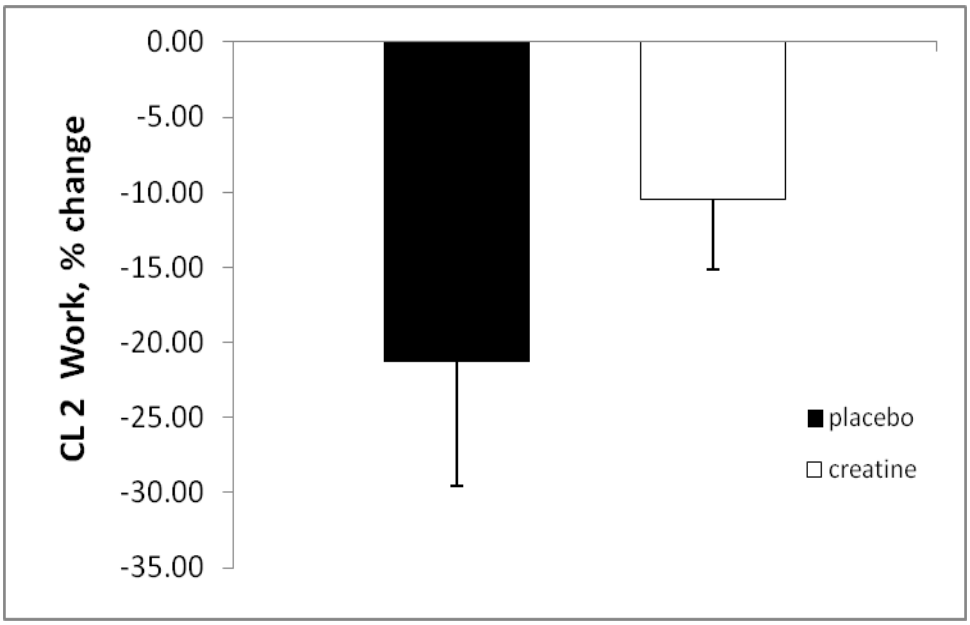

Figure 13. Percentage change in constant load bout 2 work (J) after immobilization from creatine and placebo. Values are mean ± *SE*.

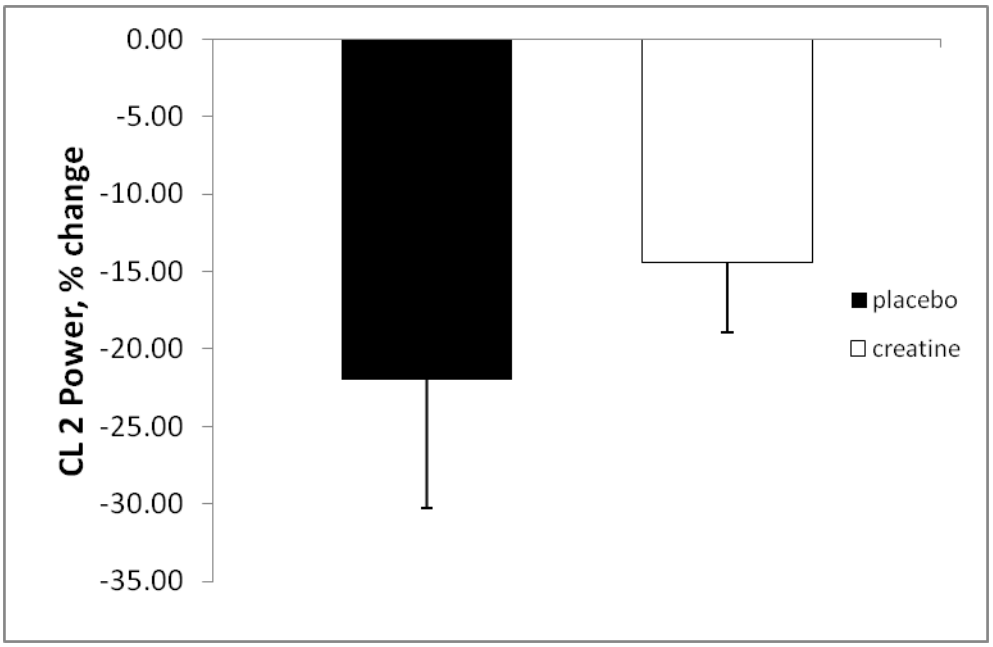

Figure 14. Percentage change in constant load bout 2 power (W) after immobilization from creatine and placebo. Values are mean ± *SE*.

Discussion

Consistent with our hypothesis and prior research (17), CR attenuated the reduction in total work and average power during the first endurance bout. There could be several reasons why CR supplementation slowed the loss in muscle endurance. Following incremental exercise to exhaustion, CR supplementation could have increased or maintained muscle creatine phosphate (CP) content (13). An increase in CP stores and/or free CR could enhance the reversibility of the CK reaction during the first rest period following the incremental protocol thus improving CP recovery kinetics (7, 12, 35). However, no improvement in endurance occurred during the second endurance bout. This may be due to the limited potential for a small muscle group like the forearms to store sufficient CP to enhance performance on multiple exercise bouts. Perhaps the CP depletion was severe enough to limit the CP recovery kinetics between the first and second endurance bouts or there was insufficient time to recover the CP stores. Fatigue could also have been influenced by the increased involvement of other energy pathways (e.g. glycolysis) during the endurance bouts. Finally, neural impairments such as decreased central activation and increases in long-interval intracortical inhibition may have played a more prominent role in fatigue during the final endurance bout (8). It is well established that neuromuscular changes occur during immobilization that contribute to post-immobilization decrements in muscle strength and endurance (21).

Deficits in muscle strength and endurance following immobilization cannot entirely be accounted for by decreases in muscle CSA and neural alterations. Metabolic factors, including changes in inorganic phosphate (Pi), have been shown to contribute to altered

muscle contractile function (9). Pathare et al. (2005) studied the effects of 7 weeks of immobilization of the ankle joint on skeletal muscle using ^{31}P MRS and muscle biopsy. Following immobilization, there was a significant decrease in plantar flexor torque and a significant increase in the Pi concentration. Single fiber measurements demonstrated an inverse relationship between Pi concentration and relative force production. The alterations in resting Pi concentration may contribute to strength deficits during immobilization independent of decreases in CSA and neuromuscular impairments (26). These results suggest that CR supplementation may be able to play a role in maintaining muscle function during immobilization by maintaining a lower Pi/PCr ratio and thus mitigating the decrease in muscle force production.

No difference between groups in work and power decrement during the incremental protocol post-immobilization was a surprising result of this study. Previous research has shown a 29.3% drop in isometric wrist flexion strength following 9 d of wrist/forearm immobilization and suspension (25). Research by Clark et al. (2010) reported a 43.2% decrease in maximal voluntary isometric contraction for the wrist flexors following 3 weeks of wrist/hand immobilization (8). In this study, wearing a cast for one week modestly decreased the total work and average power that could be produced during dynamic forearm flexion. The inconsistent results between the present study and the previous studies could be due to the type of muscle contraction. The previous studies measured isometric wrist strength instead of the dynamic wrist flexion measured in the present study (8, 25). Changes in dynamic muscle endurance may be affected at a different rate than isometric muscle actions. Another possible explanation could be the

length of the immobilization period. It is well established that muscle dysfunction increases as a function of the length of the immobilization period (11, 33). One week may be too short a time period to demonstrate the magnitude of performance decrements in the wrist flexors as reported for larger muscle groups. Perhaps two or three weeks of immobilization would have resulted in a larger decrease in work and power performance, and allowing the possibility that CR supplementation may offset the decrements we found during the incremental protocol and the CL2 exercise bout.

Improvement in endurance in the first CL1 endurance bout agrees with Johnston et al. (17). However, the improvement did not continue into the second CL2 endurance bout. One possible explanation for the initially positive endurance effects of Cr could be related to an effect on the phosphocreatine energy system. This suggestion could be resolved using ^{31} P-MRS to quantify the CP, CR, inorganic phosphate (Pi), and pH of the muscle during exercise and the recovery periods (23, 24, 29). One of the main benefits of CR supplementation is its ability to increase CP stores (12), which can be measured with ^{31}P-MRS (28, 36). Changes in CP stores, the CP/Pi ratio, as well as CP recovery kinetics following the incremental bout and between CL1 and CL2 may help explain the differences in exercise endurance (2, 9, 19, 30).

This study grouped males and females together based on prior research demonstrating similar decreases in isometric and isokinetic wrist flexion strength in male and female subjects (25). Although peak load during the incremental protocol along with total work and average power were higher in male subjects, females responded with similar relative

changes in strength and endurance during the immobilization period. In the present study, there were no obvious sex differences in the responses of the CR and PL groups.

This study was unique in that it was the first to identify work and endurance decrements during maximal and subsequent constant load dynamic wrist flexion exercise following one week of immobilization. Cr supplementation during 7 days of immobilization minimized the loss of endurance during the first constant load exercise bout. This could have important applications to patients recovering from limb immobilization, the elderly who experience muscle mass, strength, and endurance loss, and astronauts living in microgravity. The muscle mass and joint function measured (18), the type of muscle contraction measured (isometric versus dynamic) (31), and the exercise test protocol (16), may all affect the effectiveness of CR to support muscle performance (22). Future research should measure muscle high-energy phosphate compounds (e.g. CP) and acidity to explain the underlying mechanisms contributing to fatigue, along with recovery kinetics during subsequent bouts of exercise. A further understanding of the mechanisms behind this phenomenon (ability of CR to enhance endurance after immobilization) may allow additional improvement in our ability to prescribe ergogenic agents to enhance recovery of immobilized individuals.

ACKNOWLEDGMENTS

The authors would like to thank Joan Iverson for subject scheduling and randomization, and Roy Salgado, Matt DuSold, and Jason Beam for technical support.

GRANTS

Partial funding for this study was provided by a graduate research and development (GRD) grant from the University of New Mexico's Graduate and Professional Student Association.

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CHAPTER 4

SUMMARY, CONCLUSION, AND RECOMMENDATIONS

Summary

The review manuscript titled “Exploring the Potential of Creatine to Maintain Muscle Function During Immobilization” made a unique contribution to Exercise Physiology by providing insight regarding the effectiveness of Cr supplementation on muscle performance following short duration immobilization. The biochemistry of Cr, the effectiveness of Cr on short intense exercise and long-duration exercise, mechanisms behind the ergogenic benefits, and safety were addressed. In addition, the review manuscript discussed limited data demonstrating the ergogenic nature of Cr supplementation during immobilization. The manuscript also discussed the muscle metabolic effects of Cr and immobilization on muscle size and performance. Further, the manuscript addressed how ^{31}P-MRS research during exercise and recovery may be able to determine the effect of Cr supplementation has on muscle metabolic function.

The research manuscript titled “Effect of Creatine Supplementation on Wrist Flexion Work and Power after Immobilization” contributed to Exercise Physiology by providing evidence that short duration wrist immobilization results in significant decreases in dynamic forearm ergometry exercise performance. Moreover, Cr supplementation during the week of immobilization mitigated the drop in exercise endurance during the first constant load exercise bout following a brief rest following incremental exercise to fatigue. This finding is consistent with other research that has demonstrated Cr to be effective at slowing the loss of muscle performance during immobilization. This study is unique in that it was the first to measure dynamic wrist

flexion exercise performance following immobilization. In addition, this study measured forearm ergometry during an incremental protocol to fatigue as well as subsequent constant load muscle endurance.

Conclusions

The significant findings presented in the research manuscript were; a) 7 days of wrist immobilization significantly decreased exercise endurance, b) this decrement was slowed with creatine supplementation for an initial constant load exercise bout, c) there was no significant difference between the creatine or placebo groups for the incremental protocol, and, d) although there was an initial improvement in endurance during the first endurance exercise bout, there was no significant difference between creatine or placebo for the second constant load exercise bout. These results suggest that creatine ingestion during conditions of muscle wasting may help maintain at least short-term muscle endurance and power, which could be helpful during the initial phases of rehabilitation.

Recommendations

In hindsight, there are a few things that could have been done differently to improve upon this research. First, using an electronic ergometer with a radial encoder to quantify work and power for each wrist flexion repetition would have improved the accuracy of the ergometry data. Second, increasing the duration of the constant load exercise bouts to fatigue would have better reflected muscle endurance, as some subjects completed all of the repetitions prior to complete fatigue. Finally, due to the large deviations in muscle work and power during the exercise tests, increasing the number of

subjects in each group would have enhanced the statistical power and improve sensitivity of detecting significant differences between groups.

It is recommended that future research studies; a) investigate the metabolic effects of immobilization and the role of high energy phosphate compounds during exercise and recovery, b) increase the duration of the immobilization period when examining muscle performance decrements at the wrist, c) increase the number of intermittent constant load exercise bouts when studying the effectiveness of Cr supplementation on muscle performance, d) investigate the effectiveness of Cr supplementation on muscle performance during longer duration (> 2 weeks) immobilization protocols, e) determine if Cr is an effective countermeasure during other muscle disuse scenarios (e.g. bed rest), f) investigate the effectiveness of supplementing Cr before the immobilization period.

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APPENDICIES

A. Informed Consent

B. HIPPA

C. Health History Questionnaire

D. Subject Data

E. Supplement Protocol

F. Data Collection Form

G. Participant Payment Form

APPENDIX A

The University of New Mexico Health Sciences Center Consent to Participate in Research

Version Date: 2/22/2011

Effect of Creatine Supplementation on High Energy Phosphate Kinetics after Immobilization

Introduction

You are being asked to participate in a research study that is being done by Suzanne Schneider Ph.D., who is the Principal Investigator and Jeremy C. Fransen, a Ph.D. Candidate, from the Department of Health Exercise and Sport Sciences. This research is studying the effectiveness of creatine supplementation on muscle energy metabolism following wrist immobilization.

You are being asked to participate in this study because of your status as someone that engages in a regular exercise training program of at least 2 times per week of resistance exercise for more than 30 minutes per session. Twenty-four people will take part in this study at the University of New Mexico.

This form will explain the research study, and will also explain the possible risks as well as the possible benefits to you. We encourage you to talk with your family and friends before you decide to take part in this research study. If you have any questions, please ask one of the study investigators.

What will happen if I decide to participate?

If you agree to participate, the following things will happen:

Procedures

If you decide to participate, you will be asked to report to New Mexico Resonance located at 2301 Yale Blvd. SE, Albuquerque, NM on 3 separate occasions for testing that will take approximately 2 hours each. Prior to the first testing session, you will be asked to complete a health history questionnaire. Female participants will be asked to take a pregnancy test. Pregnant women will be excluded from the study.

1) For the first testing session, you will perform a familiarization exercise and recovery test, which consists of wrist/forearm flexion exercise to fatigue with your non-dominant arm inside a MRI/MRS magnet to measure muscle metabolism. The

incremental protocol will be accomplished by adding 0.5 to 1.0 kg weights attached to a wall pulley exercise device located inside the bore of the magnet. Following exercise to fatigue, your forearm will remain inside the magnet for 5 minutes to obtain muscle metabolism recovery data. You will then perform 2 minutes of continuous exercise followed by 20 minutes of recovery data. Your arm will remain inside the magnet during the entire exercise and recovery bouts.

2) The second session will begin with a measure for muscle volume. Your non-dominant hand/forearm will be submerged to the elbow in a tank of water to measure water displacement, and thus limb size. You will then perform the exercise session, where you will perform incremental forearm flexion to fatigue, followed by 5 minutes of recovery. You will then perform 2 minutes of forearm flexion exercise with a load equal to the maximal load achieved during the incremental test. You will then rest 5 minutes. After the rest, you will once again perform 2 minutes of forearm flexion exercise with the same load as the last 2 min exercise bout. You will then rest for 30 minutes to obtain complete recovery data. Your forearm will remain inside the magnet during the entire exercise testing and recovery sequence.

Following the second testing session with the magnet, your non-dominant arm will be placed in a plaster cast covering the forearm below the elbow to the mid palm of the hand. You will then be instructed to consume 5 grams of a powdered supplement, either creatine or placebo, mixed with water twice on that day with meals, for a total of 10 grams. The cast will remain on for the next 7 days during which time you will be instructed to consume 5 grams of creatine or placebo four times per day mixed with water during meals for a total of 20 grams per day.

3) The third testing session will begin on day 8, following the 7 days of casting. You will be instructed to ingest 5 grams of supplement at breakfast the morning before testing. Once you arrive at NMR, the forearm/hand cast will be removed. Your forearm will then be placed into a tank of water to measure limb size. Next, the exercise testing will be conducted in the magnet. The protocol will be identical to the pre-casting sequence as described above.

How long will I be in this study?

Participation in this study will take a total of 6 hours over a period of 3 sessions of 2 hours each. Participation will also include the non-dominant arm being in a wrist/forearm cast for 7 days.

What are the risks or side effects of being in this study?

- Possible side effects of maximal forearm exercise may include brief feelings of muscle fatigue and discomfort while performing wrist flexion exercise. In addition, you may have localized discomfort with the forearm and hand while it rests inside the magnet during the rest periods. Because the exercise bouts consist of muscle contractions, there is a risk of developing delayed muscle soreness in the forearm

muscles. The forearm muscle may lose strength and size during the week of cast immobilization. However, these changes will most likely small and only measureable by using the sensitive testing methodology of the magnet. Muscle recovery following short-term cast immobilization is rapid. Finally, there is a possibility of pain or discomfort if the cast becomes tight as a result of water weight gain associated with creatine. All personnel conducting the test have previous exercise testing experience and are aware of the signs and symptoms that are associated with possible adverse reactions during muscle exercise.

- An MRS machine acts like a large magnet, so it could move iron-containing objects in the room during your examination. Precautions have been taken to prevent any such event from happening and injuring you. You cannot have an MRS if you have a pacemaker or any metal in your body, such as an aneurysm clip, ear implant, nerve stimulator or any other metal permanent metal implants. It is unknown what, if any, the risks of a MRS are to a fetus, therefore women who are pregnant should not participate in this study.

- There is a risk of gastrointestinal discomfort; specifically, upset stomach, vomiting and diarrhea related to creatine.

  You will be ingesting a supplement that may cause a 2 to 4 lbs gain in body weight. This gain in body weight is associated with an increase in body water. The body weight gain is temporary and you should lose this weight within 2 weeks following the research study. However, there is a possibility that the water gain may cause the cast to become tight. If the tightness causes severe pain or numbness in the hand/forearm, please contact Jeremy Fransen at 702-917-3724 immediately to have the cast removed.

- There is a risk that the limited range of motion may compromise your ability to drive an automobile. There is a possibility you may not be able to drive during the 7 days in a cast. If you do drive, there is a possibility that you may not be covered by your insurance company. Please refer to your current automobile insurance policy for specific information.

- There are risks of stress, emotional distress, inconvenience and possible loss of privacy and confidentiality associated with participating in a research study.

For more information about risks and side effects, ask your study doctor.

What are the benefits to being in this study?

You may receive no benefits from this study. However, results from the incremental exercise test will give you information concerning your forearm muscle power and metabolism during exercise and recovery. Furthermore, information gained from using sensitive, state-of- the-art equipment to measure muscle metabolism and function following short-term immobilization will enhance the scientific knowledge concerning

muscle disuse. Finally, if the creatine supplement intervention slows changes in muscle during immobilization, this could result in a new therapy that would have wide- range application to patients that are immobilized following injury.

What other choices do I have if I do not want to be in this study?

The only alternative is to not participate in this study.

How will my information be kept confidential?

We will take measures to protect your privacy and the security of all your personal information, but we cannot guarantee confidentiality of all study data.

The University of New Mexico Health Sciences Center Human Research Review Committee (HRRC) that oversees human subject research, along with the PI and co-investigator, will be permitted to access your records. There may be times when we are required by law to share your information. However, your name will not be used in any published reports about this study. A copy of this consent form will be kept in your medical record.

What are the costs of taking part in this study?

You will not be charged for any of the study's procedures.

What will happen if I am injured or become sick because I took part in this study?

If you are injured or become sick as a result of this study, UNMHSC will provide you with emergency treatment, at your cost. No commitment is made by the University of New Mexico Health Sciences Center (UNMHSC) to provide free medical care or money for injuries to participants in this study.

In the event that you have an injury or illness that is caused by your participation in this study, reimbursement for all related costs of care will be sought from your insurer, managed care plan, or other benefits program. If you do not have insurance, you may be responsible for these costs. You will also be responsible for any associated co-payments or deductibles required by your insurance.

It is important for you to tell your study doctor immediately if you have been injured or become sick because of taking part in this study. If you have any questions about these issues, or believe that you have been treated carelessly in the study, please contact the Human Research Review Committee (HRRC) at the University of New Mexico Health Sciences Center, Albuquerque, New Mexico 87131, (505) 272-1129 for more information.

Will I be paid for taking part in this study?

Yes, you will be compensated $100.00 for your participation in this study. Compensation will be pro- rated based on time of participation. You will be compensated for each of the three sessions as follows: $20 for the first session, $40 for the second and $40 for the final session.

How will I know if you learn something new that may change my mind about participating?

You will be informed of any significant new findings that become available during the course of the study, such as changes in the risks or benefits resulting from participating in the research or new alternatives to participation that might change your mind about participating.

Can I stop being in the study once I begin?

Your participation in this study is completely voluntary. You have the right to choose not to participate or to withdraw your participation at any point in this study without affecting your future health care or other services to which you are entitled.

Whom can I call with questions or complaints about this study?

If you have any questions, concerns or complaints at any time about the research study, Suzanne Schneider, Ph.D., or her associates will be glad to answer them at (505) 277-2658, Monday through Friday 8:00am – 5:00pm. If you need to contact someone after business hours or on weekends, please call at 272-4751 (pager), and ask for Dr. Akshay Sood (Medical Director of the EPL). If you would like to speak with someone other than the research team, you may call the UNMHSC HRRC at (505) 272-1129.

Whom can I call with questions about my rights as a research subject?

If you have questions regarding your rights as a research subject, you may call the UNMHSC HRRC at (505) 272-1129. The HRRC is a group of people from UNM and the community who provide independent oversight of safety and ethical issues related to research involving human subjects. For more information, you may also access the HRRC website at http://hsc.unm.edu/som/research/hrrc/.

CONSENT

You are making a decision whether to participate in this study. Your signature below indicates that you read the information provided. By signing this consent form, you are not waiving any of your legal rights as a research subject.

I have had an opportunity to ask questions and all questions have been answered to my satisfaction. By signing this consent form, I agree to participate in this study. A copy of this consent form will be provided to you.

_____ _____ _____

Name of Subject (print) Signature of Subject Date

INVESTIGATOR SIGNATURE

I have explained the research to the subject and answered all of his/her questions. I believe that he/she understands the information described in this consent form and freely consents to participate.

_____

Name of Investigator/ Research Team Member (type or print)

_____ _____

(Signature of Investigator/ Research Team Member) Date

APPENDIX B

UNIVERSITY OF NEW MEXICO HEALTH SCIENCES CENTER HIPAA[1] AUTHORIZATION TO USE AND DISCLOSE PROTECTED HEALTH INFORMATION FOR RESEARCH PURPOSES

Title of Study: Effect of Creatine Supplementation on High Energy Phosphate Kinetics after Immobilization

Principal Investigator:	**Suzanne Schneider**
UNMHSC Department:	**Health, Exercise & Sport Sciences**
Mailing Address:	**sschneid@unm.edu**
Co-Investigators:	**Jeremy C. Fransen, Micah Zuhl, Nicole Vargas, Robert Robergs**

Sponsor: N/A

1. **What is the purpose of this form?** You have been asked to take part in a research study. The consent form for this study describes your participation, and that information still applies. This extra form is required by the federal Health Insurance Portability and Accountability Act (HIPAA). The purpose of this form is to get your permission (authorization) to use health information about you that is created by or used in connection with this research.

2. **What if I don't want my personal health information (PHI) to be used in this research study?** You do not have to give this permission. Your decision not to sign this form will not change your ability to get health care outside of this research study. However, if you do not sign, then you will not be allowed to participate in the study.

3. **What PHI am I allowing to be used for this research?** The information that may be used -------- includes: Health history and current health status, height, weight, age, gender, forearm muscle power, forearm muscle volume, and muscle metabolites (ATP, Pi, PCr, pH) as measured by magnetic resonance spectroscopy (^{31}P MRS) during exercise and recovery from exercise. Muscle strength, size, and metabolism measurements before and after 7 days of forearm/wrist casting will be compared to a supplement intervention versus placebo.

4. **Where will researchers go to find my PHI?** We may ask to see your personal information in records at hospitals, clinics or doctor's offices where you may have received care in the past, including but not limited to facilities in the UNM health care system.

5. **Who will be allowed to use my information for this research and why?** The researchers named AccIt may be used to check on your progress during the study, or analyze it along with information from other study participants. Sometimes research information is shared with collaborators or other institutions. Your records may also be reviewed by representatives of the research sponsor or funding agency, the Food and Drug Administration (FDA) to check for quality, safety or effectiveness, or the Human Research Review Committee (HRRC) for the purposes of oversight and subject safety and compliance with human research regulations.

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[1]HIPAA is the Health Insurance Portability and Accountability Act of 1996, a federal law related to privacy of health information.

6. **Will my information be used in any other way?** Your information used under this permission may be subject to re-disclosure outside of the research study and be no longer protected under certain circumstances such as required reporting of abuse or neglect, required reporting for law enforcement purposes, and for health oversight activities and public health purposes.

7. **What if I change my mind after I give this permission?** You can change your mind and withdraw this permission at any time by sending a written notice to the Principal Investigator at the mailing address listed at the top of this form to inform the researcher of your decision. If you withdraw this permission, the researcher may only use and share your information that has already been collected for this study. No additional health information about you will be collected by or given to the researcher for the purposes of this study.

8. **What are the privacy protections for my PHI used in this research study?** HIPAA regulations apply to personal health information in the records of health care providers and other groups that share such information. There are some differences in how these regulations apply to research, as opposed to regular health care. One difference is that you may not be able to look at your own records that relate to this research study. These records may include your medical record, which you may not be able to look at until the study
is over. The HIPAA privacy protections may no longer apply once your PHI has been shared with others who may be involved in this research.

9. **How long does this permission allow my PHI to be used?** If you decide to be in this research study, your permission to access and use your health information in this study may not expire, unless you revoke or cancel it. Otherwise, we will use your information as long as it is needed for the duration of the study.

I am the research participant or the personal representative authorized to act on behalf of the participant. By signing this form, I am giving permission for my personal health information to be used in research as described above. I will be given a copy of this authorization form after I have signed it.

Name of Research Subject	Signature of Subject	Date

Name of Person Obtaining Authorization	Signature	Date

APPENDIX C

HEALTH HISTORY QUESTIONNAIRE

Subject #________ Date___/___/___ Phone (H) _________ (Cell) ___________

Date of Birth ___/___/___ Age____ Gender____ Ethnicity______

Address (home) ____zip_________

♦♦♦

MEDICAL HISTORY

Self-reported: Height______ Weight______

Physical injuries:____

Limitations____

Have you ever had any of the following cardiovascular problems? Please check all that apply.

Heart attack/Myocardial Infarction	____	Heart surgery	____
Chest pain or pressure	____	Swollen ankles	____
Arrhythmias/Palpitations	____	Heart murmur	____
Valve problems	____	Dizziness	____
Congestive heart failure	____		

Have you ever had any of the following? Please check all that apply.

Hepatitis/HIV	_____	Cancer (specify type)	____
Rheumatic fever	_____	High blood pressure	____
Kidney/liver disease	_____	Obesity	____
Diabetes (specify type)	_____	Asthma	____
Emphysema	_____	Stroke	____
Depression	_____	Thyroid problems	____
Total cholesterol >200 mg/dl	_____	HDL cholesterol <35 mg/dl	____
LDL cholesterol >135 mg/dl	_____	Trigylcerides >150 mg/dl	____
Neuromuscular disease	_____		

(e.g. myasthenia gravis, rheumatoid arthritis, multiple sclerosis, muscular dystrophy, etc.)

If yes, describe _______________

Do immediate blood relatives (biological parents & siblings **only**) have any of the conditions listed above? If yes, list the problem, and family member age at diagnosis.

____

Do you have any metal implants or metallic objects in your body? Y N

Do you have any tattoos? Y N

Do you currently have any condition not listed that may influence test results? Y N

Details____

Indicate level of your overall health. Excellent ____ Good ____ Fair ____ Poor_____

Are you taking any medications, vitamins or dietary supplements now? Y N
If yes, what are they?____

Have you ever taken creatine monohydrate? Y N

If yes, when was the last time you took creatine? ____________________

Are you allergic to latex? Y N

Have you ever experienced any adverse effects during or after exercise (fainting, vomiting, shock, palpitations, hyperventilation)? Y N If yes, elaborate._____________

♦♦♦

LIFESTYLE FACTORS

How often do you drink the following?

Caffeinated coffee, tea, or soda _____oz/day

What do you do for physical activity/exercise now? ____________________________

How often do you exercise? ____________ How long per session?________

Do you regularly perform resistance exercise or weight training exercise? Y N

If yes, how many days per week do you train with weights? ______________

Estimate your exercise intensity level or exercise heart rate.________________

Indicate your current level of emotional stress. High____ Moderate ____ Low____

♦♦

WOMEN ONLY

Are you pregnant or could you be pregnant? Y N

Please check the response that most closely describes your menstrual status:

_____ Post-menopausal (surgical or absence of normal menstrual periods for 12 months)

_____ Eumenorrheic – Normal menstrual periods (~every 28 days)

_____ Amenorrheic – Absence of normal menstrual periods for at least 3 months

_____ Oligomenorrheic – Irregular menstrual periods with occasional missed cycles.

APPENDIX D

Table 5. Pre- Immobilization PL

Subject	Cr/PL	Gender	Incr J	Incr W	CL1 J	CL1 W	CL2 J	CL2W
1	PL	F	321.78	4.29	141.22	0.98	138.27	0.98
5	PL	F	248.23	3.31	123.56	0.86	123.56	0.86
7	PL	F	183.87	2.45	105.91	0.74	105.91	0.74
11	PL	F	248.23	3.31	123.56	0.86	123.56	0.86
16	PL	F	248.23	3.31	123.56	0.86	123.56	0.86
19	PL	F	404.52	5.39	158.87	1.10	158.87	1.10
22	PL	F	321.78	4.29	141.22	0.98	138.27	0.98
3	PL	M	643.56	8.58	282.43	1.96	282.43	1.96
6	PL	M	809.05	10.79	317.74	2.21	317.74	2.21
8	PL	M	496.46	6.62	247.13	1.72	247.13	1.72
14	PL	M	496.46	6.62	247.13	1.72	247.13	1.72
26	PL	M	496.46	6.62	247.13	1.72	247.13	1.72
Mean			409.89	5.47	188.29	1.31	187.80	1.31
Std Dev			186.22	2.48	74.26	0.52	74.60	0.52
n			12	12	12	12	12	12

Table 6. Post- Immobilization PL

Subject	Cr/PL	Gender	Incr J	Incr W	CL1 J	CL1 W	CL2 J	CL2 W
1	PL	F	321.78	4.29	141.22	0.98	129.45	0.90
5	PL	F	188.63	2.52	58.64	0.41	102.97	0.72
7	PL	F	183.87	2.45	105.91	0.74	105.91	0.74
11	PL	F	248.23	3.31	123.56	0.86	123.56	0.86
16	PL	F	248.23	3.31	44.62	0.31	0.00	0.00
19	PL	F	404.52	5.39	39.72	0.28	97.09	0.67
22	PL	F	321.78	4.29	125.53	0.87	133.37	0.93
3	PL	M	533.24	7.11	247.13	1.72	219.67	1.53
6	PL	M	809.05	10.79	308.91	2.15	300.08	2.08
8	PL	M	496.46	6.62	15.69	0.11	141.22	0.91
14	PL	M	496.46	6.62	185.35	1.29	233.40	1.62
26	PL	M	496.46	6.62	219.67	1.53	212.80	1.48
Mean			395.73	5.28	134.66	0.94	149.96	1.04
Std Dev			181.53	2.42	90.92	0.63	79.32	0.55
n			12	12	12	12	12	12

Table 7. Pre-Immobilization CR

Subject	Cr/PL	Gender	Incr J	Incr W	CL1 J	CL1 W	CL2 J	CL2 W
2	Cr	F	321.78	4.29	141.22	0.98	138.27	0.98
12	Cr	F	183.87	2.45	105.91	0.74	105.91	0.74
17	Cr	F	404.52	5.39	158.87	1.10	158.87	1.10
21	Cr	F	248.23	3.31	123.56	0.86	123.56	0.86
4	Cr	M	643.56	8.58	282.43	1.96	282.43	1.96
10	Cr	M	321.78	4.29	141.22	0.98	138.27	0.98
13	Cr	M	496.46	6.62	247.13	1.72	247.13	1.72
15	Cr	M	643.56	8.58	282.43	1.96	282.43	1.96
18	Cr	M	643.56	8.58	282.43	1.96	282.43	1.96
20	Cr	M	643.56	8.58	282.43	1.96	282.43	1.96
23	Cr	M	496.46	6.62	247.13	1.72	247.13	1.72
24	Cr	M	367.75	4.90	211.82	1.47	211.82	1.47
25	Cr	M	643.56	8.58	282.43	1.96	282.43	1.96
Mean			466.05	6.21	214.54	1.49	214.09	1.49
Std Dev			168.71	2.25	70.18	0.49	70.70	0.49
n			13	13	13	13	13	13

Table 8. Post-Immobilization CR

Subject	Cr/PL	Gender	Incr J	Incr W	CL1 J	CL1 W	CL2 J	CL2 W
2	Cr	F	321.78	4.29	141.22	0.98	138.27	0.91
12	Cr	F	138.68	1.85	94.14	0.65	88.26	0.61
17	Cr	F	404.52	5.39	150.04	1.04	141.22	0.98
21	Cr	F	248.23	3.31	123.56	0.86	123.56	0.86
4	Cr	M	619.04	8.25	258.90	1.79	243.20	1.69
10	Cr	M	321.78	4.29	137.29	0.95	137.29	0.95
13	Cr	M	496.46	6.62	199.07	1.38	205.94	1.43
15	Cr	M	643.56	8.58	251.05	1.74	258.90	1.78
18	Cr	M	643.56	8.58	251.05	1.74	282.43	1.91
20	Cr	M	643.56	8.58	251.05	1.74	258.90	1.78
23	Cr	M	496.46	6.62	247.13	1.16	247.13	1.16
24	Cr	M	367.75	4.90	188.29	1.31	211.82	1.41
25	Cr	M	608.32	8.11	266.74	1.85	109.83	0.76
Mean			457.98	6.11	196.89	1.32	188.21	1.25
Std Dev			170.51	2.27	61.17	0.41	66.99	0.44
n			13	13	13	13	13	13

Table 9. Pre to Post % Change for PL

Subject	Cr/PL	Gender	Incr J	Incr W	CL1 J	CL1 W	CL2 J	CL2 W
1	PL	F	0	0.00	0.00	0.00	-6.38	-8.27
5	PL	F	-24.01	-24.01	-52.54	-52.56	-16.67	-16.67
7	PL	F	0	0.00	0.00	0.68	0.00	-0.07
11	PL	F	0	0.00	0.00	0.00	0.00	0.00
16	PL	F	0	0.00	-63.89	-63.87	-100.00	-100.00
19	PL	F	0	0.00	-75.00	-74.98	-38.89	-38.89
22	PL	F	0	0.00	-11.11	-11.02	-3.55	-5.51
3	PL	M	-17.14	-17.14	-12.50	-12.48	-22.22	-22.25
6	PL	M	0	0.00	-2.78	-2.81	-5.56	-5.57
8	PL	M	0	0.00	-93.65	-93.65	-42.86	-47.15
14	PL	M	0	0.00	-25.00	-25.01	-5.56	-5.55
26	PL	M	0	0.00	-11.11	-11.14	-13.89	-13.88
Mean			-3.17	-3.43	-28.96	-28.90	-21.30	-21.98
Std Dev			7.85	8.14	33.31	33.36	28.58	28.70
n			12	12	12	12	12	12

Table 10. Pre to Post % Change for CR

Subject	Cr/PL	Gender	Incr J	Incr W	CL1 J	CL1 W	CL2 J	CL2 W
2	Cr	F	0	0.00	0.00	0.00	0.00	-7.52
12	Cr	F	-24.58	-24.58	-11.11	-11.08	-16.67	-16.66
17	Cr	F	0	0.00	-5.56	-5.53	-11.11	-11.06
21	Cr	F	0	0.00	0.00	0.00	0.00	0.00
4	Cr	M	-3.81	-3.81	-8.33	-8.94	-13.89	-13.89
10	Cr	M	0	0.00	-2.78	-2.76	-0.71	-2.82
13	Cr	M	0	0.00	-19.44	-19.47	-16.67	-16.68
15	Cr	M	0	0.00	-11.11	-11.13	-8.33	-9.25
18	Cr	M	0	0.00	-11.11	-11.13	0.00	-2.42
20	Cr	M	0	0.00	-11.11	-11.13	-8.33	-9.25
23	Cr	M	0	0.00	0.00	-32.29	0.00	-32.29
24	Cr	M	0	0.00	-11.11	-11.09	0.00	-4.09
25	Cr	M	-5.48	-5.48	-5.56	-5.58	-61.11	-61.10
Mean			-2.61	-2.61	-7.48	-10.01	-10.52	-14.39
Std Dev			6.83	6.83	5.83	8.59	16.60	16.37
n			13	13	13	13	13	13

APPENDIX E

Supplement Protocol:

Day 1: Get casted. Take 2 servings (1a, 1b) spaced out that day (i.e. lunch, dinner)

Day2: Take 4 servings (2a, 2b, 2c, 2d) spaced out evenly (i.e. breakfast, lunch, dinner, nighttime snack)

Day 3 – Day 7: Same as day 2 (3a, 3b, 3c, 3d; 4a, 4b, 4c, 4d; etc.)

Day 8: Take one serving (8a) with breakfast. Cast comes off. Do Post-test at NMR

Mixing procedure:

1. Place mixing jug on a plate or large bowl to catch any powder that may fall out of the container.
2. Pour powder contents into container being careful not to spill.
3. Take empty baggy and put that back into the large plastic bag.
4. Add warm (room temp) water to the 300 ml line on side of container.
5. Mix thoroughly making sure all powder is dissolved.
6. Drink

Notes:

-Take the supplement with meals. This helps absorption.

-Try to drink more water throughout the day.

-Please use at least room temperature or slightly warm water. This helps the powder dissolve and improves absorption.

-KEEP ALL THE BAGGIES!!! This is important, as I will count up all the baggies when you return them in the big plastic bag.

-If you forget a dose, just make sure to get it in at the next meal. You want to make sure you get all the required doses on that day.

-Do not take supplement with caffeine. If you must, you can have a coffee or soda, just do it between meals when not consuming the supplement.

APPENDIX F

Trial_____ Subject # _____

Cycle	**Load (kg)**	**Reps**
1		
2		
3		
4		
5		
6		
7		
8		
9		

SS Load_____ SS#1 reps _____ SS#2 reps_____

APPENDIX G

UNM Participant Form:

This form is used to set up all participants as venders in the UNM System for payment purposes. After the participant has been processed the participant will be assigned banner ID # and this form will be destroyed. Thank you for participating in this study.

Project Name: **Effect of Creatine Supplementation on High Energy Phosphate Kinetics after Immobilization**

Participant Legal Name:

_____

Address:

_____

_____

Phone #:

(_____)_____

Social Security number:

_____

Amount to be Paid:

$___100.00_____