1-1-1999

The effect of dietary creatine supplementation on compensatory muscle hypertrophy and performance characteristics of specific fiber types

Robert Earl Young
University of Nevada, Las Vegas

INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

UMI®

Bell & Howell Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

THE EFFECT OF DIETARY CREATINE SUPPLEMENTATION ON COMPENSATORY MUSCLE HYPERTROPHY AND PERFORMANCE CHARACTERISTICS OF SPECIFIC FIBER TYPES

by

Robert Earl Young

Bachelor of Science
University of Nevada, Las Vegas
1997

A thesis submitted in partial fulfillment
of the requirements for the

Master of Science Degree
Department of Kinesiology
Exercise Physiology
College of Health Sciences

Graduate College
University of Nevada, Las Vegas
December 1999

UNLV

Thesis Approval

The Graduate College
University of Nevada, Las Vegas

August 3, 19 99

The Thesis prepared by

Robert Young

Entitled

The effect of dietary creatine supplementation on compensatory muscle hypertrophy and performance characteristics of specific fiber types.

is approved in partial fulfillment of the requirements for the degree of

Master of Science, Exercise Physiology

Examination Committee Chair

Dean of the Graduate College

Examination Committee Member

Graduate College Faculty Representative

Graduate College Faculty Representative

ABSTRACT

The Effect of Dietary Creatine Supplementation on Compensatory Muscle Hypertrophy and Performance Characteristics of Specific Fiber Types

by

Robert Earl Young

Dr. Lawrence A. Golding, Examination Committee Chair
Distinguished Professor of Kinesiology
University of Nevada, Las Vegas

An animal model was used to determine the effect of dietary creatine supplementation on compensatory muscular hypertrophy and electrically stimulated performance of those muscles. Thirty-six rats were divided evenly into six groups. Two groups were surgical controls, two groups had the tibialis anterior ablated, to hypertrophy the extensor digitorum longus (EDL), and two groups the gastrocnemius and the plantaris ablated, to hypertrophy the soleus. One of each of these groups had their diet supplemented with creatine monohydrate. The size of the EDL and soleus were measured following surgical removal of the synergistic muscle and a 5-week supplementation period. Peak force, total tension over two 30 second intervals and time to one-half peak force were measured from the electrically stimulated muscle. Results indicated no difference in muscle size, peak force or tension due to the supplement. Time to one-half fatigue was increased in hypertrophied, non-supplemented soleus muscle.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGMENTS

I would like to dedicate this thesis to my wife, Jennifer, thank you for the support and encouragement. Thank you for allowing me to pursue my dreams. I would also like to thank my parents Jack and Susan for their support and guidance throughout life. If it were not for you none of this would have been possible. Special thanks to Dr. Carl Reiber for the use of his lab space and equipment. Thank you to Dr. Lawrence Golding for acting as chair of my committee, and advisor these last two years. I would like to thank the other members of the committee, Dr. Richard Tandy, Dr. Bryan Spangelo and Dr. Susan Meacham for their unique knowledge, which made this project possible. A very special thank you to Marsha Moon, Jewel Sutten and the rest of the staff at the Lab Animal Care Facility, for all of your help with the little ones.

CHAPTER 1

INTRODUCTION

Creatine (Cr) is a naturally occurring compound found within muscle cells (Figure 1). It is obtained in the diet from meat and fish, or it can by synthesized endogenously in the liver, pancreas and kidneys from methionine, glycine, and arginine. Cr is phosphorylated, via creatine kinase, to Phosphocreatine (PCr). PCr is a high-energy compound that supports the re-synthesis of ATP during the initial stages of intense muscular activity (Greenhaff, 1995; Hirvonen, Rehunen, Rusko & Harkonen, 1987; Hultman, Bergstrom & Anderson, 1967). Studies have shown that dietary supplements of Cr will result in increased Cr and PCr in the skeletal muscle. A typical supplement protocol of 5g Cr 4 times a day, for 5 to 10 days, will increase total Cr and PCr (Balsom, Soderlund & Ekblom, 1994; Balsom, Soderlund, Sjodin & Ekblom, 1995; Green, Simpson, Littlewood, MacDonald & Greenhaff, 1996; Greenhaff, Bodin, Soderlund & Hultman, 1994; Harris, Soderlund & Hultman, 1992; Hultman, Soderlund, Timmons, Cederblad & Greenhaff, 1996; Kreider, 1995). PCr stores are normally depleted within the first 20-seconds of high intensity exercise (Hultman, Greenhaff, Ren & Soderlund, 1990). Within one minute of exercise termination PCr levels are restored to 50% of pre-exercise values (Tesch, Thorsson & Fujitsuka, 1989). Complete restoration of PCr occurs about 20-minutes post exercise, however after 6-minutes PCr stores have returned to 80 to 90% of resting levels (Harris, Edwards, Hultman, Nordesjo, Nylind & Sahlin,

1976). For this reason Cr supplementation is thought to have an ergogenic effect, that will enhance athletic performance in activities that consist of short duration, high intensity muscular activity (i.e. sprinting, cycling, swimming, as well as, weight lifting). Cr supplementation may also affect those activities that feature repeated bouts of intense activity (i.e. hockey, soccer, basketball and tennis). Recent studies have yielded mixed results regarding the effect of Cr supplementation on athletic performance (Balsom, Harridge, Soderlund, Sjodin & Ekblom, 1993; Rossiter, Cannell & Jakeman, 1996; Mujika & Padilla, 1997; Mujika, Chatard, Lacoste, Barale & Geyssant, 1996; Kreider, Ferreira, Wilson, Grindstaff, Plisk, Reinardy, Cantler & Almada, 1998). The vast majority of studies have focused on humans and their performance in various running and cycling protocols, but the total amount of research involving Cr supplementation is still too small and the results too varied to draw strong conclusions. A few more recent studies have focused on Cr supplementation and weight training. Cr supplementation may have an ergogenic effect on muscle hypertrophy (Kreider, 1995; Earnest, Snell, Rodriguez, Almada & Mitchell, 1995; Kreider, et al, 1998), but the results are still inconclusive. The purpose of this study is to investigate the effects of Cr supplementation on muscle hypertrophy and the performance of those muscles, using an animal model. The use of an animal model will eliminate many problems inherent in human studies, namely placebo effect and the variability of athletic performance. Animal models have long been used in physiological studies to provide greater control of the variables involved with the studies.

In rats, muscle hypertrophy has been produced through surgical removal, or ablation, of a synergistic muscle, with more success than many other methods including tenotomy, passive muscle stretch, and exercise induced enlargement (Timson, 1990).

Ablation of the gastrocnemius and plantaris results in compensatory hypertrophy of the soleus, by 60 to 90% over control muscle size (Young, Kandarian & Kurowski, 1992; Kandarian, Young & Gomez, 1992; Kandarian & White, 1990). Ablation of the tibialis anterior (TA) results in hypertrophy of the extensor digitorum longus (EDL), by approximately 60% over control muscles (Young, Kandarian & Kurowski, 1992). In this study the soleus and EDL were chosen to represent two different types of muscle fiber. The soleus is predominantly slow twitch, while the EDL is predominantly fast twitch.

If Cr supplementation has an ergogenic affect, the animals that receive Cr should exhibit a greater degree of hypertrophy. Those hypertrophied muscles should produce a higher peak force, higher total tension and have an increased time to fatigue when exercised (electrically stimulated) maximally to exhaustion.

CHAPTER 2

REVIEW OF LITERATURE

Creatine is synthesized by the liver, pancreas and kidneys from the amino acids arginine, glycine and methionine (Figure 2) (Balsom, Soderlund & Ekblom, 1994; Bessman & Carpenter, 1985). Cr is also obtained through the dietary intake of meat and fish (Balsom et al 1994; Delanghe, De Slypere, Buyzere, Robbrecht, Weime & Vermeulen, 1989). The vast majority, 95%, of Cr is transported, via the blood stream (Jacobson, Christensen, Mogensen, Andresen & Heilskov, 1979), from sites of production to the skeletal muscle; the rest is distributed between the brain, testes and cardiac tissue (Balsom et al, 1994). Cr will react, nonenzymatically, to form creatinine (Bessman & Capenter, 1985), a waste product that is processed by the kidneys and excreted (Delanghe et al, 1989). The hydrolysis of Cr to creatinine takes place at a constant rate, approximately 2g/day in the human body (Balsom et al, 1994).

PCr is involved in muscular energetics as a high-energy phosphate donor. PCr has a large free energy of hydrolysis, about 10 Kcal/mol (Meyerhof & Lohman, 1932). Lipmann and Meyerhoff (1930) demonstrated that creatine was released during muscular contraction, and concluded that the hydrolysis of PCr was coupled to muscular contraction. While adenosine triphosphate (ATP) was discovered around the same time (Fiske & Subbarow, 1929), it was not until the early 1960's that ATP was shown to be the direct source of energy for muscular contraction (Cain & Davies, 1962). ATP can be

formed in one of three different ways: 1. Oxidative Phosphorylation in the mitochondria. 2. Transphosphorylation of PCr, or 3. Transphosphorylation of adenosine diphosphate (ADP) (Hultman, Bergstrom & Anderson, 1967). In 1978 Bessman proposed the "Creatine Phosphate Shuttle"(CPS). The CPS is diagramed in Figure 3. PCr is synthesized by the mitochondria. It then diffuses to the myofibrils where the enzyme creatine kinase is bound. As ADP is generated during contraction, creatine kinase catalyzes the formation of ATP through the following reaction: PCr + ADP $\Leftrightarrow$ Cr + ATP. The free Cr then diffuses back to the mitochondria, where it can be rephosphorylated. (Bessman & Carpenter, 1985) The CPS is also supported by the observation that adenine nucleotides are compartmentalized to the mitochondria or the peripheral ATPases (i.e. myofibrils) (Perry, 1954; Mommaerts, 1954; Hill, 1960).

While PCr is not directly responsible for muscular contraction, it is a significant determinant for muscular activity. After short duration, high intensity exercise to fatigue, ATP levels remain relatively normal, while PCr levels are nearly depleted (Hultman, Bergstrom & Anderson, 1967; Fitts, 1994; Westerblad, Lee, Lannergren & Allen, 1991; Sahlin, Tonkongi & Sodelund, 1998). An enlargement of the phosphocreatine pool should enhance the cells ability to regenerate ATP during periods of extremely high energy requirements.

Cr has been shown to stimulate myofibril protein synthesis (Sipila, Rapola, Simell, 1981). This finding was limited to the treatment of choroid retina atrophy, and has yet to be duplicated. One hypothesized explanation of this finding may be that Cr stimulates GTP production, which is required to initiate protein synthesis. Alternately it has been hypothesized that Cr may induce cellular swelling through its osmotic activity. This swelling or volumizing may influence hormones and amino acids that regulate

metabolic control and in turn modulate anabolic activity or protein synthesis (Plisk & Kreider, 1999). Muscles depleted of Cr atrophy (Fitch & Chevli, 1980; Fitch, Chevli, Petrofsky & Kopp, 1978; Fitch, Jellinek, Fitts, Baldwin & Holloszy, 1975). Possible mechanisms of atrophy include the loss of protein bound Cr, reduced Cr-cofactor effect on myosin synthesis, or impaired energy supply (Plisk & Kreider, 1999). If normal resting Cr levels are low, increasing Cr may result in an augmented response in protein synthesis.

Dietary supplementation of Cr monohydrate has been shown to increase muscle total Cr by approximately 20% over a one-week period (Harris, Soderlund & Hultman, 1992; Hultman, et al, 1996), however initial levels of muscle Cr are a factor in the amount of increase observed (Casey, Constantin-Teodosiu, Howell, Hultman & Greenhaff, 1996). Individuals with lower resting concentrations will demonstrate a greater increase then individuals with higher resting levels (Delanghe, et al, 1989). Normal resting total Cr concentrations in non-supplemented humans are approximately 125 mmol/Kg of dry muscle (Harris, Hultman & Nordjesjo, 1974). It does appear, in humans, however that an upper limit of Cr concentration of 150 to 160 mmol/ Kg can be reached with prolonged supplementation (Mujika & Padilla, 1997). Supplementation, however, is not without adverse side effects. Anecdotal claims of minor gastrointestinal distress, nausea and muscle cramping have been reported, although the number of cases is very small compared to the total number of participants in all studies. Renal function, as measured by glomerular filtration rate and protein and albumen excretion rates, does not appear to be affected (Poortmans, Auquier & Renaut, 1997). Endogenous creatine synthesis is reduced during periods of supplementation, but returns to normal when supplementation is stopped (Hultman, et al, 1996).

Dietary supplementation of Cr has been affected by caffeine and carbohydrate consumption, although the mechanism of Cr uptake, into the cell, has yet to be determined. Caffeine consumption in conjunction with Cr supplementation negates the ergogenic effect of Cr (Vandenberghe, Gillis, Van Leemputt, Van Hecke, Vanstapel & Hespel, 1996). Muscle Cr and PCr was increased by supplementation of Cr in conjunction with caffeine, but Vandenberghe, et al found that muscular performance is not enhanced. This may account for some of the negative or non-significant results seen in some performance studies. Ingestion of carbohydrates along with Cr increases the retention of Cr within the cell (Green, et al, 1996). Green, et al, found that ingesting 5 g of Cr with 93 g of a simple carbohydrate solution augmented creatine retention, when compared to ingesting 5 g Cr with a sugar free solution. Cr retention was not further enhanced by exercise prior to ingestion of the Cr and carbohydrate solution.

Cr supplementation has been shown to enhance performance in cycling (Casey, et al, 1996; Jacobs, Bleue & Goodman, 1997; Birch, Noble, Greenhaff, 1994; Balsom, et al, 1995), swimming (Grindstaff, Kreider, Bishop, Wilson, Wood, Alexander & Almada, 1997), running (Harris, Viru, Greenhaff & Hultman, 1993) and kayaking (McNaughton, Dalton & Tarr, 1999). Cr has also been shown to increase measures of strength and anaerobic power (Kreider, et al, 1998; Kreider, 1995; Earnest, et al, 1995; Greenhaff, Casey, Short, Harris, Soderlund & Hultman, 1993). These studies have utilized maximal exercise (100 to 125 % VO2 max) over very short periods of time (less than 3 minutes).

Several studies have failed to show any difference between Cr and Placebo supplemented groups in cycling (Barnett, Hinds & Jenkins, 1996; Cooke, Grandjean & Barnes, 1995; Febbraio, Flangan, Snow, Zhao & Carey, 1995; Cooke & Barnes, 1997), Swimming (Mujika, et al, 1996; Burke, Pyne & Telford, 1996), running (Balsom, et al,

1993; Redondo, Dowling, Graham, Alamada & Williams, 1996; Terrillion, Kolkhorst, Dolgener & Joslyn, 1997; Stroud, Holliman, Bell, Green, MacDonald & Greenhaff, 1994), rowing (Rossiter, Cannell & Jakeman, 1996) and isometric exercise (Greenhaff, et al, 1994; Greenhaff, Bodin, Harris, Hultman, Jones, McIntyre, Soderlund & Turner, 1993).

Jacobs, Bleue and Goodman, in 1997, showed that a 5 day Cr supplementation program increased the maximum accumulated oxygen deficit (MAOD) and time to exhaustion during cycling. Participants peddled at 125% of their VO2 max, following a 3-minute warm up, until exhaustion, before and after the 5-day period of supplementation. MAOD and time of exhaustion were increased in the Cr group as compared to the placebo group. While no change was seen in the placebo group, a relative increase in MAOD of about 10% (0.4 L) and a 9% (about 10 s) increase in time to exhaustion was seen in the Cr group. The lingering effects of Cr supplementation were measured as participants repeated the test 7 days later, following the removal of the Cr supplement from the diet. MAOD and time to exhaustion remained elevated in the Cr group following 7 days without supplementation.

Harris, Viru, Greenhaff and Hultman in 1993, demonstrated that a 5-day Cr supplementation program decreased times in repeated bouts of running. Trained middle distance runners were divided into two groups, placebo and Cr. Participants ran 4X300 meter, and 4X1000 meter runs on separate days, with 4 and 3-minute rest intervals, respectively, before and after 5 days of Cr or placebo supplementation. Total time for the 4 trials as each distance was decreased in the Cr group, as was the time for the last trial of each distance. Best 300 and 1000-meter times were also significantly decreased in the Cr group.

Grindstaff, Kreider, Bishop, Wilson, Wood, Alexander and Almada in 1997 found that 9 days of Cr supplementation decreased times in 3 100-meter swim sprints. Participants performed 3 100-meter sprints with 1-minute rest in between, before and after 9 days of Cr or placebo supplementation. Following supplementation, times for the first two of the three trials were significantly faster in the Cr group. When compared to pre-supplement trials the Cr group swam only slightly faster in the first two trials. The difference between the placebo and Cr groups was mainly due to the slower times of the placebo group. No significant differences were detected in the third trial.

Kreider, Ferreira, Wilson, Grindstaff, Plisk, Reinardy, Cantler and Almada in 1998 determined the effects of a 28-day Cr supplementation program on body composition, strength and sprint performance. Results indicated that Cr supplementation had no effect on the percentage of total body water, while total body weight increased in the Cr group. The increase was in part due to an increase in fat/bone free mass. Measures of strength, including bench press lifting volume and the sum of bench press, squat and power lifting volumes, increased in the Cr group. Bench press volume increased by an average of 225 kg while a minimal decrease, -5 kg, was seen in the placebo group. The sum of the three lifting volumes increased 1,558 kg in the Cr group compared to a 1,105 kg increase in the placebo group. No difference was seen in the volumes of the squat or power lift between the two groups. Total work for the first 5 of 12 6-sec cycling sprints was increased in the Cr group, resulting in a significant difference in the total work for the 12 sprints.

Birch, Noble and Greenhaff, in 1994, studied the effects of Cr supplementation on maximal isokinetic cycling. Participants were tested before and after a 5-day, Cr or placebo, supplementation regime. Testing consisted of 3 30-sec bouts of maximal

cycling, separated by 4-min of passive recovery. Peak power output (PPO) and mean power output (MPO) were measured during each bout. Peak power was significantly increased by Cr supplementation in bout 1 and MPO was significantly increased in bouts 1 and 2. There was no difference in bout 3.

Balsom, Soderlund, Sjodin and Ekblom, in 1995, determined the effect of Cr supplementation on high-intensity cycling. Participants were tested before and after a 6-day Cr supplementation intervention. The exercise protocol consisted of 5 6-sec ergometric cycling sprints with 30-sec of recovery after each sprint, followed by 1 10-sec sprint, 40-sec after the final 6-sec sprint. The 10-sec period was used to evaluate the participant's ability to maintain power output. Results indicated that target speed was better maintained following Cr supplementation. Target speed was maintained during each of the 6-sec periods. The total work done in the 6-sec periods was the same after supplementation as it was before. Muscle biopsies indicated that total Cr was increased by supplementation. PCr concentrations were higher in post exercise measurements following supplementation. Increased performance in the 10-sec bout is attributed to this higher post-exercise PCr concentration. The increase in total Cr was accompanied by 1.1-kg increase in body weight over the 6 days of supplementation.

Casey, Constantin-Teodosiu, Hultman and Greenhaff, in 1996, reported the effects of Cr supplementation on maximal ergometric cycling. Participants performed 2 30-sec bouts of maximal cycling before and after 5-days of Cr ingestion. Total work production during post-supplement bouts 1 and 2 increased by about 4%, as did peak work output during each bout. This increase was positively correlated with the increase in total muscle Cr, measured by muscle biopsy. Any change in body weight was not mentioned.

Greenhaff, Casey, Short, Harris, Soderlund and Hultman, in 1993, investigated the effects of Cr supplementation on maximal isokinetic knee extensions. Muscle torque production was measured during each contraction in a series of 5 bouts of 30 unilateral knee extensions, with 1-min rest between bouts. Participants were tested before and after 5-days of Cr or placebo supplementation. No difference was seen in the performance of the placebo group. Muscular torque in the Cr group was increased in the last 10 contractions of bout 1, all contractions in bout 2, 3 and 4, and the middle 10 contractions of bout 5, when compared to corresponding values in pre-supplement trial.

Earnest, Snell, Rodriguez, Almada and Mitchell, in 1995, demonstrated the effect of Cr supplementation on measures of anaerobic power, muscular strength and body composition. Anaerobic power was measured by 3 30-sec Windgate bike tests. Muscular strength was measured by a one repetition maximum (1 RM) bench press, and total bench press lifting volume calculated from repetitions of 70% 1 RM until fatigue. Body composition was measured by hydrostatic weighing. A group of strength trained athletes performed a series of workouts before and after 14 days (windgate tests) and 28 days (weight lifting and body composition) of Cr or placebo supplementation. Results indicated an increase in total anaerobic work for the windgate tests for the Cr group, while no change was seen in the placebo group. The increase was 13, 18 and 18% for bouts 1, 2 and 3 respectively. Bench-press max was higher in the Cr group, but the increase was negated when corrected for body weight. The Cr group demonstrated a significant increase in body weight. Total lifting volume was also significantly increased in the Cr group, whether expressed in absolute or relative terms. The Cr group performed an average of 26% more repetitions than the placebo group. The significant increase in

body weight, of the Cr group, was accompanied by a non-significant increase in fat free mass. No changes in body composition were noted within the placebo group.

Kreider, in 1995, illustrated the effects of Cr supplementation on strength training and weight gain. Participants in the 28-day study were randomly assigned to one of three groups, pure carbohydrate; a mixture of carbohydrate, protein, fat and creatine; and a mixture of carbohydrate, protein, fat, chromium and boron. The Cr group gained an average of 2 kg while the other groups only gained about 0.6 kg. No significant differences were seen in total body water, so increased body weight was attributed to an increase in lean body mass. Participants also under went strength testing. Results indicate that Cr has a slight affect on peak power, peak force and total work during isokinetic multijoint exercises, after 7 days of supplementation. Further supplementation does not appear to have an affect on performance.

McNaughton, Dalton and Tarr, in 1999, illustrated the effects of 5 days of Cr supplementation on kayak ergometry. Participants performed 3 maximal kayak ergometer tests of 90, 150 and 300 sec duration, before and after 5 days of Cr or placebo supplementation. Participants were then re-tested following a 4-week "wash out" period before and after 5 days of cross over supplementation. Results showed that the group re-tested after Cr supplementation performed significantly greater in all trials. Work completed was approximately 3 kJ more in all trials (90, 150 and 300 s) following Cr supplementation. Body mass was significantly greater in the Cr group.

Õõpik, Paasuke, Timpmann, Medijainen, Ereline and Smirnova, in 1999, reported the effects of a 5-day Cr supplementation regime on isokinetic power when coupled with rapid weight loss. Peak torque and work at peak torque of the knee extensors were determined in each participant before and after Cr or placebo supplementation.

Intermittent high intensity working capacity of the knee extensors was also measured by a series of sub-maximal isokinetic knee extensions, at specific angular velocities, for 45s at a rate of 30 contractions per minute, followed by 15 seconds of maximal effort. This series was repeated 3 times for a total testing period of 3 minutes. Results indicated that Cr supplementation maintained peak torque and work at peak torque when compared to the placebo group. Cr supplementation had no effect on maximal work or on the rate of fatigue during maximal work. Results also indicated that Cr supplementation had an adverse effect on sub-maximal work. Total sub-maximal work in the Cr group was significantly lower in the second and third minute of the post supplement test period when compared to pre-supplement values.

Smith, Stephens, Hall, Jackson and Earnest, in 1999, investigated the effect of Cr supplementation on work and time to fatigue in exhaustive constant power exercises at different intensities. Participants preformed cycle ergometer tests to determine the work x time relationship, then 4 bouts of exercise at an intensity selected to elicit fatigue in 90 to 600 s, before and after 5 days of Cr or placebo supplementation. Results indicated an improved time to fatigue in the Cr group for all but the lowest intensity.

Terrillion, Kolkhorst, Dolgener and Joslyn in 1997 investigated the effect of a 5-day Cr supplementation period on 2 maximal 700-meter runs. No significant differences were seen in the performance between the Cr and placebo groups. Placebo group participants ran 110.2±3.5 and 110.4±3.0 sec in the pre-supplement trial and 108.5±2.9 and 108.0±1.7 in the post-supplement trials. Cr group times were 109.9±3.2 and 110.4±3.6 for pre-supplement trials and 109.7±3.3 and 107.8±2.2 sec in the post-supplement trials. Differences between the placebo and Cr group were not significant. It

was also noted that no differences in weight gain were seen. It was concluded that maximal bouts of exercise 90 to 120 sec in duration might be too long to see the effects of Cr supplementation.

Redondo, Dowling, Graham, Almada and Williams, in 1996, tested the effect of Cr supplementation on maximal running velocity. Participants performed three 60-meter sprints prior to and following a 7-day Cr or placebo supplementation protocol. Maximal running velocities were calculated from video taped performances of the sprints. Results indicated that Cr supplementation did not influence the running velocities of the two groups. Also no difference in weight gain was reported between the two groups.

Barnett, Hinds and Jenkins, in 1996, reported the effects of Cr supplementation on repeated 10-sec sprint cycle performance. Participants were pre-tested for Max VO2 and 7 maximal 10-sec cycle ergometer sprints. Each sprint was separated by 30 sec of passive rest except for sprints 5 and 6, which were separated by 5 minutes. Peak power output (PPO), mean power output (MPO), end power output (EPO) and percent power decline were measured for each subject. Participants were cohort matched following the pre-test. Cr or placebo was supplemented in the diet for 4 days. Following supplementation Max VO2 and sprint cycle performance were again measured. Results indicated that Max VO2 and all aspects of sprint cycle performance were unchanged after 4 days of supplementation. Body weight also remained unchanged after supplementation.

Mujika, Chatard, Lacoste, Barale and Geyssant, in 1996, reported the effects of Cr supplementation on sprint swim performance. A group of highly trained swimmers was pre-tested for time and blood ammonia and lactate after 25, 50 and 100 meter sprints in their best stroke. The group was then divided into 2 groups, Cr and placebo. Participants

were re-tested following 5 days of Cr or placebo supplementation. Results failed to show any difference in performance times at each distance, however post exercise blood ammonia levels were lower in the Cr group following the 50 and 100 meter sprints. Blood ammonia levels were only decreased in the placebo group following the 50-meter sprint. No changes in lactate were seen. A significant increase in body weight (1kg) was seen in the Cr group as compared to the placebo group.

Greenhaff, Bodin, Soderlund and Hultman, in 1994, investigated the affect of Cr on phosphocreatine resynthesis and isometric contractions. Muscle biopsies were obtained from participants at 0, 20, 60 and 120-sec following electrically evoked isometric knee extensions, before and after 5 days of Cr supplementation. Results indicate that Cr supplementation had a positive affect on PCr resynthesis in 5 of the 8 participants. Supplementation increased recovery by about 35% after 2 minutes, compared to pre-supplement values in those individuals. This response was coupled with a 25% increase in total muscle Cr. The other 3 participants failed to produce significant increases in total Cr, and showed no change in PCr resynthesis.

Vandenberghe, Van Heck, Van Leemputte, Vanstapel and Hespel, in 1999, also studied the affects of Cr supplementation on PCr resynthesis. Participants under went ^{31}P NMR spectroscopy of the m. gastrocnemius, before during and after isometric plantar flexion, before and after 2 and 5 days of Cr or placebo supplementation. Resting PCr concentrations were increased by 11% after 2 days and 16% after 5 days. However, PCr breakdown and resynthesis were not significantly different from the placebo group. Participants also under went a series of isokinetic knee extensions to evaluate dynamic strength and fatigability. Torque production during maximal knee extensions was increased in the Cr group during the first of five bouts of 30 extensions.

Stroud, Holliman, Bell, Green, MacDonald and Greenhaff, in 1994, studied the effects of Cr supplementation on steady state, incremental treadmill exercise and recovery. Participants performed treadmill running at 10 km/h at predetermined grades to elicit workloads between 50 and 90% of VO2 max, before and after 5 days of Cr supplementation. Each workload lasted 6 minutes in duration and respiratory gas exchange and blood lactate concentrations were measured during the last 30 s of each stage, and every 5 minutes during a 15-minute recovery. Results indicated that Cr supplementation had no measurable effect on respiratory gas exchange or blood lactate concentrations, during exercise or recovery. Oxygen consumption and respiratory exchange ratio were not significantly different from pre to post supplement measures. A significant gain in body weight was seen from pre to post, with participants gaining about 1 kg over the 5 days of supplementation.

Balsom, Harridge, Soderlund, Sjodin and Ekblom, in 1993, reported the effects of Cr supplementation on endurance performance. Participants performed treadmill runs of 12% VO2 max and 6 km terrain runs before and after 6 days of Cr or placebo supplementation. Results indicated that Cr had no positive effect on performance in either the treadmill or terrain runs. The time on the post-supplement terrain run was significantly longer in the Cr group. This increase in time is thought to in part be due to a significant increase in body mass, seen in the Cr group over the 6 days of supplementation.

Cooke and Barnes, in 1997, reported the effects of Cr supplementation on high intensity exercise following a recovery period. Participants performed two maximal cycle ergometer sprints with one of four recovery intervals (30, 60 90 or 120 s), before and after a 5 day Cr or placebo supplementation. Results failed to demonstrate a

significant effect for Cr in any case. No difference in peak power or time to fatigue of the first or second trial was seen from pre to post supplementation. Peak power and time to fatigue were decreased from trial 1 to 2, but not significantly different from placebo to Cr.

Cooke, Grandjean and Barnes, in 1995, demonstrated the effects of Cr supplementation on power output and fatigue in cycle ergometry. Participants performed 2, 15 s consecutive maximal power tests before and after 5 days of Cr or placebo supplementation. No differences in peak power, time to peak power, total work and fatigue index were observed between Cr and placebo groups.

Febbraio, Flanagan, Snow, Zhao and Carey, in 1995, reported the effects of Cr supplementation on intermittent supramaximal exercise. Participants performed four cycling trials at 115 to 125 % VO2 max with one minute rest in between, followed by a fifth bout to fatigue, before and after 5 days of Cr supplementation. Participants were also re-tested after a 28-day washout period, the last 5 days of which were supplemented with placebo ingestion. No difference in the duration of the fifth bout of cycling was observed, even thought total Cr was increased following the 5 days of Cr supplementation.

Burke, Pyne and Telford, in 1996, investigated the effects of Cr supplementation on maximal sprint efforts in elite swimmers. Participants completed 25 m, 50 m and 100 m sprints in their best stroke, with 10 minutes of recovery between sprints, before and after 5 days of Cr or placebo supplementation. Participants were divided in to two groups and matched for sex, stroke and time. The groups were then randomly assigned to Cr or placebo supplementation, prior to the second trial. Results revealed no significant differences in sprint times between groups.

Rossiter, Cannell and Jakeman, in 1996, reported the effects of Cr supplementation on rowing performance. Participants completed a 1000 m simulated row before and after 5 days of Cr or placebo supplementation. Results revealed an overall decrease, about 1% or 2.3 s, in rowing time for the Cr group, however, and this difference was not significantly different form the placebo group.

One study has investigated the effects of Cr supplementation on rat skeletal muscle. Brannon, Adams, Conniff, and Baldwin, in 1997, reported the effect of Cr loading on running performance in rats. Running performance was assessed by duration and interval performance on treadmill runs, following 10 days of either a normal diet or a Cr supplemented diet. The duration test consisted of running at 60 m per min, on a 15% grade. This was designed to fatigue the animals in less than 2 minutes. After a two-day recovery period the rats then performed an interval test which involved 30 s of running with 30 s recovery periods. Running was performed at 13 m per min also at 15% grade. The test was stopped when the rat could no longer complete a 30 s run. Following this initial performance testing the rats were further divided in to groups to assess the effect of prolonged supplementation and training. After a 4 week training period the rats were again tested for duration and interval results. Results indicated that Cr supplementation enhanced running performance after 10 days, but no further gains were seen after 28 days of Cr supplementation. Cr animals ran significantly longer in the duration test after 10 days, but no difference in interval performance was seen. Following 4 weeks of training the Cr/training group performed significantly better in duration and interval performance than training alone and both the non-training groups.

Many studies have been conducted to determine the ergogenic effects of Cr, but results are still inconclusive. Only one of these studies has used an animal model, the rest

have focused directly on humans. The studies that have shown a positive effect generally utilize untrained or moderately trained participants. Protocols in these studies usually consist of highly intense activity, 100 to 125% VO2 max, in short duration, less than 3-minutes; or repeated bouts of high intensity exercise, with moderate recovery periods, 1 to 5-minutes. Studies that have failed to show any effect of Cr supplementation usually involve well-trained individuals, aerobic activities, very short or very long recovery periods, or have used tea or coffee to administer the Cr supplement.

CHAPTER 3

METHODS AND PROCEDURE

Design

The study was a 2 (Supplement) X 2 (Muscle Fiber Type) X 2 (Surgical Treatment) X 2 (Time) mixed design. The independent variable (IV) of supplement, muscle fiber type and surgical treatment were between groups, and the IV time was within. The IV of Supplement had 2 levels, control and Cr. The IV of Muscle Fiber Type had 2 levels, type I (soleus) and type II (EDL). The IV of Surgical Treatment had 2 levels, control and ablation. The IV of Time had 2 levels, initial period of stimulation and recovery period of stimulation. The dependent variables of interest were size of the muscle, peak force produced, total tension produced and time to one half peak force.

Animals

Male Sprague-Dawley rats (n=36, 183 ± 1.64 g) were randomly divided into 6 groups (n=6). Rats were housed individually and provided with food (Lab Chow) and water ad libitum. Cr Monohydrate (Sigma, St Louis, MO) was administered to one half of all rats by way of JellO cubes, at a dose of 300-mg/Kg-body weight per day. The dose is equivalent to 20-g creatine per day for a 70 kg human. Groups 1 and 2 were surgical controls, Group 2 received Cr Monohydrate. Groups 3 and 4 underwent tibialis anterior

(TA) ablation, Group 4 received Cr Monohydrate. Groups 5 and 6 underwent gastrocnemius and plantaris ablation, Group 6 received Cr Monohydrate.

Ablation Procedure

Rats were anesthetized with Pentobarbital Sodium (5mg/100g body weight, ip). Bilateral ablations were performed. The lateral aspect of each hindlimb was shaved and a longitudinal incision was made through the skin and fascia, along the lateral aspect of the Tibia. To ablate the gastrocnemius and plantaris, the lateral and medial heads of the muscle were isolated with careful blunt dissection. The distal 1/2 of the gastrocnemius and the plantaris were removed with care not to disturb the vasculature and nerves associated with the soleus. To ablate the TA, the distal tendon was cut and the muscle was removed in to-to. The fascia and skin were then sutured, distal to proximal, with 5-O silk. Rats were monitored during recovery for signs of pain, distress or discomfort. Any observed pain was alleviated by Butorphanol (0.1 - 0.5 mg/Kg body weight). (Kandarian & White, 1990; Young, Kandarian & Kurowski, 1992)

Urinary Analysis

Four weeks following the ablation surgery, 12 rats (6 Creatine and 6 controls) were randomly selected and placed in metabolic cages for 24 hours. Urine was collected and analyzed for Creatinine using a standard colorimetric assay (Hawk Practical Physiological Chemistry). Total Creatinine output was compared using an independent t-test, the accepted level of significance was 0.05.

Electrical Stimulation Procedure

Four to six weeks after the ablation surgery rats were again anesthetized with pentobarbital sodium (5mg/100g body weight). The sciatic nerve was exposed in the region of the thigh, before the separation of the tibial and common peroneal branches, but not cut. A dastre electrode was placed around the nerve, proximal to the bifurcation. The limb was fixed at the knee and ankle by steel pins inserted under the tibio-patellar ligament and the achilles tendon, respectively. The muscle was tied, via the distal tendon, to a force transducer (Narco Systems). The muscle was made to contract by stimulating the sciatic nerve electrically for 3 minutes, allowed to rest for 1 minute and stimulated again for 2 minutes, with 500ms trains of supramaximal voltage (70V) at a rate of 1 train/sec (Grass Scientific Instruments). Pulses were delivered at 100 Hz, with each pulse in the train having a duration of 0.1 ms. Force of contractions were captured via force transducer and feed into a computerized data acquisition system (SABLE). Muscles were carefully removed following stimulation, all visible connective tissue was removed and the muscles were weighed. In EDL hypertrophy animals the soleus was removed by freeze clamping, and in soleus hypertrophy animals the EDL was removed by freeze clamping. The samples were frozen and stored at -80°C. Following the stimulation the rats were euthanitized by an intercardial injection of pentobarbital sodium. (Young & Balon, 1997)

In ablation animals only one hindlimb was stimulated, but muscles were removed for wet weight from each limb. Control animals had the EDL simulated in one limb and the soleus stimulated in the other. Both soleus and EDL from each limb were removed for wet weight.

Data Analysis

The data for dependent variables of peak force, tension generated, time to one-half peak force and size corrected force were analyzed by 2 (Supplement) X 2 (Muscle) X 2 (Surgical Treatment) X 2 (Time) mixed modal ANOVA with repeated measures on the last factor. The data for the dependent variable of muscle size, percent recovery of area and percent recovery of tension were analyzed by 2(Supplement) X 2 (Muscle) X 2 (Surgical Treatment) ANOVA. The accepted level of significance for all tests was 0.05. All values reported are mean ± standard error.

CHAPTER 4

RESULTS AND DISCUSSION

Animal Growth and Muscle Hypertrophy

Animal growth rates for each group were statistically different. Analysis revealed that the soleus hypertrophy, Cr supplement animals (Group 6) were significantly smaller than all other groups at the time of stimulation (Table 1). Although the animals were smaller, the size of the hypertrophied soleus was no different than the non-supplemented hypertrophy group (Table 2). Muscle hypertrophy induced by the ablation surgery was unaffected by the supplementation. The EDL hypertrophied muscles in the control animals were slightly smaller, but not significant, 272.8 ± 24.5 and 299.3 ±38.5 mg for non-supplemented and Cr supplemented respectively. No difference in the size of the non-surgical controls was seen either. Surgical control EDL's were 188.5 ± 6.4 mg without Cr, and 177.1 ± 6.2 mg with Cr added to the diet. In the soleus hypertrophied muscles the control animals were slightly, but not significantly, larger than the Cr animals, 504.8 ± 77.1 and 419.2 ± 64.3 mg respectively. No difference was seen in the size of the soleus in the surgical control groups. Surgical control soleus muscles were 172.9 ± 7.7 mg and 176.9 ± 7.6 mg, for Cr and non-Cr animals. The main difference between the EDL and soleus was the nature of the ablation surgery. The tibialis anterior

was removed in to-to, while in the gastrocnemius and plantaris only the distal 50% were removed. Cr supplementation had no effect on the muscle's ability to hypertrophy as some (Plisk and Kredier, 1999) have previously suggested.

Urine Analysis

Cr supplemented animals had about 30% higher urinary creatinine outputs than control animals, 1.67 ± 0.15 mg and 1.29 ± 0.07 mg respectively (Figure 4). This difference can be attributed to the dietary supplement of Cr. An increase in total muscle Cr is seen in association with an increase in urinary creatinine (Delanghe, 1989; Rossiter, et al, 1995; Vandenberghe, et al, 1999). Increased urinary creatinine output is also associated with reaching an upper limit in skeletal muscle (Balsom, Soderlund and Ekblom, 1994; Haff and Potteiger, 1997; Juhn, 1999; Rossiter, Cannell and Jakeman, 1995).

Peak Force

Peak force produced by electrical stimulation was also unaffected by Cr supplementation (Table 3, Figures 5,6). Differences between Cr and non-Cr groups were not statistically significant. The hypertrophied animals produced significantly greater force than surgical controls (41.43 ± 3.06 g and 26.76 ± 2.67 g, respectively). Peak force produced by each group was also significantly greater in the initial period of stimulation compared to the second period (45.77 ± 2.58 g and 22.42 ± 2.53 g, respectively). In the initial stimulatory period, surgical control EDL's were able to produce 41.1 ± 3.66 g without Cr and 36.01 ± 9.40 g with Cr. Hypertrophied EDL's produced 54.18 ± 4.27 g without Cr and 51.16 ± 8.26 g with Cr. During the recovery or second stimulatory period

surgical controls produced 10.81 ± 3.98 g and 6.20 ± 0.69 g, with and without Cr respectively. Hypertrophied EDL's produced 31.18 ± 9.87 g with Cr and 22.67 ± 2.16 g without Cr in the second stimulatory period. Surgical control soleus muscles produced 41.61 ± 3.23 g without Cr and 37.11 ± 9.13 g with Cr, in the initial period and 19.93 ± 4.88 g and 21.23 ± 6.03 g respectively in the second stimulatory period. Hypertrophied soleus muscles in the initial stimulation period produced 48.58 ± 10.35 g without Cr and 56.38 ± 6.16 g with Cr. In the second period hypertrophied soleus muscles produced 36.16 ± 10.18 g without Cr and 30.51 ± 7.68 g with Cr. Hypertrophied muscles, as expected, produced significantly greater force than controls, but no difference was observed between the Cr and non-supplemented animals.

Percent Recovery of Peak Force

The percent recovery was also unaffected by Cr supplementation. The soleus recovered to a significantly greater extent than the EDL (61.5 ± 6.2% and 38.7 ± 5.6%, respectively). Also the hypertrophied muscles recovered to a significantly greater extent than the surgical controls (60.2 ± 6.7% and 40.0 ± 5.2% respectively). This result is to be expected given that the soleus is predominantly slow twitch, fatigue resistant, while the EDL is a fast twitch muscle, and less fatigue resistant than the soleus. Within the EDL groups, Cr appeared to have a slight although not significant effect. The two groups receiving Cr in their diet had slightly greater percent recoveries than the two groups without Cr. The non-hypertrophied Cr animals recovered 38.7 ± 13.5% of their peak force, while the non-hypertrophied control animals recovered only 15.4 ± 1.5%. In the hypertrophied animals the Cr group recovered to 57.5 ± 14.5% while the non-supplement

group recovered to only 43.2 ± 5.6% of their peak power. Within the soleus group, the effects of Cr were less visible. The non-hypertrophied animals recovered 45.7 ± 9.1% without Cr and 60.3 ± 6.6 % with Cr. In the hypertrophied animals the Cr group recovered 54.9 ± 14.0% while the non-Cr group recovered to 85.0 ± 15.7% of their initial stimulation period peak force.

Size Corrected Peak Force

Peak force produced was corrected for the size of the muscle to determine if any intrinsic changes occurred along with hypertrophy or Cr supplementation (Table 4, Figures 7,8). The results indicate that the hypertrophied soleus muscles produced less force per 100-mg muscle than the surgical controls (8.33 ± 1.18g and 17.71 ± 2.09 g, respectively), but no difference was seen in the EDL's. Results also indicated that surgical controls produced significantly greater force in the initial period of stimulation than hypertrophied muscles (22.24 ± 1.89 g and 14.48 ± 1.58 g, respectively). No difference was seen in the recovery period of stimulation. In the initial period of stimulation the surgical control EDL's produced 22.04 ± 1.90 and 20.54 ± 5.16 g per 100-mg muscle, without and with Cr respectively. Corrected peak force during the recovery period was 3.25 ± 0.25 and 6.37 ± 2.40 g per 100 mg muscle, without and with Cr, respectively. Hypertrophied EDL's produced 21.00 ± 1.66 g per 100 mg muscle without Cr and 15.34 ± 3.33 g per 100 mg with Cr. In the recovery period hypertrophied EDL's produced 9.13 ± 3.07 and 8.85 ± 1.01 g per 100 mg muscle, with and without Cr, respectively. Soleus surgical controls generated 22.51 ± 5.38 and 23.85 ± 2.34 g per 100-mg muscle with and without Cr respectively. Hypertrophied soleus muscles

generated only 14.58 ± 3.21 and 7.01 ± 1.36 g per 100-mg muscle with and without Cr respectively. The trend continued in the second stimulatory period as surgical controls generated 12.90 ± 3.59 and 11.57 ± 2.90 g, with and without Cr respectively. Compared to 6.73 ± 1.25 and 5.00 ± 0.98 g per 100 mg respectively. This may due, in part to scar and connective tissue in the soleus that could not be separated out from the muscle fibers. The presence of scar tissue, connective tissue, or the hydration status of the muscle may effect the size of the hypertrophied soleus muscles and decrease the force per unit muscle. No difference was seen in the EDL, which may be due to the "cleanliness" of the ablation surgery.

Tension for First 30 Seconds

Force of the contractions was calculated every 10 s. The force of the contractions was then used to calculate the fatigue curve (Figures 9,10,11,12) and the area under that curve. Areas for the first 30 s of stimulation were compared for the initial and second stimulation periods (Table 5). No difference in the tension produced was seen due to supplement. Differences were observed between the hypertrophied and surgical control muscles and between initial and recovery stimulation periods (Figures 13,14). Hypertrophied muscles produced significantly greater tension (828.57 ± 79.45 g) than surgical controls (448.53 ± 54.58 g). All muscles produced significantly more tension in the initial simulation (941.64 ± 70.85 g) than in the second period of stimulation (336.02 ± 44.41 g). The hypertrophied EDL's produced about twice as much tension as the surgical controls, 1143.98 ± 246.84 g and 1224.12 ± 151.27 g with and without Cr respectively, compared to 555.52 ± 139.06 and 601.07 ± 66.59 g, with and without Cr respectively. Hypertrophied EDL's in the second stimulation period produced a tension,

484.29 ± 174.59 g and 363.97 ± 65.48 g, with and without Cr respectively for the first 30 seconds, compared to 154.24 ± 62.63 g and 74.44 ± 9.26 g, with and without Cr respectively, in the surgical controls. The hypertrophied soleus muscles also generated greater tension in the first 30 seconds of the initial period, 1148.15 ± 17.34 g and 1219.58 ± 277.71 g with and without Cr, respectively than the controls, 815.02 ± 220.19 g and 677.72 ± 191.20 g, with and without Cr respectively. In the second stimulation period hypertrophied soleus muscles produced 444.85 ± 129.15 g and 599.45 ± 206.07 g, with and without Cr respectively. The surgical controls produced 290.38 ± 71.87 and 276.57 ± 78.29 g with and without Cr respectively.

Percent Recovery of Tension

The soleus muscles in general demonstrated a higher degree of recovery than the EDL (44.40 ± 5.69% to 28.70 ± 4.12%); similar to the recovery of peak force. Overall the non-supplemented, non-hypertrophied animals showed a significantly smaller percent recovery than all other groups, 21.57 ± 3.2% compared to 43.35 ± 9.3% for the non-hypertrophied, Cr group, 37.09 ± 6.3% for the hypertrophied, non-supplemented group, and 44.19 ± 7.4% for the hypertrophied, Cr group. In this case Cr has a positive effect, by increasing the percent recovery within the surgical controls. No difference was seen in the hypertrophied muscles, which suggests that the training effect demonstrated by the hypertrophied muscles was greater than the effect Cr has on skeletal muscle. Within the EDL the surgical controls recovered to 33.7 ± 11.3% and 12.6 ± 1.0% with and without Cr respectively. The hypertrophied EDL's recovered to 37.4 ± 9.7% and 31.1 ± 5.4% with and without Cr respectively. The soleus surgical controls recovered to 53.0 ± 15.7%

and 30.6 ± 3.8% with and without Cr respectively. While the hypertrophied soleus muscles recovered to 36.8 ± 9.8% with Cr and 57.2 ± 13.0% without Cr.

Tension for the Second 30 Seconds

Area for the second 30 seconds of stimulation (Table 6) showed that same relationship as that of the first 30 seconds (Figures 15,16). Results indicated that the hypertrophied muscles produced significantly greater tension in the second 30 second of stimulation than surgical controls (382.10 ± 42.25g and 166.54 ± 26.08 g, respectively). The muscles also generated significantly greater tension in the initial period of stimulation when compared to the recovery period (399.32 ± 39.39 g and 150.62 ± 27.41 g, respectively). In the initial period of stimulation the hypertrophied EDL's produced greater tension, 483.58 ± 135.78 g with Cr and 566.36 ± 105.83 g without Cr, than the non-hypertrophied EDL's, 189.73 ± 65.67 g with Cr and 146.37 ± 22.32 g without Cr. Cr supplementation had no effect on the tension generated during the second 30 seconds of stimulation. Tension in the initial period of stimulation was greater than the tension generated in the second stimulation period in all cases. In the second period of stimulation the hypertrophied EDL's produced 218.66 ± 86.66 g with Cr and 213.14 ± 56.83 g without Cr. The surgical controls produced 79.93 ± 29.15 g with Cr and 21.95 ± 5.18 g without Cr in the second stimulation period. In the soleus muscles the hypertrophied muscles generated 439.03 ± 88.49 g with Cr and 613.00 ± 141.55 g without Cr in the initial stimulation period. Surgical controls generated 371.19 ± 104.01 g with Cr and 374.84 ± 82.61 g without Cr. In the second stimulation period surgical controls recovered to generate 87.71 ± 24.55 g with Cr and 60.58 ± 17.65 g without Cr.

The hypertrophied muscles generated significantly greater force, although no difference was seen between supplemental controls and Cr groups. Hypertrophied soleus muscles produced 191.68 ± 62.53 g with Cr and 331.33 ± 161.15 g without Cr.

Time to One-Half Peak Force

Time to one-half peak force (T1/2) for each animal during each stimulation period was calculated from a regression equation, Y = aX^b, where Y is equal to time in seconds and X is equal to force in grams. Time to one-half peak force (T1/2) (Table 7, Figures 17,18), along with percent recovery of tension, generated the only significant results regarding supplementation. Results are similar to peak force and tension with regard to initial vs. second stimulation, hypertrophy vs. non-hypertrophy, and soleus vs. EDL. T1/2 was significantly greater in the initial stimulation period, 16.23 ± 2.03 s, compared to 10.98 ± 1.84 s for the second period. Hypertrophied muscles had significantly larger T1/2's than non-hypertrophied muscles, 17.56 ± 2.51 s and 9.66 ± 0.91 s, respectively. Soleus muscles exhibited significantly prolonged T1/2's when compared to EDLs, 17.19 ± 2.53 s to 10.02 ± 0.92 s respectively. Results of T1/2 suggest that Cr supplementation may result in a decrease time to fatigue. Non-supplemented muscles exhibited a T1/2 of 16.92 ± 2.59 s, while Cr muscles had a significantly lower T1/2 of 10.29 ± 0.78 s. An analysis of the significant muscle x supplement interaction revealed that non-supplemented soleus muscles had significantly higher T1/2s (23.34 ± 4.67 s) than Cr supplemented soleus muscles (11.04 ± 1.07 s) and both supplemented and non-supplemented EDLs (9.54 ± 1.13 s and 10.50 ± 1.46 s respectively). Further analysis of the significant supplement x surgery interaction indicated that non-supplemented, hypertrophied muscles resisted fatigue to a greater extent (24.28 ± 4.51 s) than the non-supplemented non-hypertrophied

group (9.56 ± 1.57 s) and both the Cr supplemented surgical control and Cr supplemented hypertrophied groups (9.75 ± 0.97 s and 10.83 ± 1.23 s respectively).

This result is in contrast too popularly held notions that Cr should increase time to fatigue. Fitts (1994) suggests one explanation of this result may be due to ADP and Pi level, as well as the ATP/ADP ratio within the cell. Force of contraction is regulated in large part by Ca^{2+}, which is released from the SR upon depolarization and taken back up by ATP dependent Ca^{2+}-pumps. Pi and the ATP/ADP ratio may influence these calcium pumps. If ATP levels are limited, not all Ca^{2+} is removed from the cell. The pumps do not have the energy, ATP, to continue the removal of Ca^{2+}. If Ca^{2+} is still present in the cell, complete relaxation can not occur and a summation effect takes place. If PCr is available ATP will be regenerated, and the ATP/ADP ratio will remain high, and Pi will be lower. A higher ATP/ADP ratio may allow the Ca^{2+} pumps to remove more Ca^{2+} from the cell, allowing for greater relaxation, and reducing the summation effect. A reduction in the summation effect would therefore decrease the force of contraction and give the appearance of a faster time to fatigue.

CHAPTER 5

CONCLUSION

Hypertrophy, may result in a training effect that is more pronounced than the effect of Cr supplementation, which would explain why little difference was observed in the hypertrophied muscles. With hypertrophied EDLs 57% larger and hypertrophied soleus' 165% larger than controls, the effect of Cr may have been overshadowed. Previous human studies have shown Cr to have an ergogenic effect with much smaller gains in muscle size. Cr supplementation had no effect on the size or performance of the hypertrophied muscles, with the exception of T1/2, as discussed previously. Cr supplementation may have an effect on the control muscles, however this effect is not statistically significant. There was a slight difference in the percent recovery of peak force and tension within the EDL surgical controls between the non-supplemented and Cr groups, such that the Cr group recovered to a greater extent than the non-supplemented group (Tables 3 & 5). There was also a difference in the soleus surgical controls, although not as large as the EDL. Non-supplemented, surgical control soleus muscles recovered to a lesser extent in both peak force and tension than Cr supplemented, control muscles.

Dietary Cr supplementation had no effect on muscular hypertrophy and electrically stimulated performance in rat skeletal muscle. It is possible, however unlikely, that rat skeletal muscle is affected differently than human skeletal muscle by Cr supplementation. Human studies have yielded mixed results as to Cr and it effect on

performance. Human athletic performance is subject to numerous external and internal factors, including, but not limited too, time of day, diet, motivation of the individual, environment, training state and simply how one feels that day. Dietary supplementation of Cr may have more of a psychological effect than any actually physiological effect.

It has been proposed that Cr may have an effect on Ca^{2+} removal within the muscle cell but without measurements of intracellular Ca^{2+}, ATP, ADP, Pi, Cr and PCr no firm conclusions can be drawn from this study. Future studies should focus on this possible relationship, to determine if increased intracellular Cr does assist in Ca^{2+} removal.

These findings are consistent with others who have reported that peak force, power or torque was unaffected by supplementation (Ööpik, et al, 1999; Cooke and Barnes, 1997; Cooke, Grandjean and Barnes, 1995).

APPENDIX I

DATA TABLES

Table 1

Change in Body Weight Over 5 Week Treatment Period, Post Ablation Surgery

Supplement	Surgical Conrol	EDL Hypertrophy	Soleus Hypertrophy
Control	211.0 (8.2)g n = 6	218.0 (6.3)g n = 6	221.5 (6.5)g n = 6
Creatine	210.5 (8.9)g n = 6	205.0 (7.2)g n = 6	175.8 (6.5)*g n = 6

* Significantly different from all others, $p < 0.05$.
Values presented and mean (SEM)

Table 2

Muscle Size, Following 5 Week Treatment Period

Supplement	EDL Surgical Control	EDL Hypertropy	Soleus Surgical Control	Soleus Hypertrophy
Control	188.5 (6.4)mg	272.8 (24.5)*mg	176.9 (7.6)mg	504.8 (77.1)**mg
	n = 12	n = 12	n = 12	n = 12
Creatine	177.1 (6.2)mg	299.3 (38.5)*mg	172.9 (7.7)g	419.2 (64.3)**mg
	n = 12	n = 12	n = 12	n = 12

* Significantly different from all others, $p < 0.05$.
** Significantly different from all others, $p < 0.05$.
Values presented as mean (SEM)

Table 3

Peak Force, Generated by Electrical Stimulation

EDL

	Con-Con	Con-Cr	Hyp-Con	Hyp-Cr
Initial Stim	41.1 (3.66) g	36.01 (9.40) g	54.18 (4.27)* g	51.16 (8.26)* g
	n = 5	n = 6	n = 6	n = 6
Recovery Stim	6.20 (0.69) g	10.81 (3.98) g	22.67 (2.16)" g	31/18 (9.87)" g
	n = 5	n = 6	n = 6	n = 6
% Recovery	15.4 (1.5)	38.7 (13.5)	43.2 (5.6)	57.5 (14.5)

Soleus

	Con-Con	Con-Cr	HypCon	Hyp-Cr
Initial Stim	41.61 (3.23) g	37.11 (9.13) g	48.58 (10.35)* g	56.38 (6.16)* g
	n = 5	n = 6	n = 6	n = 6
Recovery Stim	19.93 (4.88) g	21.23 (6.03) g	36.16 (10.18)" g	30.51 (7.68)" g
	n = 5	n = 6	n = 6	n = 6
% Recovery	45.7 (9.1)	60.0 (6.6)	85.0 (15.7)	54.9 (14.0)

Con-Con: Surgical control, no creatine
Con-Cr: Surgical control, creatine
Hyp-Con: Hypertrophy, no creatine
Hyp-Cr: Hypertrophy, creatine
* Significantly different from all surgical controls in initial stim, $p < 0.05$.
" Significantly different from all surgical controls in recovery stim, $p < 0.05$.
** Initial stim significantly different from recovery in all cases, $p < 0.05$.

Table 4

Size Corrected Peak Force

EDL

	Con-Con	Con-Cr	Hyp-Con	Hyp-Cr
Initial Stim	22.04 (1.90) g n = 5	20.54 (5.16) g n = 6	21.00 (1.66) g n = 6	15.34 (3.33) g n = 6
Recovery Stim	3.25 (0.25) g n = 5	6.37 (2.40) g n = 6	8.85 (1.01) n = 6	9.13 (3.07) g n = 6

Soleus

	Con-Con	Con-Cr	HypCon	Hyp-Cr
Initial Stim	23.85 (2.34)* g n = 5	22.51 (5.38)* g n = 6	7.01 (1.36) g n = 6	14.58 (3.21) g n = 6
Recovery Stim	11.57 (2.90)" g n = 5	12.90 (3.59)" g n = 6	5.00 (0.98) g n = 6	6.73 (1.25) g n = 6

Con-Con: Surgical control, no creatine
Con-Cr: Surgical control, creatine
Hyp-Con: Hypertrophy, no creatine
Hyp-Cr: Hypertrophy, creatine
* Significantly different from hypertrophied soleus in initial stim, $p < 0.05$.
" Significantly different from hypertrophied soleus in recovery stim, $p < 0.05$.
** Initial stim significantly different from recovery in all cases, $p < 0.05$.

Table 5

Tension Generated for the First 30 Seconds of Electrical Stimulation

EDL

	Con-Con	Con-Cr	Hyp-Con	Hyp-Cr
Initial Sitm	601.07 (66.59) g	555.52 (139.06) g	1224.12 (151.27)* g	1143.98 (246.84)* g
	n = 5	n = 6	n = 6	n = 6
Recovery Stim	74.44 (9.26) g	154.24 (62.63) g	363.97 (65.48)" g	484.29 (174.59)" g
	n = 5	n = 6	n = 6	n = 6
% Recovery	12.6 (1.0)	33.7 (11.3)#	31.1 (5.4)	37.4 (9.7)

Soleus

	Con-Con	Con-Cr	Hyp-Con	Hyp-Cr
Initial Stim	677.72 (191.20) g	815.02 (220.19) g	1219.58 (277.71)* g	1148.15 (147.34)* g
	n = 5	n = 6	n = 6	n = 6
Recovery Stim	276.57 (78.29) g	290.38 (71.87) g	599.45 (206.07)" g	444.85 (129.15)" g
	n = 5	n = 6	n = 6	n = 6
% Recovery	30.6 (3.8)	53.0 (15.7)#	57.2 (13.0)	36.75 (9.8)

Con-Con: Surgical control, no creatine
Con-Cr: Surgical control, creatine
Hyp-Con: Hypertrophy, no creatine
Hyp-Cr: Hypertrophy, creatine
* Significantly different from all surgical controls in initial stim, $p < 0.05$.
" Significantly different from all surgical controls in recovery stim, $p < 0.05$.
** Initial stim significantly different from recovery in all cases, $p < 0.05$.
# Significantly different from surgical control, no creatine

Table 6

Tension Generated for the Second 30 Seconds of Electrical Stimulation

EDL

	Con-Con	Con-Cr	Hyp-Con	Hyp-Cr
Initial Stim	146.37 (22.32) g n = 5	189.73 (65.67) g n = 6	566.36 (105.83)* g n = 6	483.58 (135.78)* g n = 6
Recovery Stim	21.95 (5.18) g n = 5	79.93 (29.15) g n = 6	213.14 (56.83)" g n = 6	218.66 (86.66)" g n = 6

Soleus

	Con-Con	Con-Cr	HypCon	Hyp-Cr
Initial Stim	374.84 (82.61) n = 5	371.19 (104.01) g n = 6	613.00 (141.55)* g n = 6	439.03 (88.49)* g n = 6
Recovery Stim	60.58 (17.65) g n = 5	87.71 (24.55) g n = 6	331.33 (161.15)" g n = 6	191.68 (62.53)" g n = 6

Con-Con: Surgical control, no creatine
Con-Cr: Surgical control, creatine
Hyp-Con: Hypertrophy, no creatine
Hyp-Cr: Hypertrophy, creatine
* Significantly different from all surgical controls in initial stim, $p < 0.05$.
" Significantly different from all surgical controls in recovery stim, $p < 0.05$.
** Initial stim significantly different from recovery in all cases, $p < 0.05$.

Table 7

Time to One-Half Peak Force, Calculated From the Regression Equation Y = aX^b

EDL

	Con-Con	Con-Cr	Hyp-Con	Hyp-Cr
Initial Stim	7.36 (0.53) s	8.89 (1.46) s	16.89 (2.57) s	14.69 (3.15)
r for Y = aX^b	-0.949	-0.919	-0.909	-0.925
	n = 5	n = 6	n = 6	n = 6
Recovery Stim	4.98 (0.43) s	7.93 (1.48) s	12.78 (3.88) s	6.65 (1.24) s
r for Y = aX^b	-0.956	-0.764	-0.930	-0.981
	n = 5	n = 6	n = 6	n = 6

Soleus

	Con-Con	Con-Cr	HypCon	Hyp-Cr
Initial Stim	14.23 (2.45) s	15.03 (1.80) s	39.13 (12.02)* s	13.62 (1.76) s
r for Y = aX^b	-0.880	-0.893	-0.826	-0.911
	n = 5	n = 6	n = 6	n = 6
Recovery Stim	11.68 (5.34) s	7.14 (1.41) s	28.31 (11.09)* s	8.37 (1.95) s
r for Y = aX^b	-0.928	-0.941	-0.816	-0.927
	n = 5	n = 6	n = 6	n = 6

Con-Con: Surgical control, no creatine
Con-Cr: Surgical control, creatine
Hyp-Con: Hypertrophy, no creatine
Hyp-Cr: Hypertrophy, creatine
Values presented as mean (SEM)
* Significantly different from all others, $p < 0.05$.
** Initial stim significantly different from recovery in all cases, $p < 0.05$.

APPENDIX II

FIGURES

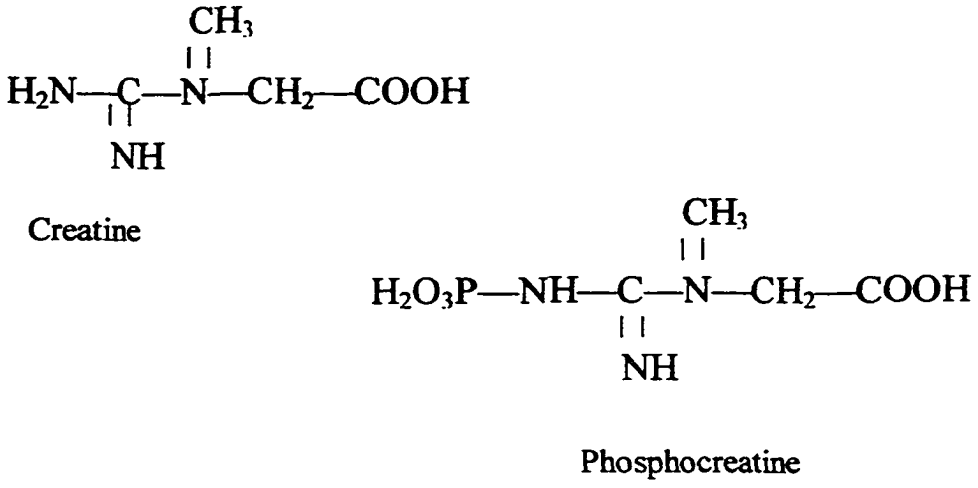

Figure 1

Structure of Creatine and Phosphocreatine

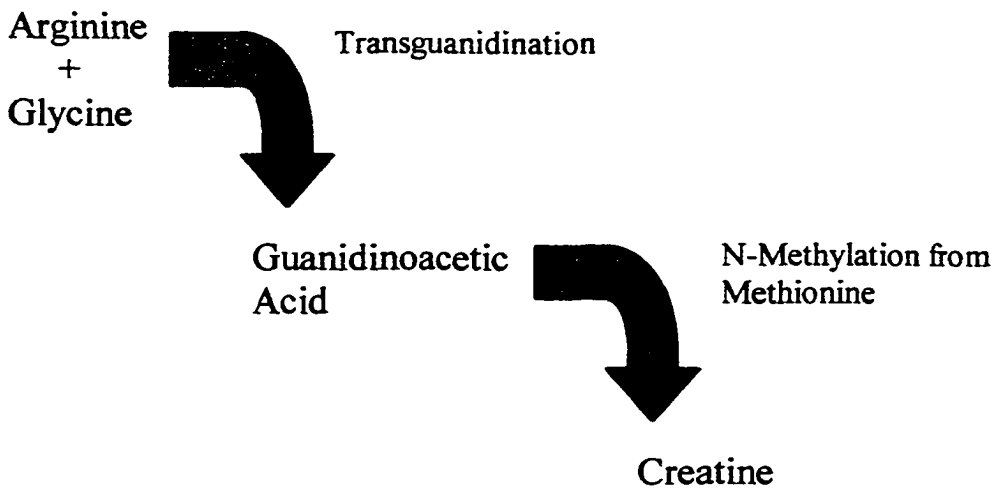

Figure 2

Synthesis of Creatine

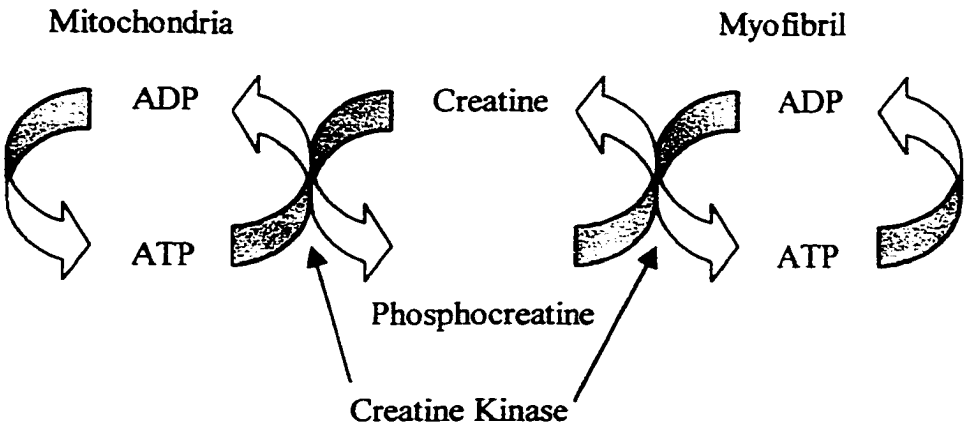

Figure 3

Phosphocreatine Energy Shuttle

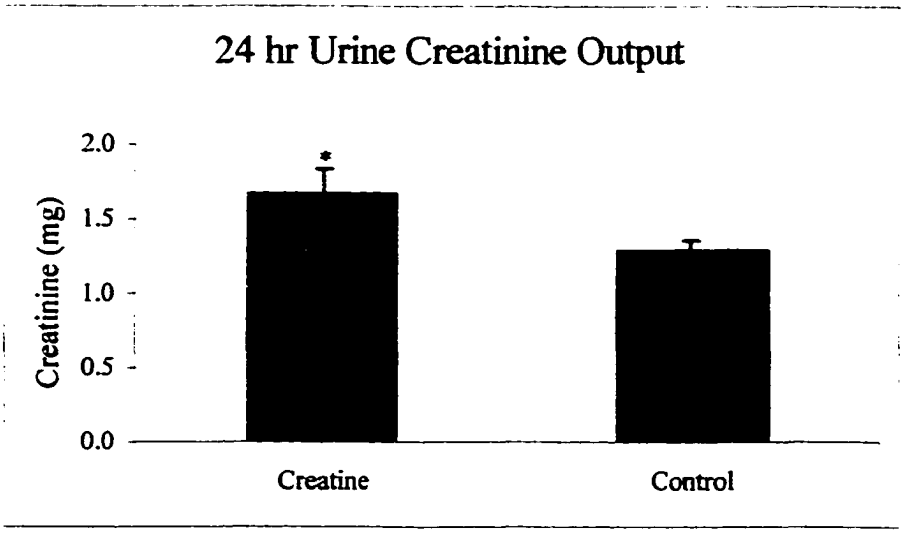

Figure 4

Urinary Creatinine

* Significantly different than non-supplement or control, $p < 0.05$.

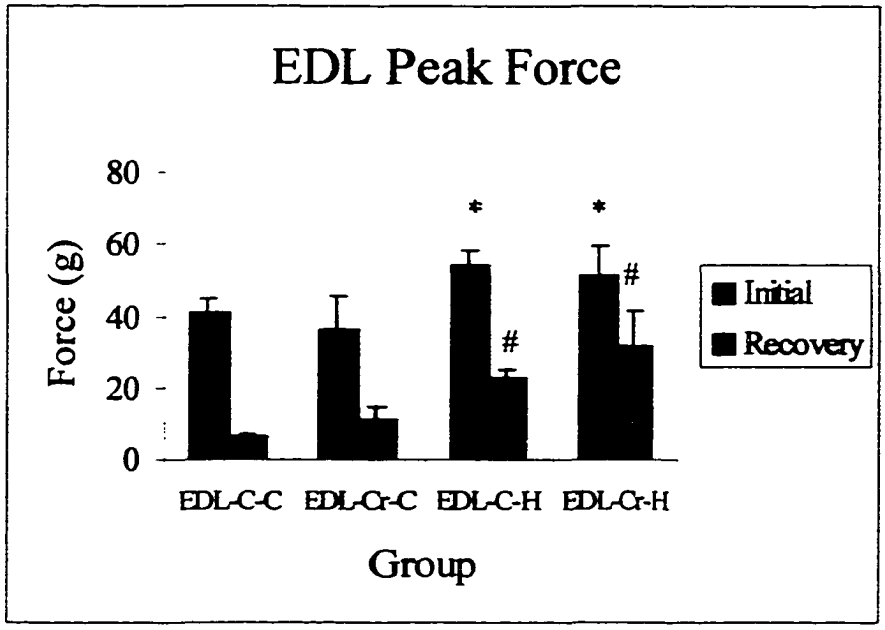

Figure 5

EDL Peak Force Produced

EDL-C-C: Surgical Control, No Creatine
EDL-Cr-C: Surgical Control, Creatine
EDL-C-H: Hypertrophy, No Creatine
EDL-Cr-H: Hypertrophy, Creatine
*Significantly different from surgical controls in initial, $p < 0.05$.
#Significantly different from surgical controls in recovery, $p < 0.05$.
**Initial significantly different from recovery in all cases, $p < 0.05$.

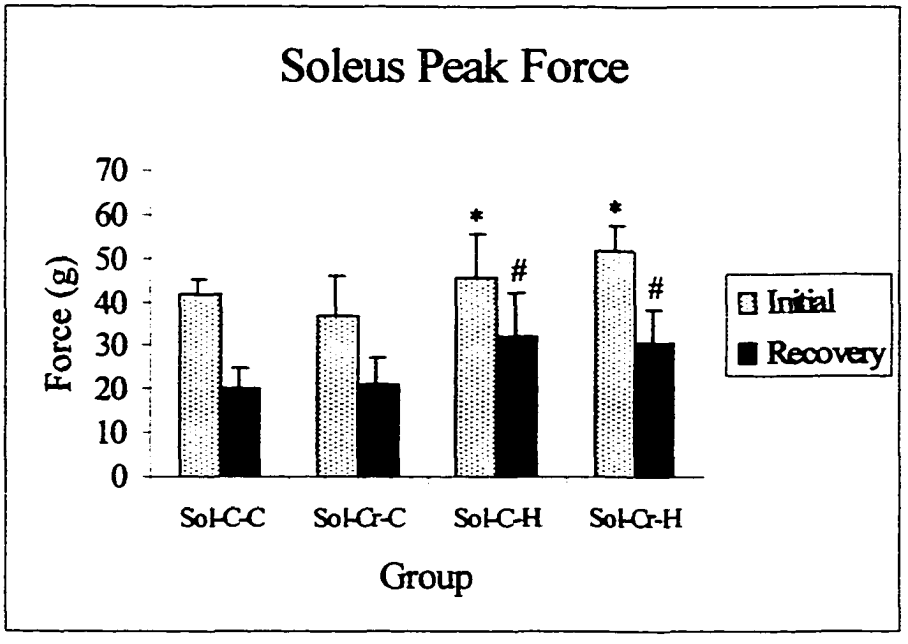

Figure 6

Soleus Peak Force Produced

Sol-C-C: Surgical control, No Creatine
Sol-Cr-C: Surgical control, Creatine
Sol-C-H: Hypertrophy, No Creatine
Sol-Cr-H: Hypertrophy, Creatine
*Significantly different from surgical controls in initial, $p < 0.05$.
#Significantly different from surgical controls in recovery, $p < 0.05$.
**Initial significantly different from recovery in all cases, $p < 0.05$.

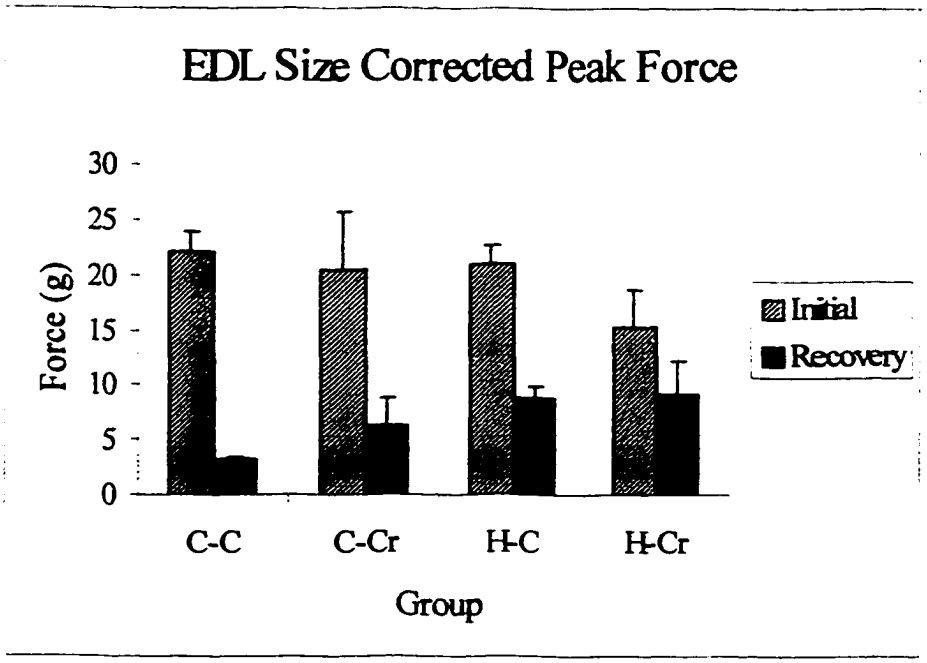

Figure 7

EDL Size Corrected Peak Force

C-C: Surgical Control, No creatine
C-Cr: Surgical Control, Creatine
H-C: Hypertrophy, No creatine
H-Cr: Hypertrophy, Creatine
**Initial significantly different from recovery, $p < 0.05$.

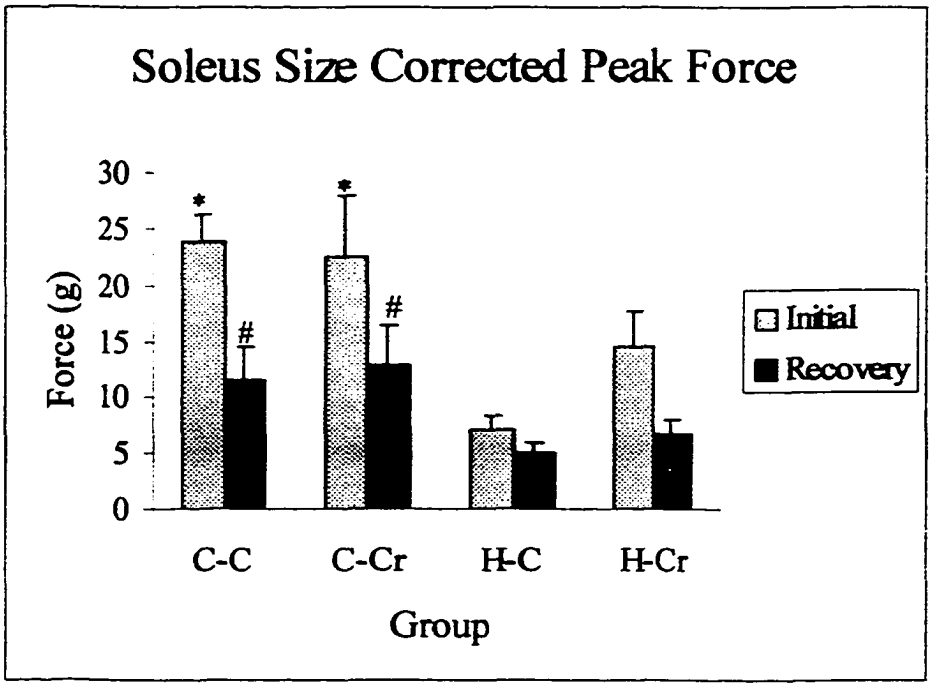

Figure 8

Soleus Size Corrected Peak Force

C-C: Surgical control, No Creatine
C-Cr: Surgical control, Creatine
H-C: Hypertrophy, No Creatine
H-Cr: Hypertrophy, Creatine
*Significantly different from hypertrophy in initial, $p < 0.05$.
#Significantly different from hypertrophy in recovery, $p < 0.05$.
**Initial significantly different from recovery in all cases, $p < 0.05$.

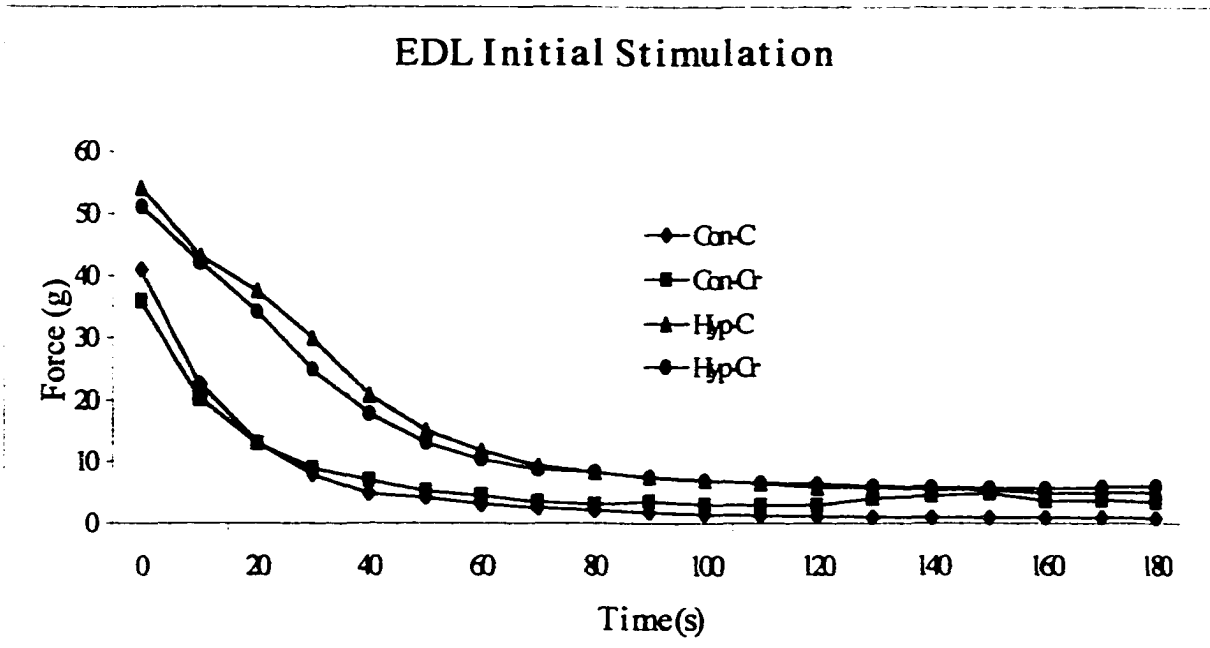

Figure 9

EDL Initial Stimulation Fatigue Curve

Con-C: Surgical control, No creatine
Con-Cr: Surgical control, Creatine
Hyp-C: Hypertrophy, No creatine
Hyp-Cr: Hypertrophy, Creatine

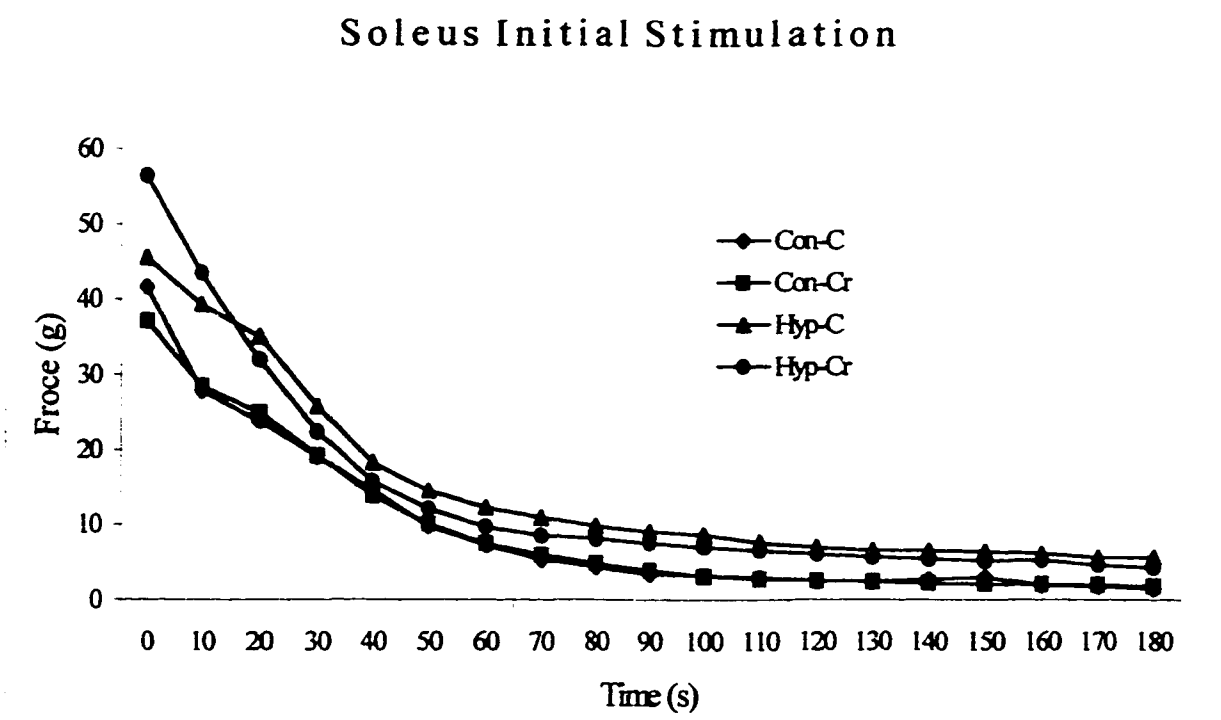

Figure 10

Soleus Initial Stimulation Fatigue Curve

Con-C: Surgical control, No creatine
Con-Cr: Surgical control, Creatine
Hyp-C: Hypertrophy, No creatine
Hyp-Cr: Hypertrophy, Creatine

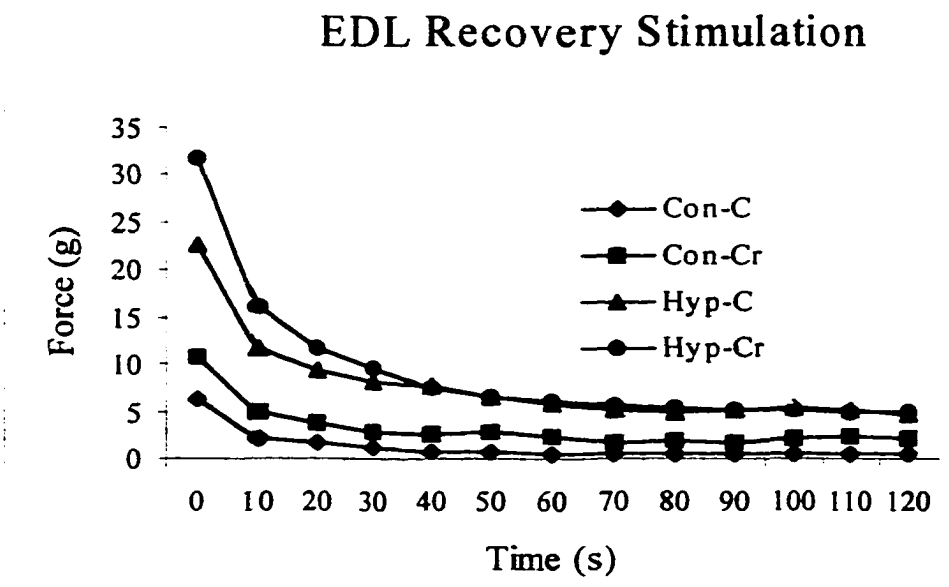

Figure 11

EDL Recovery Stimulation Fatigue Curve

Con-C: Surgical control, No creatine
Con-Cr: Surgical control, Creatine
Hyp-C: Hypertrophy, No creatine
Hyp-Cr: Hypertrophy, Creatine

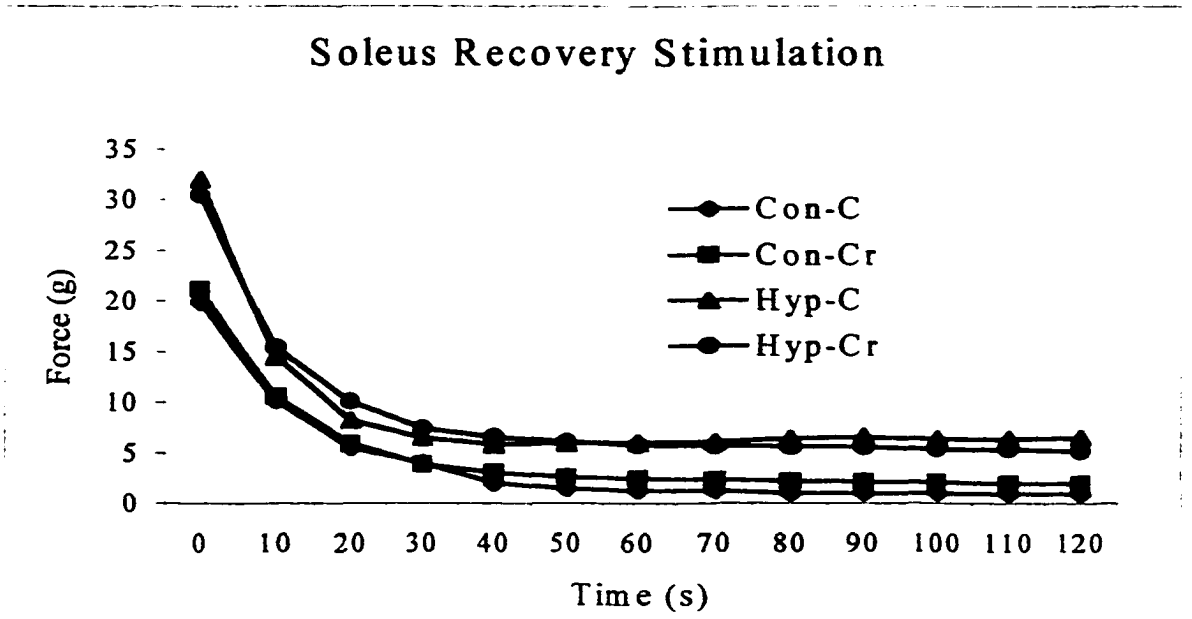

Figure 12

Soleus Recovery Stimulation Fatigue Curve

Con-C: Surgical control, No creatine
Con-Cr: Surgical control, Creatine
Hyp-C: Hypertrophy, No creatine
Hyp-Cr: Hypertrophy, Creatine

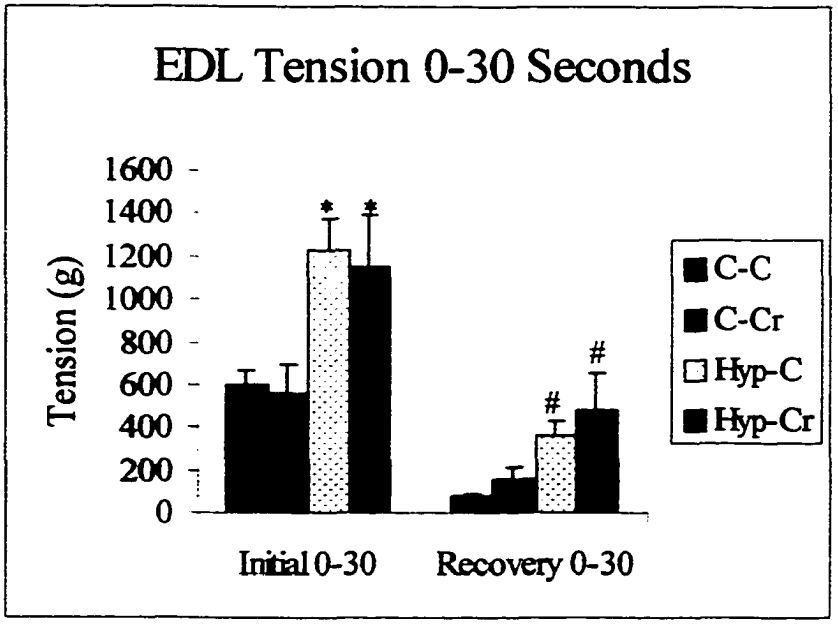

Figure 13

EDL Tension of First 30 Seconds

C-C: Surgical control, No creatine
C-Cr: Surgical control, Creatine
Hyp-C: Hypertrophy, No Creatine
Hyp-Cr: Hypertrophy, Creatine
*Significantly different from surgical controls, in initial, $p < 0.05$.
#Significantly different from surgical controls, in recovery, $p < 0.05$.
**Initial significantly different from recovery in all cases, $p < 0.05$.

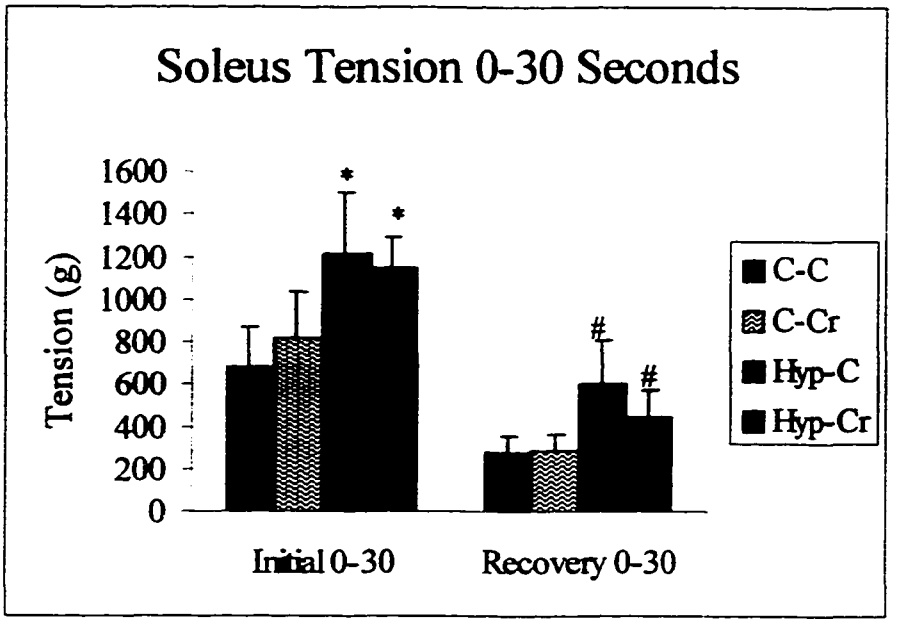

Figure 14

Soleus Tension of First 30 Seconds

C-C: Surgical control, No creatine
C-Cr: Surgical control, Creatine
Hyp-C: Hypertrophy, No Creatine
Hyp-Cr: Hypertrophy, Creatine
*Significantly different from surgical controls, in initial, $p < 0.05$.
#Significantly different from surgical controls, in recovery, $p < 0.05$.
**Initial significantly different from recovery in all cases, $p < 0.05$.

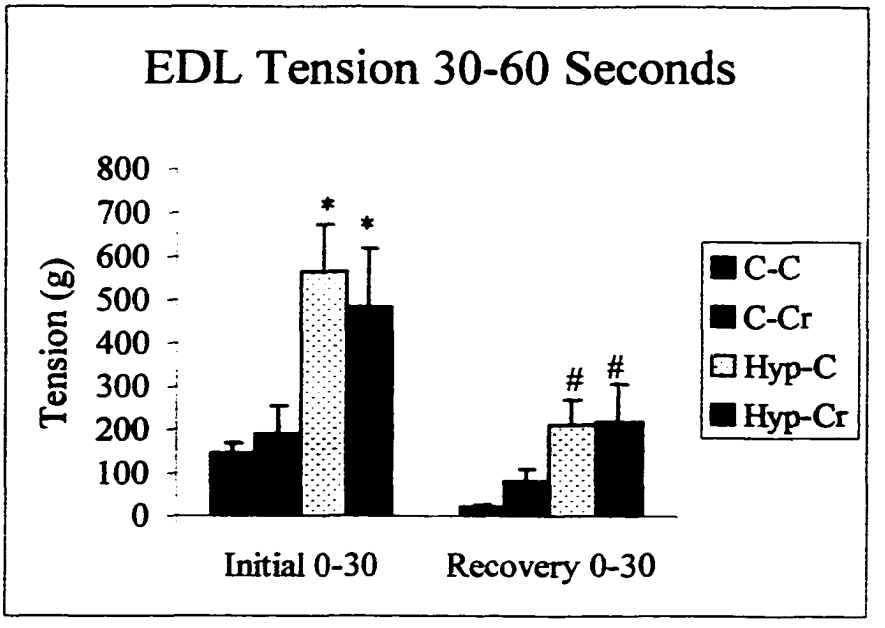

Figure 15

EDL Tension of Second 30 Seconds

C-C: Surgical control, No creatine
C-Cr: Surgical control, Creatine
Hyp-C: Hypertrophy, No Creatine
Hyp-Cr: Hypertrophy, Creatine
*Significantly different from surgical controls, in initial, $p < 0.05$.
#Significantly different from surgical controls, in recovery, $p < 0.05$.
**Initial significantly different from recovery in all cases, $p < 0.05$.

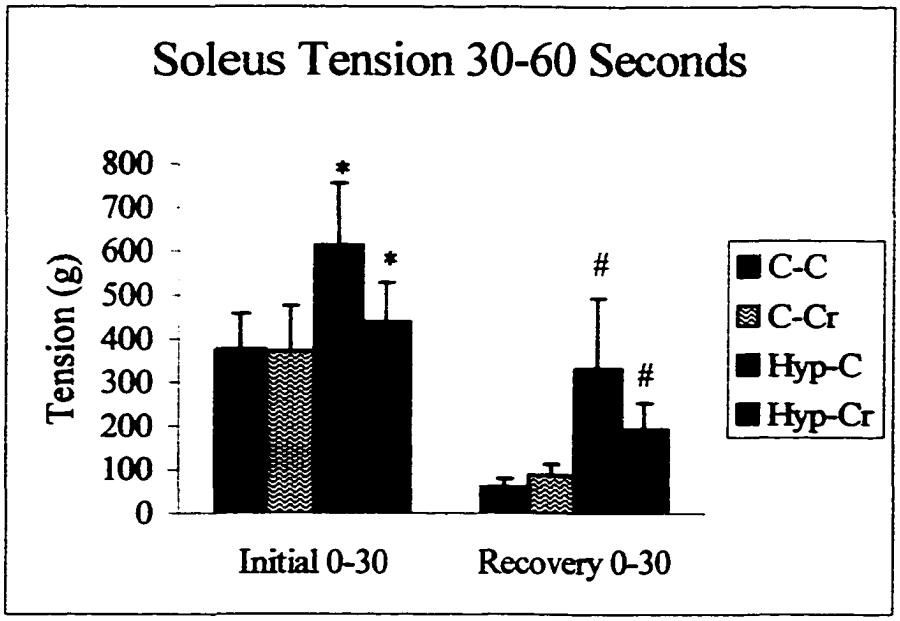

Figure 16

Soleus Tension of Second 30 Seconds

C-C: Surgical control, No creatine
C-Cr: Surgical control, Creatine
Hyp-C: Hypertrophy, No Creatine
Hyp-Cr: Hypertrophy, Creatine
*Significantly different from surgical controls, in initial, $p < 0.05$.
#Significantly different from surgical controls, in recovery, $p < 0.05$.
**Initial significantly different from recovery in all cases, $p < 0.05$.

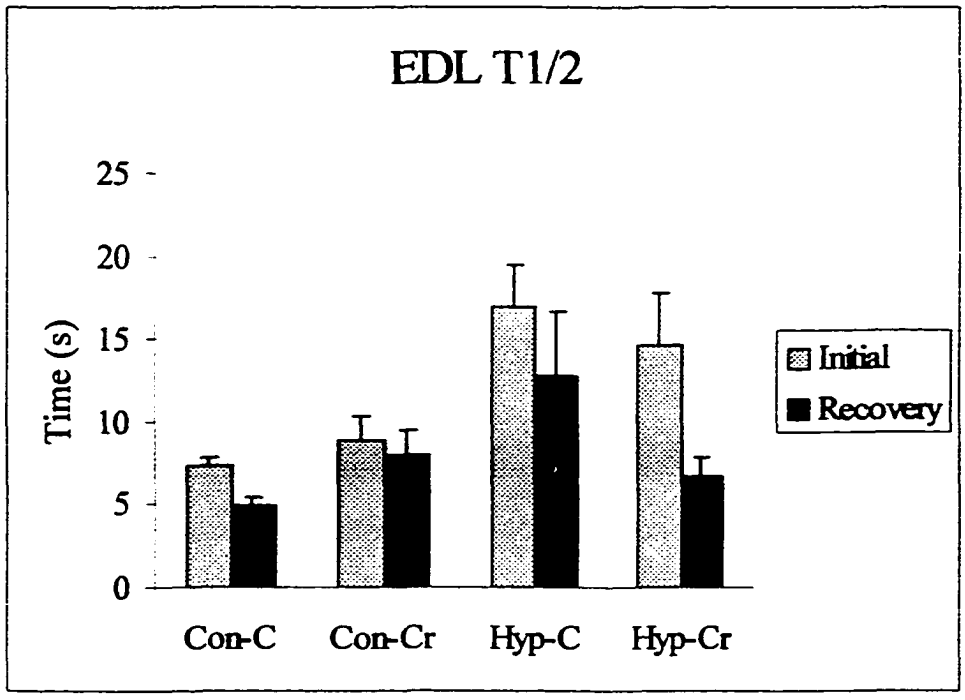

Figure 17

EDL Time to One-Half Peak Force

Con-C: Surgical control, No creatine
Con-Cr: Surgical control, Creatine
Hyp-C: Hypertrophy, No Creatine
Hyp-Cr: Hypertrophy, Creatine
**No significant differences between groups.

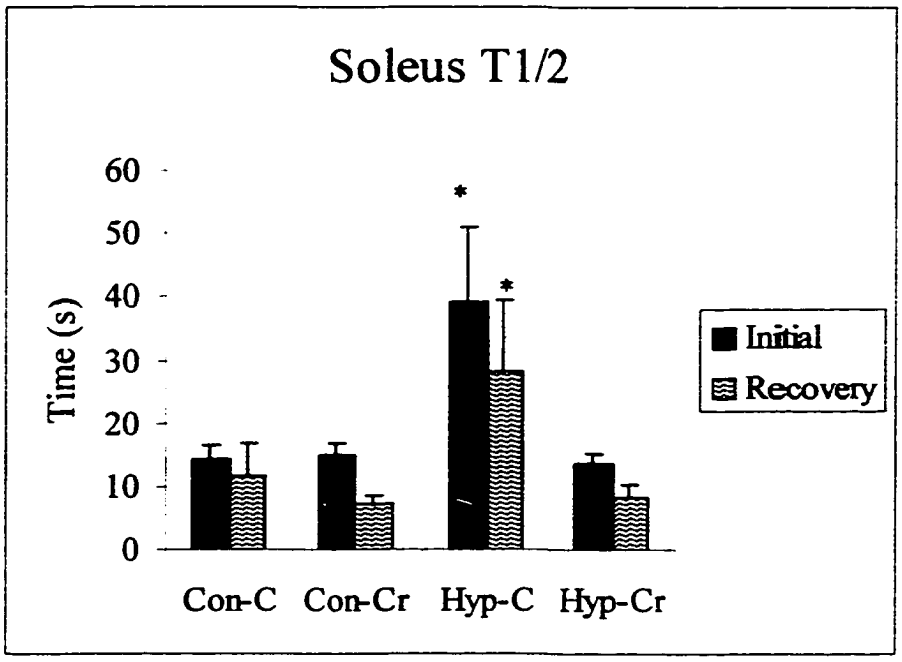

Figure 18

Soleus Time to One-Half Peak Force

Con-C: Surgical control, No creatine
Con-Cr: Surgical control, Creatine
Hyp-C: Hypertrophy, No Creatine
Hyp-Cr: Hypertrophy, Creatine
*Significantly different from all others, $p < 0.05$.

APPENDIX III

PROTOCAL FOR ANIMAL CARE AND USE

FOR COMMITTEE USE ONLY

UNIVERSITY OF NEVADA, LAS VEGAS

PROTOCOL FOR ANIMAL CARE AND USE - PROTOCOL NUMBER R701-1099-143

TITLE: The effect of dietary creatine supplementation on compensatory muscle hypertrophy and performance characteristics of specific fiber types

COMMITTEE ACTION:

___ **RECOMMENDED FOR APPROVAL AS SUBMITTED, DATE:** 15 Oct 98

__xx__ **RECOMMENDED FOR APPROVAL WITH MODIFICATIONS***

___ **DEFERRED FOR ADDITIONAL INFORMATION, DATE:** _____

___ **RETURNED TO INVESTIGATOR FOR REVISION, DATE:** _____

___ **DISAPPROVED, DATE:** _____

11/3/98
DATE

Stanley D. Hillyard
IACUC CHAIRMAN

11/3/98
DATE

*** MODIFICATIONS REQUIRED:**

On Supplement Form 2, question #3b., put that there will be supplement anesthesia given as necessary and indicate the dosage (recommended 1mg/100g). Please submit addition to either Stan Hillyard or Marsha Moon before procedures can start.

OFFICE USE ONLY: Protocol No.:_____
Date received:_____

UNIVERSITY OF NEVADA, LAS VEGAS
PROTOCOL FOR ANIMAL CARE AND USE
(form must be typewritten)

1. **TITLE:** The effect of dietary creatine supplementation on compensatory muscle hypertrophy and performance characteristics of specific fiber types.

2. **PRINCIPAL INVESTIGATOR/COURSE DIRECTOR:**

NAME & TITLE: Dr. Carl Reiber
DEPARTMENT: Biological Sciences
OFFICE PHONE: 895-1549 **EMERGENCY PHONE:** 896-5104

3. **OTHER RESPONSIBLE PERSONS:**

NAME	RESPONSIBILITY	OFFICE PHONE	EMERGENCY PHONE
Robert Young		895-1582	454-0001
John C. Young Ph.D.	(Co- P.I.)	895-4626	898-8845

4. **PROTOCOL STATUS: _X_NEW __MODIFICATION __CONTINUATION __RESEARCH __TEACHING __EXHIBITION**
START DATE: 11-1-98 **ANTICIPATED COMPLETION DATE:** 2-1-99

5. **FUNDING SOURCE: _X_DEPARTMENT _X_OTHER INTRAMURAL SOURCE ___EXTRAMURAL, SPECIFY SOURCE:** URGFC___

6. **CATEGORY OF ETHICAL CONCERN APPLICABLE TO THIS PROTOCOL:** C__
(SEE TABLE 1 FOR EXPLANATION OF CATEGORIES, IF MORE THAN ONE CATEGORY APPLIES, USE THE HIGHEST)

7. **WILL THE PROJECT OR EXERCISE INVOLVE EXPOSURE OF ANIMALS OR ANIMAL HANDLERS TO:**

BIOHAZARDS? _X_NO___YES. IF YES, SPECIFY:_____
RADIOISOTOPES? _X_NO___YES. IF YES, SPECIFY:_____
CARCINOGENS? _X_NO___YES. IF YES, SPECIFY:_____
TOXIC CHEMICALS? _X_NO___YES. IF YES, SPECIFY:_____
IF ANY ITEM ANSWERED YES, COMPLETE AND ATTACH SUPPLEMENT FORM 1

8. CHECK ALL ITEMS WHICH APPLY TO THIS PROJECT:

X Blood and/or tissue collection	X Survival surgery
__Antibody production and collection	X Non-survival surgery
__Behavioral studies	X Aseptic surgery
__Prolonged physical restraint	__Multiple surgeries on the same animal
__Food or water deprivation	
__Environmental extremes	X Alleviated pain
X Electrical stimuli	__Unalleviated pain
__Induction of trauma	X Anesthetics used
__Work to be done off campus	__Immobilizing agents without anesthesia
__Field studies	

9. ANIMAL REQUIREMENTS:

SPECIES	STRAIN	SEX	AGE/WT	TOTAL NO.
Rats	Sprague-Dawley	Male	140 - 160 g	36

USE RATE	DURATION OF STAY	SOURCE
	4 - 6 weeks	Standard Vendor

10. LOCATION OF ANIMAL HOUSING AND USE AREAS:

ANIMAL HOUSING FACILITY: Animal Care Facility - White Hall - UNLV
WILL ANIMALS BE REMOVED FROM THE HOUSING FACILITY FOR USE ELSEWHERE? __NO _X_YES. IF YES, SPECIFY LOCATION(S) (BLDG & ROOM NO.): Bigalow Health Sciences, Rm 119

11. IS SURGERY INVOLVED IN THE PROPOSED PROJECT?
__NO _X__YES. IF YES, COMPLETE AND ATTACH SUPPLEMENT FORM 2

12. ARE STRESSFUL OR PAINFUL PROCEDURES, OTHER THAN SURGERY, PART OF THIS PROJECT? _X__NO ___YES. IF YES, COMPLETE AND ATTACH SUPPLEMENT FORM 3.

13. ARE PROLONGED (MORE THAN A FEW HOURS) PHYSICAL RESTRAINT PROCEDURES PART OF THIS PROJECT? _X__NO __YES. IF YES, COMPLETE AND ATTACH SUPPLEMENT FORM 4.

14. WILL ANIMALS BE EUTHANITIZED? __NO _X__YES. IF YES, LIST THE METHOD(S) (BY SPECIES) AND, IF APPLICABLE, THE DOSE AND ROUTE OF ADMINISTRATION:
Pentobarbital Sodium. Intercardial injection upon completion of electrical stimulation procedure. (Animals will be anesthetized throughout the electrical stimulation procedure.)

15. WILL ANIMALS BE ALIVE AT THE END OF THE PROJECT? _X_NO __YES. IF YES, HOW WILL THEY BE USED?

16. **ARE THERE ANY SPECIAL REQUIREMENTS FOR ANIMAL HOUSING, DIETS, RESTRAINT, OR PROCEDURES FOR DISPOSAL? __NO _X_YES. IF NO, ANIMALS WILL BE MAINTAINED ACCORDING TO THE STANDARD OPERATING PROCEDURES. IF YES, PROVIDE (AN) EXPLANATION(S) FOR EACH PARAMETER INVOLVED (E.G., LIGHT:DARK CYCLES, HUMIDITY, AMBIENT TEMPERATURE, CAGING, BEDDING, DIETS, AND SO FORTH):**
In addition to a normal diet, animals will be fed a dietary supplement (Jello squares) which may or may not contain the nutritional supplement creatine. Half the rats will receive creatine and half will not.

17. **RESEACH OR TEACHING PLAN: IN SUFFICIENT DETAIL TO PERMIT EVALUATION BY MEMBERS OF THE INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC), ANSWER THE FOLLOWING QUESTIONS (USE CONTINUATION PAGES IF NECESSARY):**

a. **WHAT IS (ARE) THE OBJECTIVE(S) AND POTENTIAL SIGNIFICANCE OF THE ACTIVITIES INVOLVING ANIMAL USE?**

Creatine is a popular nutritional supplement used by athletes to enhance performance. Creatine is an amino acid obtained from meat and fish in the diet and synthesized from methionine, glycine, and arginine. Creatine is phosphorylated, via creatine kinase, to phosphocreatine. Phosphocreatine is a high energy compound that supports the re-synthesis of ATP during the initial stages of intense muscular activity. Studies have shown the creatine supplementation leads to increased phosphocreatine stores within skeletal muscles. However, these studies have yielded mixed results as to creatine's effect on muscular hypertrophy and intense muscular activity. The focus of this study is to investigate the effect of creatine supplementation on compensatory hypertrophy and maximal muscular activity.

b. **SPECIFICALLY, WHY ARE LIVE ANIMALS REQUIRED FOR THIS PROJECT RATHER THAN NON-ANIMAL ALTERNATIVES?**

Rats were selected as the model for this study because synergistic ablation results in relatively rapid muscle hypertrophy, which is similar to that achieved by humans over a considerably longer time period with high resistance weight training. In addition, rat skeletal muscles of nearly homogenous muscle fiber types permit the determination of creatine's effect in fast and slow twitch muscles. Computer simulations were considered but were rejected because the absence of data regarding the effects of creatine on performance measures of specific fiber types precluded this approach.

c. **IN ACCORDANCE WITH THE REQUIREMENTS OF THE FEDERAL ANIMAL WELFARE ACT (SECTION 2.31(D)(1)(II), PLEASE LIST WHAT NON-ALTERNATIVES YOU CONSIDERED USING, HOW YOU DETERMINED THAT THEY ARE NOT SUITABLE NOR THAT OTHER ALTERNATIVES ARE NOT AVAILABLE, AND YOUR SOURCES OF INFORMATION ABOUT THE AVAILABILITY OF ALTERNATIVES.**

Computer simulations were considered, but the absence of data concerning the effects of creatine on hypertrophy and performance of specific fiber types does not permit use of this alternative. The use of human subjects is impractical due to the relatively slow rate of muscular hypertrophy, and the in ability to measure performance of specific fiber types. Information was obtained from literature searches of the national library of medicine, utilizing Medline through PubMed, with the keywords creatine, muscle hypertrophy and computer simulation. Information was also obtained from previous research experience.

d. **WHY IS (ARE) THE ANIMAL MODEL(S) YOU ARE PROPOSING TO USE THE MOST APPROPRIATE MODEL(S) FOR ACHIEVING THE OBJECTIVES OF THIS PROJECT?**

Substantial literature attests to the viability of synergistic muscle ablation as a method of producing compensatory muscle hypertrophy. The relatively rapid rate of hypertrophy, in rats, ensures that the objectives of this project can be accomplished in a reasonable time frame. Also, rats will willingly ingest a palatable diet containing nutritional supplements.

e. **WHAT ARE THE REASONS FOR REQUIRING THE NUMBER OF ANIMALS REQUESTED?**

The number of animals is the minimum number judged to be necessary to demonstrate statistical differences in muscular hypertrophy and performance characteristics of individual fiber types.

f. **WHAT ARE THE SPECIFIC PROCEDURES YOU ARE PROPOSING TO USE WHICH WILL INVOLVE OR EFFECT THE ANIMALS?**

See attached page.

18. **CHECK ALL APPLICABLE ITEMS:**

INSTRUCTIONS FOR DISPOSITION OF SICK OR INJURED ANIMALS	**INSTRUCTIONS FOR DISPOSITION OF DEAD ANIMALS**
_X_ CALL INVESTIGATOR	_X_ CALL INVESTIGATOR
__ VETERINARIAN TO TREAT	__ VETERINARIAN TO TREAT
__ EUTHANITIZE	__ EUTHANITIZE

19. **IF WILD OR EXOTIC SPECIES ARE TO BE USED: ARE SPECIAL PERMITS REQUIRED? _X_ NO __ YES. IF YES, ATTACH A COPY OF APPLICABLE PERMITS OR PERMIT APPLICATIONS.**

WILL ANIMALS BE OBSERVED, MANIPULATED, OR TRAPPED IN THE WILD? _X_ NO __ YES. IF YES, AND THEY HAVE NOT ALREADY BEEN ADDRESSED, DESCRIBE THE FIELD PROCEDURE(S) AND GIVE THE GEOGRAPHIC LOCATION(S) INVOLVED:

20. **WILL ANY NONANESTHETIC SUBSTANCES BE INTRODUCED INTO THE ANIMALS AS PART OF THIS PROJECT (E.G., PHARMACOLOGICAL OR TOXICOLOGIC AGENTS, ANTIGENS, HORMONES, TUMOR CELLS, ETC.)? _X_ NO __ YES. IF YES, DESCRIBE:**

SPECIES	**SUBSTANCE**	**DOSE**	**ROUTE**	**FREQUENCY**

IS ANY ANIMAL PAIN, DISTRESS, OR DISCOMFORT EXPECTED TO BE ASSOCIATED WITH INTRODUCTION OF THIS (THESE) SUBSTANCES?
_X_ NO __ YES. IF YES, EXPLAIN:

21. **WILL BLOOD OR TISSUES BE COLLECTED FROM THE ANIMALS OTHER THAN DURING TERMINAL ANESTHESIA PROCEDURES? _X_NO __YES. IF YES, DESCRIBE THE TECHNIQUES TO BE USED:**

IF BLOOD IS TO BE COLLECTED REPEATEDLY, WHAT IS THE QUANTITY OF BLOOD TO BE COLLECTED AT EACH DRAW?___, HOW OFTEN WILL BLOOD SAMPLES BE COLLECTED? ___, AND WHAT PROCEDURES, IF ANY, WILL BE USED TO PREVENT ANEMIA?

22. **ARE BEHAVIORAL STUDIES INVOLVED IN THIS PROJECT? _X_NO __YES. IF YES, AND NOT PREVIOUSLY DESCRIBED, PROVIDE A DESCRIPTION OF ALL METHODS AND PROCEDURES TO BE USED, OR WITH, THE ANIMALS (INCLUDING ANY NUTRITIONAL DEPRIVATION OR APPLICATION OF NOXIOUS STIMULI) AND INDICATE THE DURATION OF SUCH STUDIES:**

23. **JUSTIFICATION: THIS SECTION IS REQUIRED FOR ANY PROJECT IN WHICH THERE IS A POTENTIAL FOR PAIN, DISTRESS, OR DISCOMFORT THAT CANNOT BE ALLEVIATED. EXAMPLES OF SUCH PROJECTS INCLUDE INDUCED DISEASE STATES WHICH CAUSE SEVERE SYMPTOMS OR DEATH, SURGICAL STUDIES RESULTING IN SEVERE POST-OPERATIVE DISCOMFORT OR LOSS OF FUNCTION, THE USE OF SEVERE AND UNAVOIDABLE NOXIOUS STIMULI, AND SO FORTH. (USE CONTINUATION PAGES IF NECESSARY)**

a. DESCRIBE WHAT UNALLEVIATED PAIN, DISTRESS, OR DISCOMFORT MAY BE EXPECTED TO BE ASSOCIATED WITH THIS PROJECT.

Post surgical pain, distress or discomfort is not anticipated. Previous studies using this procedure have shown that animals were locomotive upon regaining consciousness, weight gain is normal, relative to control animals (Young, Kandarian &Kurowski, 1992), and there was no gnawing of sutures or any similar behaviors that would indicate discomfort. However if it determined by the PI, Co-PI, or attending veterinarian that any animal is experiencing pain, Butorphanol will be administered (0.1 – 0.5 mg/Kg) as prescribed by the attending veterinarian. The criteria for determining whether or not an animal is experiencing pain will be failure to exhibit locomotion upon regaining consciousness, failure to consume a normal diet, and gnawing at sutures.

b. PROVIDE SPECIFIC JUSTIFICATION FOR THE NECESSITY OF ANY UNALLEVIATED PAIN, DISTRESS, OR DISCOMFORT LISTED IN THE PREVIOUS SECTION.

N/A

c. IS DEATH USED AS AN END-POINT IN THIS PROJECT?
_X_NO ___YES. **IF YES, EXPLAIN WHY SOME EARLIER END-POINT CANNOT BE USED INSTEAD (PROVIDE OBJECTIVE SUPPORTING DATA):**

17. f. **Ablation Procedure:**

Rats will be anesthetized with pentobarbital sodium (5mg/100g body weight, ip). Bilateral ablations will be performed. The lateral aspect of each hindlimb is shaved and a longitudinal incision is made through the skin and fascia along the lateral aspect of the tibia. To ablate the gastrocnemius and plantaris, the lateral and medial heads of the muscle are isolated with careful blunt dissection. The distal 2/3 of the gastrocnemius and plantaris are removed with care not to disturb the vasculature and nerves associated with the soleus. To ablate the tibialis anterior the distal tendon is cut and the muscle removed *in toto*. The fascia and skin are then sutured distal to proximal with 5-O silk. Rats will be monitored during recovery for signs of pain, distress or discomfort. Upon observation of pain, distress, or discomfort, by animal care staff, attending veterinarian, or primary investigator, rats will be given Butorphanol as prescribed by the attending veterinarian.

Kandarian, S.C. & White, T.P. Mechanical deficit persists during long-term muscle hypertrophy. *Journal of Applied Physiology*, 69(3): 861-867, 1990.

Young, J.C., Kandarian, S.C. & Kurowski, T.G. Skeletal muscle glucose uptake following overload-induced hypertrophy. *Life Sciences*, 50: 1319-1325, 1992.

Creatine Supplementation:

In addition to a normal diet, animals will be feed a dietary supplement (Jello squares) which may or may not contain the nutritional supplement creatine monohydrate. The dose for animals receiving creatine monohydrate will be 300 mg/Kg body weight, once daily. This rate is equivalent to the recommended rate for human supplementation. Unpublished lab results have shown creatine to have no significant effect on growth rate (see attached graph), or normal feeding patterns.

Electrical Stimulation Procedure:

Six weeks following the ablation surgery the rats will again be anesthetized with pentobarbital sodium (5mg/100g body weight, ip). The sciatic nerve is exposed in the region of the thigh (before the separation of the tibial and common peroneal branches) but not cut. A dastre electrode is placed around the nerve, proximal to the bifurcation. The limb is fixed at the knee and ankle by steel pins inserted under the tibio-patellar ligament and the achilles tendons respectively. The muscle is tied via the distal tendon to a force transducer, connected to a Grass polygraph. The muscles are made to contract by stimulating the sciatic nerve electrically for two 5 min periods separated by 1 min of rest, with 500 ms trains of supramaximal voltage (60 – 80V) at a rate of 1 train/sec. Pulses are delivered at 100Hz, with each pulse in the train having a duration of 0.1 ms. Immediately after stimulation the muscles are isolated, freeze clamped and removed. Following the stimulation rats will be euthanitized by an intercardial injection of pentobarbital sodium, without being allowed to allowed to regain consciousness.

Young, J.C. & Balon, T.W. *Role of dihyropyridine sensitive calcium channels in glucose transport in skeletal muscle.* Life Sciences 61(3): 335-342, 1997.

24. ASSURANCES:

a. PRINCIPAL INVESTIGATOR: I HAVE READ AND AGREE TO ABIDE BY THE PROVISIONS OF THE UNLV POLICIES AND PROCEDURES HANDBOOK FOR THE USE OF LABORATORY ANIMALS AND THE PUBLIC HEALTH SERVICE'S GUIDE FOR THE CARE AND USE OF LABORATORY ANIMALS (COPIES OF THESE DOCUMENTS MAY BE OBTAINED FROM THE SUPERVISOR OF LABORATORY ANIMAL CARE SERVICES OR THE DIRECTOR OF LABORATORY ANIMAL MEDICINE). I WILL ADVISE THE UNLV INSTITUIONAL ANIMAL CARE AND USE COMMITTEE IN WRITING OF ANY SIGNIFICANT CHANGES IN THE PROCEDURES DESCRIBED IN THIS PROTOCOL.

_____,_____,_____
PRINCIPAL INVESTIGATOR/ TITLE/RANK DATE
COURSE DIRECTOR

b. ALSO, I UNDERSTAND THAT "UNNECESSARILY DUPLICATIVE" RESEARCH INVOLVING LABORATORY ANIMALS IS NOT PERMITTED BY THE FEDERAL ANIMAL WELFARE ACT AND HEREBY PROVIDE ASSURANCE THAT THE RESEARCH PROPOSED HEREIN DOES NOT, TO THE BEST OF MY KNOWLEDGE, HAVING REVIEWED THE RELEVANT LITERATURE, UNNECESSARILY DUPLICATE RESEARCH ALREADY REPORTED IN THE PUBLIC LITERATURE.

_____,_____,_____
PRINCIPAL INVESTIGATOR/ TITLE/RANK DATE
COURSE DIRECTOR

c. DEPARTMENT CHAIRMAN/DEAN: I HAVE REVIEWED THIS PROTOCOL AND CERTIFY THAT THIS PROJECT IS SCIENTIFICALLY VALID AND THAT THE PERSONNEL CONDUCTING THE ANIMAL ASSOCIATED PROCEDURES ARE QUALIFIED AND TRAINED TO ACCOMPLISH THE PROCEDURES.

_____,_____,_____
DEPARTMENT HEAD/DEAN DEPARTMENT DATE

d. SUPERVISOR, LABORATORY ANIMAL CARE SERVICES: I HAVE REVIEWED THIS PROTOCOL AND CERTIFY THAT RESOURCES FOR HOUSING AND HANDLING THE ANIMALS, INCLUDING FOR ANY SPECIAL RE QUIREMENTS SPECIFIED HEREIN, ARE AVAILABLE OR CAN BE EASILY OBTAINED.

_____,_____,_____
SUPERVISOR, LABORATORY ANIMAL CARE, DATE

e. VETERINARY ASSURANCE: THE TYPE AND AMOUNT OF ANAL GESIC, ANESTHETIC, OR TRANQUILIZING DRUGS PROPOSED FOR USE IN THIS PROJECT ARE APPROPRIATE BY CURRENT PROFESSIONAL STANDARDS TO RELIEVE ANIMAL PAIN, DISTRESS, OR DISCOMFORT, EXCEPT AS JUSTIFIED ABOVE BY THE PRINCIPAL INVESTIGATOR. METHODS OF EUTHANASIA ARE COMPATIBLE WITH THE RECOMMENDATION OF THE AMERICAN VETERINARY MEDICAL ASSOCIATION'S PANEL ON EUTHANASIA REPORT (JAVMA, FEBRUARY 1, 1986). FURTHERMORE, IF REQUIRED BY THE FEDERAL ANIMAL WELFARE ACT REGULATIONS, THE PRINCIPAL INVESTIGATOR HAS CONFERRED WITH ME DURING THE PREPARATION OF THIS PROTOCOL.

_____, _____, _____

DIRECTOR, LABORATORY ANIMAL MEDICINE DATE
(or other Attending Veterinarian)

UNIVERSITY OF NEVADA, LAS VEGAS
PROTOCOL FOR ANIMAL CARE AND USE

SUPPLEMENT FORM 2

SURGICAL PROCEDURES
(complete only if applicable - if not, discard)

1. **a. Describe pre-operative care and selection procedures (including physical examinations, lab tests, and preconditioning procedures):**
No special requirements beyond normal care and feeding.

b. List pre-operative and inter-operative medications and anesthetics:

Species	Drug	Dose/Kg Body Wt.	Route	Freq.
Rats	Pentobarbital Sodium	5mg/100g body weight	ip. injection	1X

If gas anesthetics will be used, describe the scavenging system for removal of extraneous anesthetic:

c. Who will administer the anesthetic? J.C. Young

2. Describe the surgical procedures, attaching additional sheets if necessary. (Sterile surgical techniques MUST be used in survival surgeries involving most animals; thus, a description of the procedures to be used to ensure asepsis should be provided. Multiple survival surgeries on a single animal are generally prohibited. The only circumstances under which multiple survival surgeries may be justified are when they are related components of a single research project or to conserve rare and endangered species. Cost savings alone is not an adequate reason for performing multiple survival surgeries.)

Ablation surgeries will be performed in the LACF. A clean operating field will be established, sterile gloves will be worn, a sterile drape will be used and absorbent pads will be placed under the animals during surgery. Rats will be anesthetized with pentobarbital sodium (5mg/100g body weight, ip). Bilateral ablations will be performed. The lateral aspect of each hindlimb is shaved in an area apart from the surgical field. The shaved area will be scrubbed with betadyne, prior to the incision being made. All instruments will be sterilized in a steam autoclave prior to the first surgery. After the first surgery, all instruments will be washed and wiped with alcohol. A longitudinal incision is made through the skin and fascia along the lateral aspect of the tibia. To ablate the gastrocnemius and plantaris, the lateral and medial heads of the muscle are isolated with careful blunt end dissection. The distal 2/3 of the gastrocnemius and plantaris are removed with care not to disturb the vasculature and nerves associated with the soleus. To ablate the tibialis anterior the distal tendon is cut and the muscle removed *in toto*. The fascia and skin are then sutured distal to proximal with 5-O silk.

Will multiple survival surgeries be performed? ___X__NO _____YES
If yes, describe and justify:

3. a. Normally, it is not permissible to use paralyzing agents without general anesthetics. Will paralyzing drugs be used?
__X__NO _____YES. If yes, list:

Species	Drug	Dose/Kg Body Wt.	Route	Freq.

b. How and by whom will the level of anesthesia be assessed and how often will the assessment be made?

Level of anesthesia will be assessed by reflex response to toe pinch and tail pinch. P.I. or CO P.I. will make determination prior to initiation of surgery. If during the surgical procedure the animal is assessed as being "lightly" anesthetized, supplemental Pentobarbital Sodium (1-2 mg/100g body weight)will be administered.

c. What criteria will be used to evaluate pain/discomfort?

Criteria for adequate level of anesthesia (lack of pain or discomfort) will be the absence of pinch reflex in toes or tail.

d. What methods will be used to prevent dehydration and hypothermia during surgery?

Tissues will be moistened with physiological saline during surgery if necessary. Surgery will be performed under a lamp to reduce the risk of hypothermia.

e. For a non-survival study, what is the duration of surgery and study prior to the animal being euthanitized?

Electrical stimulation procedure (see 17 f.) will be non-survival. Duration of this procedure prior to euthanization will be approximately 30 min per rat.

4. Post-Operative Care for Survival Studies:

a. Post-Anesthesia Recovery - Describe the observations that will ensure that the animals are stable and returning to a safe level of recovery from anesthesia and indicate who will be responsible for the observations:

PI or Co-PI will check rats periodically (approximately every 15 minutes), while in their cages until, consciousness is regained and locomotion resumed.

b. Post-Surgical Recovery - Describe the frequency of examination and observation of the animal and management procedures for potential experiment-related diseases:

Rats will be observed at least twice daily at feeding times.

c. What criteria will be used to evaluate post-operative pain, distress, or discomfort?

Observation of P.I., Co P.I., attending veterinarian, or LACF staff of pain, distress, or discomfort.

d. List post-operative medications which may be used (e.g., antibiotics or analgesics). If the use of such agents would interfere with the project objectives, explain and document with objective data:

Butorphanol (0.1-0.5 mg/Kg body weight), bid (every 12 hours) by subcutaneous injection as deemed necessary by attending veterinarian.

e. How long will the animals be kept alive after the surgery?

4 to 6 weeks following initial ablation surgery.

f. Describe any long-term care required post-surgically. If animals are chronically implanted or instrumented, this section should be completed.

None

APPENDIX IV

ANOVA TABLES

ANOVA Table: Animal Growth

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
GRP	2	816.66666667	408.33333333	0.77	0.4733
SUP	**1**	**3117.36111111**	**3117.36111111**	**5.89**	**0.0228**
SUBJ	5	6107.91666667	1221.58333333	2.31	0.0747
GRP*SUP	**2**	**4206.22222222**	**2103.11111111**	**3.97**	**0.0318**
Error	25	13242.58333333	529.70333333		
Corrected Total	35	27490.75000000			

ANOVA Table: Muscle Size

Source	DF	Anova SS	Mean Square	F Value	Pr > F
MUS	**1**	**169512.04166667**	**169512.04166667**	**8.99**	**0.0037**
SUP	1	8325.37500000	8325.37500000	0.44	0.5084
SRG	**1**	**914160.66666667**	**914160.66666667**	**48.48**	**0.0001**
SUBJ	11	178610.12500000	16237.28409091	0.86	0.5810
MUS*SUP	1	16485.04166667	16485.04166667	0.87	0.3527
MUS*SRG	**1**	**202768.16666667**	**202768.16666667**	**10.75**	**0.0016**
SUP*SRG	1	2860.16666667	2860.16666667	0.15	0.6980
MUS*SUP*SRG	1	21480.16666667	21480.16666667	1.14	0.2892
Error	77	1451899.87500000	18855.84253247		
Corrected Total	95	2966101.62500000			

ANOVA Table: Peak Force

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TIM	**1**	**13090.94460000**	**13090.94460000**	**57.35**	**0.0006**
SUBJ(TIM)	5	1141.36810000	228.27362000	.	.
MUS	1	528.75093750	528.75093750	1.74	0.1920
SUP	1	7.72935000	7.72935000	0.03	0.8739
SRG	**1**	**5168.24150417**	**5168.24150417**	**16.96**	**0.0001**
MUS*SUP	1	16.66666667	16.66666667	0.05	0.8158
MUS*TIM	1	463.40881667	463.40881667	1.52	0.2216
MUS*SRG	1	72.69720417	72.69720417	0.24	0.6267
SUP*TIM	1	76.43370417	76.43370417	0.25	0.6180
SUP*SRG	1	54.00000000	54.00000000	0.18	0.6751
TIM*SRG	1	27.34935000	27.34935000	0.09	0.7654
MUS*SUP*TIM	1	327.30320417	327.30320417	1.07	0.3036
MUS*SUP*SRG	1	0.61440000	0.61440000	0.00	0.9643
MUS*TIM*SRG	1	37.35015000	37.35015000	0.12	0.7273
SUP*TIM*SRG	1	106.21833750	106.21833750	0.35	0.5568
MUS*SUP*TIM*SRG	1	176.09583750	176.09583750	0.58	0.4497
MUS*SUP*SRG*SUB(TIM)	70	21328.19458750	304.68849411	.	.
Corrected Total	95	42875.90294063			

ANOVA Table: Percent Recovery of Force

Source	DF	Anova SS	Mean Square	F Value	Pr > F
MUS	**1**	**10830.02083333**	**10830.02083333**	**6.15**	**0.0181**
SUP	1	1958.40750000	1958.40750000	1.11	0.2987
SRG	**1**	**9009.12000000**	**9009.12000000**	**5.12**	**0.0300**
SUBJ	5	12648.35250000	2529.67050000	1.44	0.2353
MUS*SUP	1	434.40333333	434.40333333	0.25	0.6224
MUS*SRG	1	197.64083333	197.64083333	0.11	0.7395
SUP*SRG	1	457.56750000	457.56750000	0.26	0.6133
MUS*SUP*SRG	1	32.67000000	32.67000000	0.02	0.8924
Error	35	61592.77750000	1759.79364286		
Corrected Total	47	97160.96000000			

ANOVA Table: Size Corrected Peak Force

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TIM	**1**	**2587.63050104**	**2587.63050104**	**75.00**	**0.0003**
SUBJ(TIM)	5	172.50071771	34.50014354	.	.
MUS	1	2.10930104	2.10930104	0.04	0.8372
SUP	1	11.47475104	11.47475104	0.23	0.6320
SRG	**1**	**469.80225938**	**469.80225938**	**9.47**	**0.0030**
MUS*SUP	1	63.91238437	63.91238437	1.29	0.2602
MUS*TIM	1	143.39925938	143.39925938	2.89	0.0935
MUS*SRG	**1**	**588.40655104**	**588.40655104**	**11.86**	**0.0010**
SUP*TIM	1	20.45183438	20.45183438	0.41	0.5229
SUP*SRG	1	2.02710937	2.02710937	0.04	0.8404
TIM*SRG	**1**	**265.83398438**	**265.83398438**	**5.36**	**0.0236**
MUS*SUP*TIM	1	70.57225104	70.57225104	1.42	0.2370
MUS*SUP*SRG	1	99.98042604	99.98042604	2.02	0.1601
MUS*TIM*SRG	1	2.46720937	2.46720937	0.05	0.8242
SUP*TIM*SRG	1	19.41300937	19.41300937	0.39	0.5336
MUS*SUP*TIM*SRG	1	36.14987604	36.14987604	0.73	0.3962
MUS*SUP*SRG*SUB(TIM)	70	3472.21008125	49.60300116	.	.
Corrected total	95	8193.95114896			

ANOVA Table: Total Tensions First 30 Seconds

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TIM	**1**	**8273008.10962604**	**8273008.10962604**	**118.91**	**0.0001**
SUBJ(TIM)	5	347866.22206771	69573.24441354	.	.
MUS	1	283903.84137606	283903.84137606	1.76	0.1885
SUP	1	0.08942606	0.08942606	0.00	0.9994
SRG	**1**	**3800330.98255105**	**3800330.98255105**	**23.60**	**0.0001**
MUS*SUP	1	8364.72012603	8364.72012603	0.05	0.8204
MUS*TIM	1	14779.56585938	14779.56585938	0.09	0.7628
MUS*SRG	1	85945.20008437	85945.20008437	0.53	0.4675
SUP*TIM	1	5325.40937605	5325.40937605	0.03	0.8562
SUP*SRG	1	51674.28805103	51674.28805103	0.32	0.5729
TIM*SRG	1	367226.95312606	367226.95312606	2.28	0.1355
MUS*SUP*TIM	1	106323.60960937	106323.60960937	0.66	0.4192
MUS*SUP*SRG	1	55031.83625105	55031.83625105	0.34	0.5607
MUS*TIM*SRG	1	14153.54085936	14153.54085936	0.09	0.7677
SUP*TIM*SRG	1	4996.67612602	4996.67612602	0.03	0.8607
MUS*SUP*TIM*SRG	1	453.92252604	453.92252604	0.00	0.9578
MUS*SUP*SRG*SUB(TIM)	70	11271372.13573950	161019.60193914	.	.
Corrected Total	95	24874304.08989580			

ANOVA Table: Percent Recovery of Tension of First 30 Seconds

Source	DF	Anova SS	Mean Square	F Value	Pr > F
MUS	**1**	**3111.32505208**	**3111.32505208**	**4.96**	**0.0325**
SUP	1	718.96860208	718.96860208	1.15	0.2918
SRG	1	884.16916875	884.16916875	1.41	0.2432
SUBJ	5	2507.27016042	501.45403208	0.80	0.5579
MUS*SUP	1	425.48475208	425.48475208	0.68	0.4158
MUS*SRG	1	80.16085208	80.16085208	0.13	0.7229
SUP*SRG	1	2363.63435208	2363.63435208	3.77	0.0604
MUS*SUP*SRG	1	525.29716875	525.29716875	0.84	0.3665
Error	35	21963.75998958	627.53599970		
Corrected Total	47	32580.07009792			

ANOVA Table: Total Tension Second 30 Seconds

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TIM	**1**	**1468855.85992605**	**1468855.85992605**	**76.92**	**0.0003**
SUBJ(TIM)	5	95484.79216771	19096.95843354	.	.
MUS	1	113286.98745938	113286.98745938	2.27	0.1362
SUP	1	26545.13877605	26545.13877605	0.53	0.4680
SRG	**1**	**1115158.70377605**	**1115158.70377605**	**22.37**	**0.0001**
MUS*SUP	1	37024.93537603	37024.93537603	0.74	0.3918
MUS*TIM	1	28240.21917604	28240.21917604	0.57	0.4542
MUS*SRG	1	49422.90420937	49422.90420937	0.99	0.3228
SUP*TIM	1	10585.47005104	10585.47005104	0.21	0.6464
SUP*SRG	1	99733.15690103	99733.15690103	2.00	0.1617
TIM*SRG	1	37257.82200937	37257.82200937	0.75	0.3903
MUS*SUP*TIM	1	536.61855104	536.61855104	0.01	0.9177
MUS*SUP*SRG	1	9418.66450105	9418.66450105	0.19	0.6652
MUS*TIM*SRG	1	76824.88992605	76824.88992605	1.54	0.2186
SUP*TIM*SRG	1	2236.19467605	2236.19467605	0.04	0.8329
MUS*SUP*TIM*SRG	1	1844.76967604	1844.76967604	0.04	0.8480
MUS*SUP*SRG*SUB(TIM)	70	3489847.79529791	49854.96850426	.	.
Corrected Total	95	13606186.85022390			

ANOVA Table: Time to One-Half Peak Force

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TIM	**1**	**661.55250104**	**661.55250104**	**19.34**	**0.0070**
SUBJ(TIM)	5	171.05426771	34.21085354	.	.
MUS	**1**	**1233.59850938**	**1233.59850938**	**9.46**	**0.0030**
SUP	**1**	**1054.89930104**	**1054.89930104**	**8.09**	**0.0058**
SRG	**1**	**1497.76100104**	**1497.76100104**	**11.48**	**0.0012**
MUS*SUP	**1**	**771.40350938**	**771.40350938**	**5.91**	**0.0176**
MUS*TIM	1	45.52637604	45.52637604	0.35	0.5566
MUS*SRG	1	142.52063437	142.52063437	1.09	0.2995
SUP*TIM	1	1.96940104	1.96940104	0.02	0.9026
SUP*SRG	**1**	**1114.59325104**	**1114.59325104**	**8.54**	**0.0047**
TIM*SRG	1	78.13845937	78.13845937	0.60	0.4416
MUS*SUP*TIM	1	2.83937604	2.83937604	0.02	0.8831
MUS*SUP*SRG	1	313.09538437	313.09538437	2.40	0.1259
MUS*TIM*SRG	1	3.79612604	3.79612604	0.03	0.8650
SUP*TIM*SRG	1	11.57175938	11.57175938	0.09	0.7667
MUS*SUP*TIM*SRG	1	99.28767604	99.28767604	0.76	0.3860
MUS*SUP*SRG*SUB(TIM)	70	9132.72512292	130.46750176	.	.
Corrected Total	95	17825.59100000			

APPENDIX V

RAW DATA

EDL	Stim	Time (s)	Force of contraction (g)																	
Rat #	Group	0	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180
19	Con-C																			
22		27.01	11.74	5.84	4.02	2.59	2.04	1.22	0.97	0.70	0.68	0.66	0.58	0.52	0.53	0.36	0.48	0.40	0.47	0.49
24		51.14	25.64	14.26	6.91	3.43	2.80	2.89	2.01	1.75	1.44	1.17	0.92	0.84	0.68	0.67	0.76	0.80	0.63	0.52
27		40.53	24.25	10.87	5.47	4.29	4.22	3.35	2.73	2.38	2.11	1.53	1.27	1.07	0.91	0.85	0.78	0.65	0.76	0.70
32		50.25	30.90	19.92	13.62	7.39	5.00	3.82	3.10	2.83	2.60	2.29	2.08	1.96	1.89	2.03	1.77	1.62	1.68	1.73
36		36.75	20.21	14.20	9.71	6.87	6.79	4.52	3.58	3.00	2.50	1.74	1.63	1.50	1.32	1.20	1.22	1.24	1.06	1.02
Mean		41.14	22.55	13.02	7.95	4.91	4.17	3.16	2.48	2.13	1.87	1.48	1.30	1.18	1.07	1.02	1.00	0.94	0.92	0.89
StErr		4.48	3.20	2.31	1.70	0.95	0.84	0.55	0.46	0.42	0.36	0.27	0.26	0.25	0.25	0.29	0.23	0.22	0.21	0.23
10	Con-Cr	37.09	28.11	20.34	14.01	10.55	8.17	6.40	5.91	5.54	4.30	3.95	3.52	2.71	4.13	6.47	7.93	4.58	4.89	4.35
16		1.74	1.14	0.57	0.27	0.17	0.16	0.18	0.08	0.00										
21		49.06	31.68	25.57	19.75	15.67	12.24	9.55	7.40	6.32	7.08	6.47	6.03	5.68	5.29	4.96	4.81	4.75	4.74	4.28
29		64.87	27.63	17.34	12.35	10.68	6.12	6.28	4.12	4.24	4.55	3.47	4.49	3.11	2.77	2.07	2.07	1.82	1.61	1.61
30		16.88	12.02	9.19	5.46	3.00	1.78	1.68	1.15	0.82	0.78	0.54	0.00							
33		46.39	20.30	4.51	1.95	1.82	3.12	2.84	1.71	1.16	1.14	0.81	0.59	0.00						
Mean		36.01	20.15	12.92	8.97	6.98	5.27	4.49	3.40	3.01	3.57	3.05	2.93	2.88	4.06	4.50	4.94	3.72	3.75	3.41
StErr		9.40	4.76	3.97	3.11	2.52	1.83	1.43	1.18	1.10	1.17	1.10	1.15	1.16	0.73	1.29	1.69	0.95	1.07	0.90
4	Hyp-C	58.72	58.54	49.03	32.97	19.44	12.59	9.14	7.28	6.32	5.36	4.40	4.03	3.52	3.72	2.62	2.40	2.36	1.90	1.78
11		36.57	32.58	33.78	21.35	15.55	13.14	11.50	11.09	9.62	9.71	9.50	9.36	7.44	8.72	8.31	7.09	6.59	7.01	6.87
12		47.98	29.83	26.80	21.60	14.69	10.98	9.05	8.55	6.50	5.87	4.62	5.03	4.71	4.23	4.05	4.06	4.00	3.83	3.81
15		57.52	34.28	20.82	20.78	15.66	10.02	6.78	5.06	4.24	3.78	3.32	2.84	2.59	2.89	3.12	3.22	2.93	2.58	2.52
20		57.67	44.15	36.73	26.66	19.40	14.43	11.55	9.55	8.43	8.18	7.72	7.25	7.15	6.88	6.72	6.51	6.41	6.41	6.26
35		66.63	59.34	58.11	56.31	39.70	28.95	22.84	14.11	14.71	12.15	12.26	10.59	9.74	8.51	8.21	8.84	7.52	8.78	8.91
Mean		54.18	43.12	37.55	29.95	20.74	15.02	11.81	9.27	8.30	7.51	6.97	6.52	5.86	5.83	5.51	5.35	4.97	5.09	5.03
StErr		4.27	5.38	5.67	5.61	3.88	2.86	2.32	1.28	1.49	1.27	1.42	1.25	1.11	1.04	1.05	1.02	0.88	1.11	1.13
7	Hyp-Cr	30.40	16.72	12.05	9.07	5.87	4.59	3.70	2.97	2.80	2.57	2.57	2.55	2.32	2.28	2.18	2.18	2.04	1.96	2.02
8		22.33	10.67	7.43	4.68	2.94	2.28	1.98	1.88	1.57	1.41	1.37	1.12	1.03	0.98	0.93	1.02	0.92	1.35	1.49
9		68.46	67.87	57.48	41.48	31.47	26.72	20.46	16.87	17.87	16.81	15.25	15.11	14.20	13.18	12.75	12.55	12.36	13.44	14.00
13		57.29	50.60	40.08	33.84	27.18	19.62	16.21	13.54	12.57	11.59	10.96	10.46	10.01	9.69	9.82	9.92	9.83	9.76	10.12
23		56.95	57.35	53.59	39.32	27.03	17.33	13.48	11.96	10.40	9.22	7.93	7.47	7.07	6.40	6.47	4.67	5.02	5.47	5.91
26		71.53	49.84	34.31	21.44	11.53	7.65	6.21	5.41	4.33	3.32	3.05	2.82	3.37	3.98	2.95	4.23	3.45	3.21	2.47
Mean		51.16	42.18	34.16	24.97	17.67	13.03	10.34	8.77	8.26	7.49	6.86	6.59	6.33	6.09	5.85	5.76	5.60	5.87	6.00
StErr		8.26	9.42	8.48	6.41	5.04	3.94	3.04	2.52	2.62	2.48	2.25	2.23	2.07	1.90	1.91	1.85	1.85	1.96	2.08

EDL	Recov	Time (s)		Force of contractions (g)										
Rat #	Group	0	10	20	30	40	50	60	70	80	90	100	110	120
19	Con-C													
22		4.76	1.35	0.83	0.42	0.33	0.27	0.19	0.16	0.17	0.18	0.17	0.18	0.26
24		6.70	2.47	1.42	0.72	0.38	0.17	0.13	0.00					
27		3.81	1.75	1.05	0.88	0.71	0.39	0.27	0.26	0.21	0.21	0.26	0.16	0.11
32		8.41	3.06	2.62	1.76	1.27	1.18	1.03	0.92	0.81	0.77	0.76	0.78	0.75
36		7.32	2.44	2.06	1.56	1.29	1.01	0.99	1.04	1.00	0.93	0.91	0.88	0.85
Mean		6.20	2.21	1.60	1.07	0.80	0.60	0.52	0.48	0.55	0.52	0.53	0.50	0.49
StErr		0.84	0.30	0.33	0.25	0.21	0.21	0.20	0.21	0.21	0.19	0.18	0.19	0.18
10	Con-Cr	17.78	6.55	5.74	3.67	3.38	4.15	2.59	2.98	2.75	2.13	2.22	2.15	2.03
16		1.63	0.86	0.32	0.48	2.85	4.69	4.53	0.35	1.65	1.71	1.61	4.55	4.10
21		25.87	14.48	10.74	8.34	7.00	6.16	5.22	5.26	4.78	4.52	4.40	4.92	5.02
29		11.50	2.99	2.36	1.67	1.27	0.93	0.84	0.74	0.76	0.78	0.70	0.67	0.59
30		0.65	0.84	0.22	0.20	0.00	0.00	0.00	0.24	0.08	0.00	2.42	1.44	0.66
33		7.45	3.92	2.91	1.99	1.35	0.99	0.85	0.72	0.65	0.63	0.58	0.54	0.53
Mean		10.81	4.94	3.72	2.73	2.64	2.82	2.34	1.72	1.78	1.63	1.99	2.38	2.16
StErr		3.98	2.10	1.63	1.23	1.00	1.02	0.88	0.82	0.71	0.66	0.57	0.78	0.80
4	Hyp-C	15.86	2.18	1.83	1.93	1.83	1.58	1.39	1.24	1.20	0.95	0.81	0.73	0.72
11		24.80	14.41	8.20	5.94	4.19	3.91	3.68	3.56	3.66	3.72	3.73	3.58	3.84
12		17.48	6.53	4.89	3.89	2.77	3.42	3.55	3.69	3.49	4.35	4.26	4.28	3.89
15		22.11	12.83	12.18	11.66	16.02	11.50	9.20	6.60	6.57	7.61	9.59	7.59	6.14
20		25.84	15.93	13.50	11.55	9.67	7.74	6.81	6.17	5.60	5.36	5.12	4.72	4.89
35		29.90	18.28	15.36	13.56	12.25	11.31	10.23	9.78	9.37	9.50	8.75	9.33	9.15
Mean		22.67	11.69	9.33	8.09	7.79	6.58	5.81	5.17	4.98	5.25	5.38	5.04	4.77
StErr		2.16	2.50	2.15	1.96	2.34	1.73	1.43	1.22	1.16	1.23	1.34	1.24	1.14
7	Hyp-Cr	8.55	3.80	3.04	2.63	2.07	1.61	1.36	1.28	1.19	1.13	1.10	1.08	1.04
8		6.90	1.75	1.01	0.80	0.66	0.47	0.41	0.41	0.36	0.34	0.32	0.34	0.32
9		50.35	31.10	22.94	18.75	15.21	13.81	13.31	12.76	12.09	11.76	11.42	11.93	11.95
13		65.87	36.79	27.27	21.46	17.09	14.94	13.79	13.07	12.46	12.09	11.63	11.19	11.09
23		40.77	18.38	12.45	11.17	8.36	6.83	5.90	5.09	4.73	4.32	4.01	3.75	3.50
26		18.41	5.27	2.92	2.05	1.74	1.68	1.82	1.61	0.92	1.72	1.66	0.93	1.16
Mean		31.81	16.18	11.61	9.48	7.52	6.56	6.10	5.70	5.29	5.23	5.02	4.87	4.84
StErr		9.87	6.14	4.60	3.70	2.95	2.63	2.48	2.37	2.30	2.19	2.12	2.17	2.16

Sol	Stim	Time (s)	Force of contractions (g)																	
Rat #	Group	0	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180
19	Con-C																			
22		46.71	32.88	28.07	21.40	15.31	11.11	8.82	6.69	5.39	4.45	3.75	3.14	2.69	2.42	2.04	1.94	1.96	1.80	1.71
24		54.03	50.69	43.27	38.22	24.07	17.53	12.76	8.70	7.00	4.33	4.26	4.93	4.41	4.69	7.19	7.55	3.99	3.37	2.86
27		31.40	21.14	19.98	15.17	13.16	9.31	5.95	4.34	3.61	2.79	3.21	2.61	1.87	1.63	1.04	1.20	1.09	0.84	0.70
32		39.20	3.03	2.23	1.85	1.48	1.21	0.90	0.92	0.78	0.55	0.52	0.41	0.53	0.62	0.30	1.97	0.66	0.89	0.83
36		36.75	31.68	25.91	18.56	18.56	9.82	8.09	6.14	4.84	4.88	4.29	3.73	2.99	2.68	2.60	1.95	1.96	1.83	1.52
Mean		41.62	27.88	23.89	19.04	14.52	9.80	7.30	5.36	4.32	3.40	3.21	2.96	2.50	2.41	2.63	2.92	1.93	1.75	1.52
StErr		3.96	7.82	6.64	5.85	3.74	2.60	1.94	1.31	1.04	0.80	0.70	0.75	0.64	0.67	1.21	1.17	0.57	0.46	0.39
10	Con-Cr	0.91	0.62	0.22	0.40	0.55	0.59	0.53	0.54	0.62	0.65	0.60	0.58	0.68	0.63	0.64	0.66	0.69	0.75	0.69
16		22.69	13.32	7.70	4.04	3.03	2.37	1.90	1.44	0.96	0.86	0.66	0.53	0.49	0.49	0.44	0.39	0.42	0.44	0.44
21		62.53	49.08	48.23	35.35	22.91	15.13	12.15	10.55	9.19	7.77	6.47	5.81	5.23	4.92	4.54	4.29	4.10	3.90	3.63
29		53.99	40.97	33.44	23.27	15.71	11.34	9.15	7.54	5.43	4.39	3.53	2.93	2.61	2.38	2.25	2.06	2.05	1.92	1.89
30		44.22	36.19	34.72	30.88	25.78	20.48	13.29	9.20	7.10	5.21	3.68	3.10	2.58	2.18	1.97	1.68	1.56	1.45	1.38
33		38.31	30.61	25.14	21.13	14.99	10.02	7.54	6.32	5.40	4.52	3.97	3.61	3.30	3.62	3.40	3.53	3.21	3.30	2.54
Mean		37.11	28.47	24.91	19.18	13.83	9.99	7.43	5.93	4.78	3.90	3.15	2.76	2.48	2.37	2.21	2.10	2.01	1.96	1.76
StErr		9.13	7.42	7.35	5.78	4.18	3.08	2.14	1.67	1.38	1.11	0.91	0.81	0.72	0.70	0.65	0.63	0.59	0.57	0.49
2	Hyp-C	63.98	59.71	60.10	60.42	37.24	29.07	24.87	21.63	21.15	20.27	22.24	22.98	24.16	25.54	26.20	28.62	31.20	30.83	32.01
3		71.74	65.38	68.32	45.77	26.15	18.57	13.51	10.68	9.60	9.00	8.29	8.87	7.32	6.99	6.81	6.04	5.52	5.65	5.82
5		45.72	35.07	29.95	24.48	18.60	15.16	13.20	12.13	10.73	9.71	8.91	8.21	7.42	7.63	7.96	7.83	8.57	5.49	4.90
6		1.79	2.65	1.02	1.97	2.19	2.37	2.29	2.61	1.55	1.29	1.09	0.93	0.86	0.55	0.56	0.73	0.72	0.75	0.58
18		44.54	35.61	34.68	34.74	28.36	22.78	20.05	18.67	18.03	17.53	17.89	14.27	13.51	12.80	12.32	12.51	12.22	12.48	12.95
31		63.71	57.75	41.17	21.82	15.96	13.69	12.20	10.54	8.85	7.67	6.67	5.85	5.45	4.87	5.35	4.78	3.62	3.57	3.53
Mean		45.50	39.29	35.03	25.76	18.25	14.51	12.25	10.93	9.75	9.04	8.57	7.63	6.91	6.57	6.60	6.38	6.13	5.59	5.56
StErr		10.35	9.56	9.75	8.30	4.93	3.70	3.14	2.75	2.86	2.83	3.17	3.11	3.32	3.56	3.62	4.03	4.48	4.49	4.72
1	Hyp-Cr	63.30	32.44	16.07	10.84	8.94	7.61	6.32	5.75	5.02	4.37	3.72	3.29	3.08	2.60	2.42	2.32	1.97	1.86	1.73
14		52.49	50.68	30.20	16.87	10.31	7.10	4.78	3.72	2.95	2.36	2.25	2.14	1.94	1.98	2.04	2.36	2.91	3.76	4.24
17		28.17	21.17	17.33	11.77	7.96	5.40	4.50	3.17	3.12	2.99	2.84	2.55	2.14	1.95	2.11	2.11	2.81	3.42	3.68
25		66.19	45.17	40.68	32.64	24.46	18.83	14.20	12.05	10.61	9.10	8.50	7.32	7.68	7.23	6.66	6.11	5.85	5.76	5.89
28		58.11	56.15	43.99	31.04	19.56	12.48	9.43	9.01	9.85	10.34	9.53	7.91	7.47	6.90	6.54	6.01	5.59	5.70	5.67
34		69.69	55.49	43.19	31.25	23.53	21.02	18.80	17.51	16.76	16.07	15.41	16.45	14.12	13.40	12.42	11.87	12.56	6.99	4.40
Mean		56.33	43.52	31.91	22.40	15.79	12.07	9.67	8.54	8.05	7.54	7.04	6.61	6.07	5.68	5.37	5.13	5.28	4.58	4.27
StErr		6.15	5.71	5.22	4.22	3.10	2.68	2.35	2.26	2.20	2.17	2.08	2.21	1.92	1.83	1.66	1.55	1.59	0.77	0.62

Sol	Recov	Time (s)	Froce of contractions (g)											
Rat #	Group	0	10	20	30	40	50	60	70	80	90	100	110	120
19	Con-C													
22		28.01	14.12	5.26	2.88	2.00	1.63	1.63	1.41	1.14	1.07	0.88	1.01	1.01
24		36.43	22.69	13.32	11.43	3.98	2.77	2.09	2.41	1.59	1.56	2.00	2.01	1.78
27		12.13	2.39	1.16	0.69	0.29	0.25	0.28	0.17	0.25	0.20	0.23	0.10	0.17
32		2.20	3.09	2.07	1.27	0.93	0.75	0.76	0.82	0.85	0.95	0.78	0.72	0.80
36		20.90	8.85	5.60	3.53	2.60	2.04	1.51	1.34	1.26	1.12	1.01	0.98	0.96
Mean		19.93	10.23	5.48	3.96	1.96	1.49	1.25	1.23	1.02	0.98	0.98	0.96	0.94
StErr		5.97	3.77	2.14	1.94	0.65	0.45	0.32	0.37	0.23	0.22	0.29	0.31	0.26
10	Con-Cr	0.76	0.50	0.69	0.70	0.76	0.59	0.63	0.62	0.62	0.59	0.68	0.67	0.63
16		10.06	5.89	3.51	2.31	1.96	2.52	1.74	1.81	1.83	1.57	1.03	0.84	0.55
21		43.01	13.95	7.18	5.18	4.94	4.31	5.20	4.92	4.75	4.81	5.22	5.06	5.06
29		21.76	11.31	5.02	3.38	2.43	1.87	1.59	1.44	1.39	1.40	1.37	1.36	1.36
30		29.64	12.78	6.46	2.61	1.76	1.44	1.24	1.19	1.15	1.07	1.08	1.07	1.03
33		22.16	19.14	12.62	8.79	6.35	4.90	4.22	3.80	3.34	3.12	2.85	2.62	2.69
Mean		21.23	10.60	5.91	3.83	3.03	2.61	2.44	2.30	2.18	2.09	2.04	1.94	1.89
StErr		6.03	2.67	1.64	1.16	0.87	0.69	0.75	0.69	0.64	0.65	0.71	0.69	0.71
2	Hyp-C	56.87	55.38	46.68	38.52	35.39	35.76	36.43	36.36	36.36	36.46	36.62	36.47	36.10
3		72.80	25.44	6.72	3.13	3.51	4.68	5.18	4.88	5.21	5.41	4.70	4.42	5.33
5		24.49	16.50	11.50	8.62	7.56	7.43	7.07	6.78	6.69	6.53	6.21	6.01	6.01
6		2.69	1.03	2.06	2.62	2.26	1.88	1.41	1.38	1.74	1.48	1.13	1.20	1.22
18		31.51	24.85	17.94	15.61	13.91	13.83	14.45	15.58	16.97	17.84	18.46	18.62	18.45
31		28.60	4.65	3.01	2.36	2.07	1.88	1.87	1.81	1.57	1.51	1.46	1.47	1.46
Mean		32.02	14.49	8.25	6.47	5.86	5.94	6.00	6.09	6.44	6.55	6.39	6.34	6.49
StErr		10.18	7.96	6.84	5.74	5.25	5.29	5.43	5.47	5.49	5.55	5.66	5.66	5.56
1	Hyp-Cr	16.35	1.43	0.93	0.74	0.66	0.59	0.55	0.49	0.45	0.46	0.44	0.34	0.34
14		64.04	36.48	16.84	12.56	11.18	10.18	9.06	8.34	7.85	7.57	7.48	7.85	7.48
17		11.88	3.72	3.22	2.09	1.37	1.03	1.37	2.41	3.08	3.07	2.84	2.45	2.26
25		27.14	13.40	13.25	7.56	6.40	5.87	5.37	5.30	4.74	4.49	3.79	3.85	3.52
28		25.39	13.07	7.59	6.06	5.72	5.62	5.49	5.27	5.50	5.70	5.58	5.61	5.49
34		38.28	24.46	18.75	15.45	13.82	13.11	12.61	12.28	12.03	12.05	12.14	12.22	12.01
Mean		30.51	15.43	10.10	7.41	6.53	6.07	5.74	5.68	5.61	5.56	5.38	5.39	5.18
StErr		7.68	5.38	2.99	2.35	2.13	2.02	1.87	1.72	1.63	1.63	1.67	1.72	1.70

REFERENCES

Balsom, P.D., Harridge, S.D.R., Soderlund, K., Sjodin, B. & Ekblom, B. (1993) Creatine supplementation per se does not enhance endurance exercise performance. Acta Physiol Scand, 149:521-523.

Balsom, P.D., Soderlund, K. & Ekblom, B. (1994) Creatine in humans with special reference to creatine supplementation. Sports Medicine, 18:268-280.

Balsom, P.D., Soderlund, K., Sjodin, B. & Ekblom, B. (1995) Skeletal muscle metabolism during short duration high intensity exercise: influence of creatine supplementation. Acta Physiol Scand, 154:303-310.

Barrnett, C., Hinds, M., & Jenkins, D.G. (1996) Effects of oral creatine supplementation on multiple sprint cycle performance. The Australian Journal of Science and Medicine in Sport, 28:35-39.

Bessman, S.P. & Carpenter, C.L. (1985) The creatine-creatine phosphate energy shuttle. Annual Reviews in Biochemistry, 54:831-62.

Birch, R., Noble, D. & Greenhaff, P.L. (1994) The influence of dietary creatine supplementation on performance during repeated bouts of maximal isokinetic cycling in man. European Journal of Applied Physiology, 69:268-270.

Brannon, T.A., Adams, G.R., Conniff, C.L. & Baldwin, K.M. (1997) Effects of creatine loading and training on running performance and biochemical properties of rat skeletal muscle. Medicine and Science in Sports and Exercise 29:489-495.

Burke, L.M., Pyne, D.B. & Telford, R.D. (1996) Effects of oral creatine supplementation on single-effort sprint performance in elite swimmers. International Journal of Sports Nutrition, 6:222-233.

Cain, D.F. & Davies, R.E. (1962) Biochemistry and Biophysiology Research Communications, 8: 361-366.

Casey, A., Constantin-Teodosiu, D., Howell, S., Hultman, E. & Greenhaff, P.L. (1996) Creatine ingestion favorably affects performance and muscle metabolism during maximal exercise in humans. American Journal of Physiology, 271:E31-E37.

Cooke, W.H. & Barnes, W.S. (1997) The influence of recovery duration on high intensity exercise performance after oral creatine supplementation. Canadian Journal of Applied Physiology, 22:454-467.

Cooke, W.H., Grandjean, P.W. & Barnes, W.S. (1995) Effect of oral creatine supplementation on power output and fatigue during bicycle ergometry. Journal of Applied Physiology, 78:670-673.

Delanghe, J., De Slypere, J., De Buyzere, M., Robbrecht, J., Wieme, R. & Vermeulen, A. (1989) Normal reference values for creatine, creatinine and carnitine are lower in vegetarians. Clinical Chemistry, 35:1802-1803.

Earnest , C.P., Snell, P.G., Rodriguez, R., Almada, A.L. & Mitchell, T.L. (1995) The effect of creatine monohydrate ingestion on anaerobic power indices, muscular strength and body composition. Acta Physiol Scand, 153:207-209.

Febbraio, M.A., Flanagan, T.R., Snow, R.J., Zhao, S. & Carey, M.F. (1995) Effect of creatine supplementation on intramuscular TCr, metabolism and performance during intermittent, supramaximal exercise in humans. Acta Physiol Scand, 155:387-395.

Fiske, C.H. & Subbarow, Y. (1929) Science, 70: 381-83

Fitch, C.D. & Chevli, R. (1980) Inhibition of creatine and phosphocreatine accumulation in skeletal muscle and heart. Metabolism, 29:686-90.

Fitch, C.D., Chevli, R., Petrofsky, J.S. & Kopp, S.J. (1978) Sustained isometric contraction of skeletal muscle depleted of phosphocreatine. Life Sciences, 23:1285-92.

Fitch, C.D., Jellinek, M., Fitts, R.H., Baldwin, K.M. & Holloszy, J.O. (1975) Phosphorylated β-guanidinopropionate as a substitute for phosphocreatine in rat muscle. American Journal of Physiology, 228:1123-25.

Fitts, R.H. (1994) Cellular mechanisms of muscle fatigue. Physiological Reviews, 74:49-94.

Green, A.L., Simpson, E.J., Littlewood, J.J., Macdonald, I.A. & Greenhaff, P.L. (1996) Carbohydrate ingestion augments creatine retention during creatine feeding in humans. Acta Physiol Scand, 158:195-202.

Greenhaff, P.L. (1995) Creatine and its application as an ergogenic aid. International Journal of Sport Nutrition, 5:S100-S110.

Greenhaff, P.L., Bodin, K., Harris, R.C., Hultman, E., Jones, D.A., McIntyre, D.B., Soderlund, K. & Turner, D.L. (1993) The influence of oral creatine supplementation on muscle phosphocreatine resynthesis following intense contraction in man. Journal of Physiology, 467:75P.

Greenhaff P.L., Bodin, K., Soderlund, K. & Hultman, E. (1994) Effect of oral creatine supplementation on skeletal muscle phosphocreatine resynthesis. American Journal of Physiology, 266:E725-E730.

Greenhaff, P.L., Casey, A., Short, A.H., Harris, R., Soderlund, K. & Hultman, E. (1993) Influence of oral creatine supplementation of muscle torque during repeated bouts of maximal voluntary exercise in man. Clinical Science, 84:565-571.

Grindstaff, P.D., Kreider, R., Bishop, R., Wilson, M., Wood, L., Alexander, C. & Almada, A. (1997) Effects of creatine supplementation on repetitive sprint performance and body composition in competitive swimmers. International Journal of Sports Nutrition, 7:330-346.

Haff, G.G. & Potteiger, J.A. (1997) Creatine supplementation for the strength/power athlete. Strength and Conditioning, Dec: 72-74.

Harris, R.C., Edwards, R.H.T., Hultman, E., Nordesjo, L.O., Nylind, B. & Sahlin, K. (1976) The time course of phosphorylcreatine resynthesis of the quadriceps muscle in man. Pflugers Archives, 367:137-142.

Harris, R.C., Hultman, E. & Nordesjo, L.O. (1974) Glycogen, glycolytic intermediates and high-energy phosphates determined in biopsy samples of musculus quadriceps femoris of man at rest. Methods and variance of values. Scandinavian Journal of Clinical Laboratory Investigations, 33:109-120.

Harris, R.C., Soderlund, K. & Hultman, E. (1992) Elevation of creatine in resting and exercised muscle of normal subjects by creatine supplementation. Clinical Science, 83:367-374.

Harris, R.C., Viru, M., Greenhaff, P.L. & Hultman, E. (1993) The effect of oral creatine supplementation on running performance during maximal short term exercise in man. Journal of Physiology, 467:74P.

Hill, D.K. (1960) Journal of Physiology, 153: 433-446.

Hirvonen, J., Rehunen, S., Rusko, H. & Harkonen, M. (1987) Breakdown of high-energy phosphate compounds and lactate accumulation during short supramaximal exercise. European Journal of Physiology, 56:253-259.

Hultman, E., Bergstrom, J & McLennan Anderson, N. (1967) Breakdown and resynthesis of phosphorylcreatine and adenosine triphosphate in connection with muscular work in man. Scandinavian Journal of Clinical Laboratory Investigations, 19:56-66.

Hultman, E., Greenhaff, P.L., Ren, J. & Soderlund, K. (1990) Energy metabolism and fatigue during intense muscle contraction. Biochemical Society Transactions, 19:347-353.

Hultman, E. Soderlund, K. Timmons, J.A., Cederblad, G. & Greenhaff, P.L. (1996) Muscle creatine loading in men. Journal of Applied Physiology, 81:232-237.

Jacobs, I., Bleue, S. & Goodman, J. (1997) Creatine ingestion increases anaerobic capacity and maximum accumulated oxygen deficit. Canadian Journal of Physiology, 22:231-243.

Jacobsen, F.K., Christensen, C.K., Mogensen, C.E., Andreasen, F. & Heilskov, N.S.C. (1979) Pronounced increase in serum creatinine concentration after eating cooked meat. British Medical Journal, April 21:1049-1050.

Juhn, M.S. (1999) Oral creatine supplementation, Separating fact from hype. The Physician and Sportsmedicine, 27(5): 47-56.

Kandarian, S.C. & White, T.P. (1990) Mechanical deficit persists during long-term muscle hypertrophy. Journal of Applied Physiology, 69:861-867.

Kandarian, S.C., Young, J.C. & Gomez, E.E. (1992) Adaptation in synergistic muscles to soleus and plantaris muscle removal in the rat hindlimb. Life Sciences, 51:1691-1698.

Krieder, R.B. (1995) The effect of creatine loading on muscular strength and body composition. Strength and Conditioning, Oct:72-73.

Krieder, R.B., Ferreira, M., Wilson, M., Grindstaff, P., Plisk, S., Reinardy, J., Cantler, E. & Almada, A.L. (1998) Effects of creatine supplementation on body composition, strength, and sprint performance. Medicine & Science in Sports & Exercise, 30:73-82.

Lipmann, F. & Meyerhof, O. (1930) Biochemistry, 227: 84-109.

McNaughton, L.R., Dalton, B. & Tarr, J. (1998) The effects of creatine supplementation on high-intensity exercise performance in elite performers. European Journal of Applied Physiology, 78:236-240.

Meryerhof, O. & Lohmann, K. (1932) Biochemistry, 252: 431-461.

Mommaerts, W.F.H.M. (1954) Nature, 174: 1083.

Mujika, I., Chatard, J., Lacoste, L., Barale, F. & Geyssant, A. (1996) Creatine supplementation does not improve sprint performance in competitive swimmers. Medicine and Science in Sports and Exercise, 28:1435-1441.

Mujika, I. & Padilla, S. (1997) Creatine supplementation as an ergogenic aid for sports performance in highly trained athletes: A critical review. International Journal of Sports Medicine, 18:491-496.

Õõpik, V., Pääsukem, M., Timpmann, S., Medijainen, L., Erelin, J. & Smirnova, T. (1998) Effect of creatine supplementation during rapid body mass reduction on

metabolism and isokinetic muscle performance capacity. Eurpoean Journal of Applied Physiology, 78:83-92.

Perry, S.V. (1954) Biochemistry Journal, 57: 427-434.

Plisk, S.S. & Kreider, R.B. (1999) Creatine Controversy? Strength and Conditioning Journal, 21(1): 14-23.

Poortmans, J.R., Auquier, H. & Renaut, V. (1997) Effect of short-term creatine supplementation on renal responses in men. European Journal of Applied Phsiology, 76: 566-567.

Redondo, D.R., Dowling, E.A., Graham, B.L., Almada, A.L. & Williams, M.H. (1996) The effect of oral creatine monohydrate supplementation on running velocity. International Journal of Sport Nutrition, 6: 213-221.

Rossiter, H.B., Cannell, E.R. & Jakeman, P.M. (1996) The effect of oral creatine supplementation on the 1000-m performance of competitive rowers. Journal of Sports Sciences, 14:175-179.

Sahlin, K., Tonkonogi, M. & Soderlund, K. (1998) Energy supply and muscle fatigue in humans. Acta Physiol Scand, 162:261-266.

Sapila, I., Rapola, J., Simell, O. & Vamas, A. (1981) Supplementary creatine as a treatment for gyrate atrophy of the choroid and retina. New England Journal of Medicine, 304: 867-870.

Smith, J.C., Stephens, D.P., Hall, E.L., Jackson, A.W. & Earnest, C.P. (1998) Effect of oral creatine ingestion on parameters of the work rate-time relationship and time to exhaustion in high-intensity cycling. European Journal of Applied Physiology, 77: 360-365.

Stroud, M.A., Holliman, D., Bell, D., Green, A.L., Macdonald, I.A. & Greenhaff, P.L. (1994) Effect of oral creatine supplementation on respiratory gas exchange and blood lactate accumulation during steady-state incremental treadmill exercise and recovery in man. Clinical Science, 87:707-710.

Terrillion, K.A., Kolkhorst, F.W., Dolgener, F.A. & Joslyn, S.J. (1997) The effect of creatine supplementation on two 700-m maximal running bouts. International Journal of Sport Nutrition, 7: 138-143.

Tesch, P.A., Thorsson, A. & Fujitsuka, N. (1989) Creatine phosphate in fiber types of skeletal muscle before and after exhaustive exercise. Journal of Applied Physiology, 66:1756-1759.

Timson, B.F. (1990) Evaluation of animal models for the study of exercise-induced muscle enlargement. Journal of Applied Physiology, 69:1935-1945.

Vandenberghe, K., Gillis, N., Van Leemputte, M., Van Hecke, P., Vanstapel, F. & Hespel, P. (1996) Caffeine counteracts the ergogenic action of muscle creatine loading. Journal of Applied Physiology, 80:452-457.

Vandenberghe, K., Van Hecke, P., Van Leemputte, M., Vanstapel, F. & Hespel, P. (1999) Phosphocreatine resynthesis is not affected by creatine loading. Medicine and Science in Sports and Exercise, 31: 236-242.

Westerblad, H., Lee, J.A., Lannergren, J. & Allen, D.G. (1991) Cellular mechanisms of fatigue in skeletal muscle. American Journal of Physiology, 261:C195-C209.

Young , J.C. & Balon, T.W. (1997) Role of dihydropyridine sensitive calcium channels in glucose transport in skeletal muscle. Life Sciences, 61:335-342.

Young, J.C., Kandarian, S.C. & Kurowski, T.G. (1992) Skeletal muscle glucose uptake following overload-induced hypertrophy. Life Sciences, 50:1319-1325.

VITA

Kinesiology
University of Nevada, Las Vegas

Robert Earl Young

Home Address:
1924 Magnolia Dr
Henderson, NV 89014

Degrees:
Bachelor of Science, Kinesiology, 1997
University of Nevada, Las Vegas

Thesis Title: The Effect of Dietary Creatine Supplementation on Compensatory Muscle Hypertrophy and Performance Characteristics of Specific Fiber Types.

Thesis Examination Committee:
Chairperson, Dr. Lawrence A. Golding, Ph. D.
Committee Member, Dr. Richard Tandy, Ph. D.
Committee Member, Dr. Carl Reiber, Ph. D.
Committee Member, Dr. Susan Meacham, Ph. D.
Graduate Faculty Representative, Dr. Bryan Spangelo, Ph. D.