TIME COURSE OF CHANGES IN NEUROMUSCULAR FUNCTION DURING AND FOLLOWING CREATINE LOADING

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ABSTRACT

Eric J Sobolewski: The Time Course of Changes in Neuromuscular Function during and following Creatine Loading
(Under the Direction of Eric D. Ryan)

The purposes of the present study were to determine the effect of Creatine (Cr) supplementation on 1) voluntary isometric and isokinetic strength and 2) active and passive range of motion (ROM) during loading and the subsequent washout period. The secondary purpose was to address any possible underlying neuromuscular mechanisms that might influence these changes. Using a double-blinded, placebo-controlled, matched-paired, randomized design, 40 males were assigned to a Cr (n=20, age: 20.3 ± 2.1 yrs,) or a placebo group (Pl; 20.4 ± 2.3 yrs) group. Participants supplemented four times daily for five days with 5g Cr + 20g dextrose or 20g dextrose. Testing was conducted prior to supplementation, during the loading phase (days 2, 4 & 6), and during the washout period (day 20 & 35). Muscular strength was examined with a maximal isometric and isokinetic (30, 90 & 120°·sec^{-1}) muscle action of the plantar flexors on a calibrated isokinetic dynamometer. Muscle activation was determined by examining percent voluntary activation (%VA) and normalized electromyographic (EMG) amplitudes. Passive ROM , common and relative passive stiffness values were determined from a slow passive ROM assessment on a dynamometer. Active ROM was determined as the maximal dorsiflexion possible. Total body water, extracellular water, and intracellular water were measured with Bioimpedance Spectroscopy. Panoramic ultrasound imaging was used to address architectural changes in muscle cross sectional area, pennation angle, and fascicle length. Resting evoked twitch properties were performed to examine Cr-induced changes in Ca^{2+} kinetics. Magnetic

resonance spectroscopy (MRS) was used to evaluate Cr uptake by the muscle in a subset of participants (Cr = 4; Pl = 4). Mass and fluid distribution for the Cr group did not significantly ($P \geq 0.11$) differ from the Pl group over the course of the study. There was no significant interactions for all strength measures ($P \geq 0.36$) or active and passive ROM ($P \geq 0.15$), or any of the possible underlying mechanism ($P > 0.05$). There were no changes in Cr stores ($P > 0.05$). Overall, the results of this study suggest that Cr supplementation alone does not influence neuromuscular function or any of the underlying factors that could improve strength or limit ROM.

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CHAPTER I

INTRODUCTION

Physiological Role of Creatine

Creatine (Cr) is one of the most popular ergogenic aids on the market. Its popularity could be contributed to the large number of studies demonstrating its positive influence on high intensity exercise performance.[102] Cr is a naturally occurring organic compound usually consumed in the diet as it is found in meat and fish. Cr is also synthesized in the liver and pancreas from the amino acids glycine, arginine, and methionine.[109] The majority of Cr (95%) is stored in skeletal muscle, with the remaining 5% found in the heart, brain and testes. Cr is primarily (~60%) found bound to inorganic phosphate in the form of phosphocreatine (PCr) with the remaining 40% stored as the free form of Cr. Cr's role in metabolism was first discovered by Hutlman et al,[49] who identified Cr and its ability to re-phosphorylate adenosine diphosphate (ADP) to adenosine triphosphate (ATP) via the creatine kinase reaction. It was not until 1992 when Harris et al[42] demonstrated Cr supplementation could increase muscle Cr stores both as PCr and free form Cr. Since this ground breaking study, Cr research has further evaluated the possible implications of Cr supplementation as an ergogenic aid.

Dietary supplementation with Cr has been shown to increase PCr stores in muscle, increasing the cells ability to re-phosphorylate ATP.[38,63,84] Previous authors have suggested that Cr may have many other positive functions inside the cell which may be influenced by Cr supplementation. For example, Cr along with PCr act as a buffer by shuttling ATP from inside

the mitochondria to the cytosol,[74] and during times of low pH, PCr and ADP buffer hydrogen ions (H^+) by the formation of Cr and ATP.[83] Cr may also have additional metabolic effects due to its potential involvment in regulating glycolysis.[81] For example, low levels of Cr promote the release of oxidative enzymes that in turn increase glycolysis. Cr may also indirectly increase protein synthesis. An increase in Cr concentrations through the breakdown of PCr to Cr during muscle contractions has been shown to stimulate protein synthesis.[50] Swelling of muscle cells has also been suggested to increase protein synthesis.[43] Since Cr has known osmotic properties and results in the cells pulling water from the extracellular fluid into the cells causing them to swell,[9] it is likely this may lead indirectly to increases in protein synthesis. Lastly, Cr in the form of PCr acts as a membrane stabilizer by binding to the phospholipid heads thus not allowing for the cytoplasmic contents to dissipate through the cell wall which could lead to an increase in cell size.[93]

The influence of creatine supplementation on muscle creatine stores

Traditionally, Cr is "loaded' by consuming 20 g a day for the first five days followed by a maintenance dose of 3-5 g per day.[62,94,106,107] The "loading" of Cr in the system is done to rapidly saturate muscle cells with Cr.[26] In the classic study by Harris et al,[42] Cr stores increased in as little as two days during loading and by the fifth day had increased by more than 20% from pre values. The maintenance dosage was determined by Hultman et al[48] who compared a loading period followed by either the cessation of Cr supplementation or two grams for the next 28 days. They observed a steady decline in Cr stores following cessation, but Cr levels remained elevated through 35 days with the two gram/day dosage. Cr may also be taken without loading (2-5 $g \cdot day^{-1}$) but in order to saturate the muscle it may take up to 14 days.[48] The results of these studies suggest that Cr stores can be rapidly increased (20-40%)[26,41,48,63,87,88] during the

traditional Cr loading period or gradually increased with lower dosages[48,89] and once saturated, Cr stores can be maintained with 3-5 g·day^{-1}.

The influence of creatine supplementation on performance

The most commonly supplemented form of Cr is Cr monohydrate, consumed by a variety of athletes ranging from high school football players to professional track athletes.[61,97,102] A recent review[97] has suggested that the prevalence of Cr usage is greatest in elite power lifters and high school and college-aged football players. Cr supplementation improves maximal strength,[5,6,45,58] vertical jump height,[60] sports specific skills,[54,82] running capacity,[63,77] training volume[30] and delays fatigue.[89,96,99] The performance enhancing effects of Cr vary depending the type of performance (strength vs endurance) and if training is coupled with supplementation. The majority of the research[62] examining the effects of Cr supplementation on performance include supplementation protocols lasting between 5-28 days[39,42,48,70,89,107] and is often combined with training. These increases in performance have been attributed to the ergogenic effects of Cr and overall greater training volume.[108] In theory, if a more readily available energy source is present, this will allow for greater training loads and volumes; which may subsequently improve performance. For example, a meta-analysis by Branch et al[13] has indicated Cr supplementation improves work capacity (ES: 0.29), force output (0.29), 1-RM strength (0.32), power output (0.20) and sprint times (0.36). However, it is important to note that fewer studies have examined Cr supplementation without training. Cr supplementation alone has demonstrated improvements in 1-RM[22,33,100,108,110], isokinetic[5,39,55,90] and isometric[34,70,72,103] strength, sprint times,[17,24] vertical jump height,[51] and power[116]. However, future work is needed to fully understand the potential changes in neuromuscular function that may occur following Cr supplementation.

The influence of creatine supplementation on neuromuscular function

Fewer studies have examined the influence of Cr supplementation on neuromuscular function[5,52,72,95,104] without training. Collectively, the body of literature has reported that Cr loading does not improve voluntary isometric strength of the bicep brachii,[7,52,53,104] plantarflexors,[95] or leg flexors,[7,98] except for the Medeiros et al[72] who observed a 7% increase in leg extension strength. The discrepancy between the changes in isometric strength may be explained in the different muscles being tested, as larger muscles have been shown to respond better to Cr supplementation mainly due to their larger mass and demand for Cr.[103] Cr supplementation does however appear to enhance isokinetic strength at fast velocities (180 and 240 °/sec).[5,39,55] Previous authors have suggested that improvements in voluntary strength following Cr supplementation may be due to (1) increases in muscle activation, (2) increases in intracellular water (ICW) content which may increase muscle size[102] potentially stimulating protein synthesis,[103] and/or altering pennation angles,[52] and (3) Ca^{2+} kinetics that may improve as Cr buffers ADP allowing for Ca^{2+}-ATPase to improve actin-myosin unbinding.[5,104] Ca^{2+} sensitivity may also improve with an increase in ICW as it requires less Ca^{2+} to produce the same force in a reduced ion environment.[78] Ca^{2+} kinetics can be indirectly evaluated during evoked twitch properties (peak twitch torque and half-relaxation time).

Muscle activation has been reported to be improved with Cr supplementation as Mederios et al. [72] observed by an increase in surface EMG amplitude of the leg extensors following Cr loading. However, although they attributed the increase in EMG amplitude to an improved neural drive, they did not provide any details regarding their normalization procedures. On the

contrary, other studies[52,104] have indicated Cr loading does not improve percent voluntary activation, but may improve conduction velocity[104] through recruiting faster motor neurons or facilitating the uptake of neurotransmitters.[5] Bazzucchi et al.[5] previously demonstrated that five days of Cr loading improved the torque-angular velocity curve, mean fiber conduction velocity at all angular velocities, electrically induced peak twitch force, and the time to reach peak twitch force. However, Smith-Ryan et al[95] reported no changes in peak torque (PT), peak rate of torque development (PRTD) and half relaxation time (HRT) following five days of supplementation in women. Bazzuchi et al.[5] speculated that the change in neuromuscular function after Cr supplementation was contributed to the increased cross bridge cycling rate. It is possible that more readily available ATP, mainly due to the enhanced PCr energy system, increases the Ca^{2+}-ATPase pump therefore increasing cycling rates.[5,104] These changes may result in a faster detachment of actomyosin bridges increasing the capacity to produce force rapidly.[5] The results are still inconclusive since other studies[52,95,104] have found no significant changes in isometric strength, relaxation time, or rate of force development. It is speculated that Ca^{2+} kinetics maybe dependent on the load and type of stimulus[32], thus in an isometric contraction; where cross bridge cycling rate is not a limiting factor, may not be affected.[85] This would explain why Bazzucchi et al. [5] observed improvements in isokinetic strength and not isometric strength following Cr loading. Additional mechanisms for Cr induced increases in performance may be the increase in muscle size[102] and architectural changes.[52] For example, Terjung et al[102] speculated that "if Cr supplementation was to enlarge muscle volume and this in turn could in some manner alter the force/power development; there could be a real enhancement in muscle performance." Jakobi et al[52] also suggests that with the increase in cell size from swelling, pennation angles could be altered and potentially improve force production. These findings

highlight the need for future studies to further evaluate the influence of Cr loading on neuromuscular function.

The influence of creatine supplementation on range of motion and stiffness

Even less research has addressed the effect of Cr loading on the extensibility of muscle. The only study to date that has examined the influence of Cr loading on flexibility is a recent paper by Sculthorpe et al.[92] They addressed Cr loading on active range of motion and concluded that Cr loading limits range of motion during shoulder extension and abduction and ankle dorsiflexion movements. The authors attributed the decreases in flexibility to increases in cell size as a result of an increase in intracellular water (ICW) content. The authors speculate that the increase in cell size may have led to increases in muscle stiffness and/or altered muscle spindle neural outflow. In theory, altered muscle spindles would result in the stretch reflex being initiated earlier in the range of motion and potentially decrease maximum range of motion. Cr has the potential to bring water into the cell via its osmotic properties and restrain cytoplasm in the cell due to the membrane stabilization of PCr. This increase in cell size is commonly thought to reflect the increase in body mass (1 – 3 kg) seen during Cr loading.[97] This increase in body mass is most likely due to the Cr induced changes in total body water (TBW). The majority of literature[28,56,57,86,97,112,114] attributes the increase in TBW to increases in intracellular water (ICW) credited to Cr pulling water into the cell. Another possible explanation attributes the weight gain to an increase in dry matter growth.[28,31] In theory, increases in muscle cell volume from increases in ICW or dry matter would result in a change in muscle viscosity and/or stiffness. This was previously suggested by Grazi et al.[35,36] who noted that any increase in osmotic pressures will lead to increases in viscosity and stiffness. Thus, it is possible that if Cr supplementation

alters ICW, there would be an increase in passive stiffness which may lead to a decreased range of motion and increased risk of soft tissue injury.[113]

We are aware of only one study that has examined changes in stiffness following Cr loading. Watsford et al[111] measured active stiffness using the oscillation technique with loads ranging from 50-200% of body weight, and found no significant increases in stiffness. This could be due to the variability of how the oscillation technique is performed and/or the stiffness contributed to muscle cell volume does not play a role in active stiffness. Recent studies [91] have employed techniques utilizing powered dynamometers and surface electromyographic measures to examine passive stiffness and maximal passive range of motion (ROM). EMG onset (point of which muscle activity becomes detachable during a passive stretch)[11] is believed to indicate muscle spindle activity in response to excessive stretch or load. If the stretch reflex is altered due to increases in muscle size as speculated by Sculthorpe et al,[92] it is possible EMG onset may occur earlier during a passive ROM assessment following Cr loading. Overall, passive stiffness and passive ROM have not been evaluated after Cr loading. The limited research addressing changes in viscosity and stiffness does not allow for a conclusion to be made if the osmotic properties of Cr influence ROM and stiffness. Furthermore, Sculthorpe et al. [92] and Watsford et al [111] failed to determine if changes in fluid distribution actually occurred and if these changes may account for the alterations in ROM and/or stiffness. Recent studies have demonstrated that bioelectrical impedance spectroscopy (BIS) is a valid assessment of total body water (TBW) and extracellular water [20,66,76] (r= 0.80-.97) and can be used to examine changes in fluid distribution during Cr supplementation.[114] To further evaluate the possible changes in muscle cell size as a result of Cr supplementation, non-invasive diagnostic tools have been utilized. For example, using magnetic resonance imaging (MRI), Ziegenfuss et al [116] reported muscle volume increased

after just three days of Cr loading and attributed these changes to an increase in TBW. Ultrasonography has been reported to be a valid and reliable measure of muscle size.[1] Thus, ultrasound measurements may offer a unique alternative to MRI techniques to examine the changes in muscle size during Cr supplementation.

Time Course

Research would indicate when the muscle is fully saturated with Cr[38,42] and when performance is improved[5,55,64] may occur at different time points. For example, Harris et al, [42] demonstrated that only two days of Cr loading was needed to increase Cr stores by 20% and improve maximal leg extension torque production. However, Eckerson et al [23] and Law et al [65] demonstrated that two days of loading did not improve anaerobic work capacity or anaerobic power and back squat strength, respectively. Law et al [65] reported that a five day loading scheme was necessary to enhance strength and power. Kambis et al [55] reported increases in muscle performance (isokinetic contractions) just after five days of Cr supplementation. This would imply that the mechanisms that are responsible for the improvements in performance may have an adaptation period. There may be a difference from the time that Cr is introduced into the system and saturates the cells to when improvements in neuromuscular function are observed. Future research is need to simultaneously examine changes in muscle Cr concentrations and neuromuscular function. Traditionally Cr concentrations are measured using invasive muscle biopsies.[39,42,48,107] A potential non-invasive technique is proton magnetic resonance spectroscopy (MRS)[105] which has demonstrated to be a valid and reliable assessment of total Cr content in skeletal muscle.[12]

Another aspect that has not been fully addressed is the washout period, it has yet to be determined if the effects of Cr are permanent or have a residual effect that gradually declines after Cr supplementation is discontinued. To date, there has been limited research that has evaluated changes in performance observed during the washout period,[27,48,88] which would provide insight into the possible mechanisms affected by Cr and provide practical dosing strategies for practitioners and athletes. The fundamental study for the majority of the literature that addresses the washout period is by Hultman et al,[48] who loaded with Cr for six days then ceased supplementation and tracked muscle Cr levels for 35 days. Muscle total Cr concentrations (both PCr and free Cr) were measured with muscle biopsies of the vastus lateralis prior to Cr loading, immediately following loading (day 7), at day 21 and 35. Total Cr concentration was elevated following the loading period but then gradually declined until day 35 when Cr levels were no different than pre supplementation values. Another study that examined Cr content in skeletal muscle was by Rawson et al[88] who examined PCr levels in muscle using phosphate (31P) magnetic resonance spectroscopy prior to and following a five day loading and following a 30 day washout period. A 45% increase in PCr stores was observed following Cr loading, and after the 30 day washout period PCr levels remained 23% above pre-supplementation levels. The discrepancies between these studies maybe related to the type of Cr stores examined. For example, Hultman et al[48] measured both PCr and free Cr, and it appeared in their tables that free Cr was returning to baseline but PCr remained elevated even up to day 35. This would then be in line with Rawson et al[88] who indicated that PCr will remain elevated following Cr loading.

To date minimal research has addressed the washout period and it implication on performance. For example, Febbraio et al [27] examined one minute intermittent cycling

performance following Cr loading and a 28 day washout period. Despite increases in intramuscular creatine stores following Cr loading, supplementation had no influence on performance at either time point. This study found that after 28 days, total Cr levels returned to normal, but failed to shed any light on the washout effect on performance since loading was insufficient at increasing performance. Most research only addresses Cr loading and fails to monitor the time course effects that Cr may have, thus if the possible underlying mechanisms are affected we do not know if the results are permanent or if they gradually diminish as Cr is slowly returns to baseline values. Thus, the effects of Cr loading on neuromuscular function and the possible implication with the cessation of supplementing (washout) warrants further investigation.

Statement of purpose

Although Cr is one of the most studied supplements to date, some of the fundamental questions on how Cr influences performance are still left unanswered. The underlying mechanisms that are believed to be responsible for the increases in performance have failed to be determined by the literature. The current literature only speculates Cr's effect on neuromuscular function, ROM and stiffness. One common implication is the idea of an increase fluid retention and swelling of cells. Yet the majority of the research fails to measure water distribution and muscle Cr content. Therefore, the purpose of this study was to determine the effects of Cr supplementation on muscular strength and ROM during and following a traditional loading period and subsequent washout.

Specific Aims

Specific Aim 1: To determine the effect of Cr supplementation on voluntary isometric and isokinetic strength during a traditional loading period and the subsequent washout.

We hypothesize that isokinetic strength at the fastest velocity ($180^{o} \cdot s^{-1}$) will increase after five full days of Cr loading then return to baseline by the 35^{th} day. The increase in isokinetic strength will be accompanied by a decrease in evoked twitch half-relaxation time, none of the other variables will differ from that of those observed by the control group and will remain the same throughout the washout period.

Specific Aim 2: To determine the effects of Cr supplementation on active and passive ROM during a traditional loading period and the subsequent washout.

We hypothesize that active ROM will be reduced following five days of Cr supplementation and return to baseline by the 35^{th} day. These changes will be accompanied by an increase in TBW, ECW and ICW and subsequent increase in muscle size, however, passive ROM, stiffness, and the onset of EMG activity will not differ from that of those observed by the control group and will remain the same throughout the washout period

CHAPTER II

REVIEW OF THE LITERATURE

This review of the literature is not an all-inclusive list of studies examining Cr supplementation on physiological function, but rather highlights the current literature examining Cr supplementation (without training) on neuromuscular function. This review will focus on studies that have specifically used the twitch interpolation technique, resting evoked twitches, isokinetic and isometric testing, and measures of ROM and stiffness as it pertains to Cr supplementation. In addition, key articles describing the physiological role of Cr supplementation are included. Lastly, a table is provided in the appendix that includes studies that are most relevant to the current research questions.

The influence of Cr supplementation on neuromuscular function

Bazzucchi I, Felici F, and Sacchetti M (2009)

The purpose of this study was to evaluate the effects of short term Cr supplementation on muscle contractile properties, the force-velocity relationship and muscle fiber conduction velocity. Sixteen moderately trained males (25 ± 5 yrs) were either given Cr monohydrate (n=8) or maltodextrin (5 days, 20 g/day). Isometric MVC, max twitch force, isokinetic contractions (15, 30, 60, 90, 120, 180, & 240 $^{o}\cdot s^{-1}$) and fatigue of the elbow flexors were measured prior to and following supplementation. Tests were conducted on an isokinetic dynamometer with the elbow axis of rotation aligned with the center or the lever arm. Force was measured from a load cell strapped to the wrist. EMG of the biceps brachii was recorded to evaluate conduction

velocity via the cross-correlation technique. The fatiguing protocol consisted of five sets of 30 isokinetic (180 $^{o}\cdot s^{-1}$) contractions. Following supplementation twitch peak torque increased, and time to peak torque decreased (P<0.05). Isokinetic peak torque was significantly increased at 180 and 240 $^{o}\cdot s^{-1}$ (P<0.05) from pre to post. Conduction velocity was increased following supplementation at all velocities (P<0.05). There was no difference between pre and post peak torque values during the fatiguing protocol. The authors concluded that Cr supplementation improves neuromuscular function of the elbow flexors as an increase in torque was observed at higher velocities. They speculated that the increase in performance is a result of improvement in Ca^{2+} kinetics which is contributed to the increase in available ATP. They also demonstrated that Cr may have a neurological effect that needs further study. Surprisingly the study did not observe differences in any of the fatigue measures, the authors hypothesize that the factors that are positively influenced by Cr supplementation are not the same as the ones negatively affected by fatigue.

Bembem MG, Tuttle, TD, Bembem DA, and Knehans AW (2001)

The purpose of this study was to examine the effect of Cr supplementation on the force time curve of an isometric contraction of different muscles. Nineteen healthy sedentary males consumed 20g of either Cr monohydrate (n=11) or corn starch (n=8) for five days followed by a maintenance dose of five grams for five days. All testing was done on an isometric testing cage. Elbow flexion and extension were conducted at 90º of flexion with the palm supinated for flexion and pronated for extension. Knee extension and flexion was performed at 90º of knee flexion. A load cell was appropriately placed on the wrist or ankle to measure force output. Three MVCs at each joint were held for three sec with one min rest between each contraction. Following the isometric MVC, an endurance task consisting of 14 isometric MVCs with two sec

rest were performed. All isometric tests were completed on the same muscle group before moving on to the next. The force time curve for each contraction was analyzed to determine maximal force (MF), maximal rate of force development (MRFD), time to max force (TMF), impulse of max force (IMF), total impulse (TI) and from the endurance tasks they calculated percent force decrement. No significant difference ($P > 0.05$) between groups was found for MF (pre: 246, post 232N), TMF, and MRFD (pre: 782, post 853 $N \cdot s^{-1}$). For the endurance task, there was no significant difference for TI and PFD. There was a time effect found for both groups as both groups improved from day one to day 10. The authors of this study concluded that Cr supplementation alone does not bring about any changes in isometric force production after 10 days of supplementation. They believed that the positive effects of Cr supplementation can be attributed to its ability to increase work capacity and must be paired with weight training to maximize the effects.

Deutekom M, Beltman JGM, de Ruiter CJ, de Koning JJ, and de Haan A (2000)

The purpose of this study was three fold, the first objective was to determine if short term Cr loading effects power output during whole body performance; the second objective was to investigate the effect of Cr loading evoked peak torque and rate of torque development, and the third objective was to address the influence of Cr loading on fatigue during electrically stimulated contractions of the quadriceps. Well trained rowers were given five grams of either maltodextrine (n=12) or Cr monohydrate (n=11) four times a day for six days. Testing was conducted prior to and following the supplementation period. Evoked twitch properties were conducted using surface electrodes placed on the quadriceps and were stimulated until 30% of the force generated during a maximal isometric contraction was reached. Isometric contractions were conducted using a force transducer strapped to the leg with the knee flexed to 90º. Five

maximal contractions were performed followed by the electrical stimulus at 10, 20, 50, 100, 150 & 200 Hz. The sequence of the stimulus was randomly ordered and one min of rest was given between each contraction. For total body fatigue two 30s sprints on a cycle ergometer was used to determine power output. Cr loading demonstrated no effect on maximal contractions (pre: 305, post 298 Nm), muscle activation, or power output during the cycle ergometer fatiguing task. The authors concluded that Cr supplementation did not significantly affect torque production or the rate of torque production and fatigue or recovery from fatigue.

Greenhaff PL, Casey A, Short AH, Harris R, Soderlund K, and Hutlman E (1993)

The purpose of this study was to examine the effect of Cr supplementation on torque production during repeated bouts of maximal isokinetic contraction. Twelve college students (9 males, 3 females) consumed 20g (5 x 4 serving) per day for five days of either glucose (n=6) or Cr monohydrate (n=6). Participants reported to the lab on a two occasions for initial testing and following the five days of supplementation. Testing included five sets of 30 maximal voluntary leg extensions at a constant angular velocity of 180 $^{o}\cdot s^{-1}$ with a minute rest between each set. Blood draws were also taken before testing, after the warm up, between each set, immediately post, and two, five, and 10 minutes post exercise. At baseline both groups were similar in peak torque production and both had a similar decline in toque production during the repeated isokinetic muscle actions. There was no difference ($P > 0.05$) in the decline (~50%) between pre and post supplementation with the placebo group; however the participants that supplemented with Cr maintained higher levels of torque across all five sets post supplementation. The Cr group generated significantly more torque during the second ($P < 0.001$) and third ($P < 0.05$) exercise bouts than the placebo group. There was no difference in blood lactate levels after exercise between groups, or pre and post supplementation, but the Cr group had less ammonia

accumulation post supplementation. The authors speculated that the low amount of ammonia acumination and the muscles ability to maintain higher levels of peak torque was the result of more available Cr due to supplementation and its ability to phosphorylate ATP via the PCr cycle. In conclusion Cr supplementation did not improve peak torque production but aided in maintaining torque levels through a fatiguing task.

Hamilton KL, Meyers MC, Skelly WA, and Marley RJ (2000)

The purpose of this study was to investigate the influence of Cr on upper body strength and fatigue in women who participate in overhead sports. Females were given either 25 g of Cr monohydrate (n=11) or a placebo (n=13) for seven days. All testing was conducted prior to and following supplementation. All participants performed elbow flexion and shoulder internal rotation on a powered isokinetic dynamometer. For elbow flexion the following tests were performed: concentric and eccentric isokinetic flexion at 90 $^{o}\cdot s^{-1}$, an isotonic 1RM, and a fatiguing protocol which consisted of performing as many repetitions to failure at 70% of 1RM. For shoulder internal rotation an isotonic 1RM, one isotonic max velocity test at 25% of 1RM, and the fatiguing protocol were performed. Two min of rest was given between all tests. The isokinetic testing of the elbow flexors yielded no significant change in eccentric (Pre: 62, Post 64 Nm) or concentric (Pre: 4, Post: 46Nm) torque values following supplementation. Reps to fatigue for the elbows flexors was significant increased (P<0.05, 12-16 reps) from pre to post supplementation in the Cr group. There were no significant changes in 1RM or reps to fatigue (P < 0.05) for the internal rotators. The results of this study determined that only isotonic work to fatigue was improved with Cr supplementation. None of the other variables measured showed significant changes from pre to post supplementation or differed from the placebo. In conclusion the authors determined that short term Cr supplementation with no formal training does not

improve upper extremity strength measurements, thus explosive overhead athletes may not benefit from Cr supplementation alone. The authors speculated that Cr's effects would have been larger had supplementation been part of a training program.

Jakobi JM, Rice CL, Curtin SV, and Marsh CD (2000)

The purpose of this investigation was to determine if short term Cr supplementation would alter the neuromuscular response prior to and following a fatiguing task. Fourteen young males (22 ± 3 yrs) were either given either 20 grams of Cr monohydrate (n=7) or maltodextrin for 5 days. Prior to and following supplementation, participants performed a twitch interpolation technique that consisted of an isometric contraction of the elbow flexors (90º of elbow flexion) with a superimposed twitch, followed by resting twitches. Prior to and following a fatiguing task, tetanic twitches were administered at 50Hz. The fatiguing task consisted of repeated bouts of six second isometric contractions at 50 % maximum force production with four seconds of rest until 50% of maximum force could no longer be achieved. Force output was measured via a strain gauge that was tethered to the floor and attached to the wrist. From the twitch interpolation technique, percent activation, peak torque, time to peak torque, half relaxation time, EMG amplitude, contraction duration where calculated. Tetanic tension and tetanic relaxation time were calculated prior to and following the fatiguing task. Time to fatigue was also measured as the time at which 50% of maximum force could not be achieved. The Cr group was not significantly different from the placebo group at any measure pre or post supplementation. There was a 44% reduction in peak force during the fatiguing task *(P*<0.05) combining the groups. Following the fatigue protocol, there was a 10% decrease in muscle activation and an increase in relaxation time in the resting twitches for both groups *(P*<0.05). The results of this study indicate that Cr supplementation does not alter maximum strength and evoked twitch

properties following a fatiguing bout of exercise. The authors speculate that muscle metabolism and muscle force are independent of each other; Cr supplementation positively influences the metabolic properties of muscle but may not influence the mechanical properties.

Kambis KW and Pizzedaz SK (2003)

The purpose of this study was to investigate the effect of Cr supplementation on power and torque output of the quadriceps. Twenty-two healthy college aged women were matched according to diet, menstrual phase and exercise history then given $0.05 g \cdot kg^{-1}$ a day of either a Cr monohydrate or a placebo. The supplement was consumed in four equal servings per day for five days. Participants completed all testing prior to and following supplementation. Participants completed five consecutive maximal isokinetic muscle actions of the leg extensors and flexors at $60\ ^{o} \cdot s^{-1}$. Participants then rested five minutes before completing 50 maximal contractions at $180\ ^{o} \cdot s^{-1}$. During the isokinetic testing, power output and time to peak torque were examined. There was as an increase in power output across the 50 isokinetic contractions at $180\ ^{o} \cdot s^{-1}$ for both flexion (6.0 W) and extension (9.1 W) ($P < 0.05$) following Cr supplementation. Time to peak torque for leg extension decreased 2.2ms following Cr supplementation ($P < 0.05$). In the placebo group, there were no changes in any measure during the 50 contractions pre to post. There was no difference in any of the measures at $60\ ^{o} \cdot s^{-1}$ between groups or pre or post supplementation ($P=0.05$). In conclusion, Cr supplementation improves muscle performance in women at faster velocities of leg extension but does not influence muscle performance during low velocity isokinetic muscle actions. The authors contribute these differences to the recruitment pattern of muscles at high velocities in which fast twitch fibers are recruiter more thus type II fibers, which rely heavily on the PCr systems, would more likely benefit from supplementation.

Law YLL, Ong WS, GillianYap TL, Lim SCJ, and Von Chia (2009)

This study was included in the literature review based of the fact that it is the only study to date the addresses different days within the Cr loading period on muscular function, however, the Cr supplementation was coupled with eight resistance exercises on day one and four. The purpose of this study was to address the effect of two and five days for Cr loading on muscular strength and anaerobic performance. Participants either consumed 20 g of Cr (n=8) or a placebo (n=9) for five days. One repetition maximum (1RM) bench press and squat exercises were conducted with the ascending weights on a smith machine working up to a 1RM. Power was determined from a 30s Wingate anaerobic power test. All testing was conducted prior to and following two and five days of supplementation. There was no improvement in strength ($P>0.05$) or power ($P>0.05$) after two days of supplementing. There was however, a significant increase in the 1RM squat strength following five days of supplementation ($P<0.05$). Average power also improved following five days ($P<0.05$) of Cr use. The authors concluded that it takes at least five days for Cr to influence measures of strength and power. The improvements in performance seen with Cr supplementation may take longer to manifest then it takes Cr to enter the cell. This research indicates there may be a saturation level that must be reached to improve performance or that there must be is a delay in Cr uptake by the cells to when it improves cellular function.

Medeiros RJD, Santos AAd, Ferreira AdCD, et al. (2010)

The purpose of this study was to examine the effect of Cr loading on maximal isometric strength and EMG amplitude in women. Twenty seven women (23±2 years) consumed either 20 grams of Cr monohydrate (n=13) or maltodextrin (n=14) for 6 days. Isometric leg extension strength was tested at 120º of leg extension of the dominate leg using a powered dynamometer with EMG electrodes placed on the vastus lateralis. Three maximal isometric knee extensions were performed for six seconds with 180 seconds rest between trials. For each trial, peak torque and max EMG amplitude were analyzed as well as the mean of the three trials. Comparisons were done between pre and post measures of isometric strength and root mean squared EMG amplitude. A significant increase (P= 0.002) in isometric strength from pre to post (~7.85%) was found with Cr supplementation but not the placebo. The significant increase in isometric strength of the participants who supplemented with Cr was accompanied by a significant (P= 0.026) increase in EMG amplitude (~15μV). The authors conclude that Cr supplementation alone does have the ability to increase isometric force production, and this was accompanied by an increase in EMG amplitude. The authors acknowledge that increase in EMG does not necessarily coincide with an increase in strength. The authors attribute the increase in EMG amplitude to an increase in recruitment of motor units and/or firing frequency, but failed to normalize or provide any mechanisms behind the increases in EMG amplitude and torque production.

Rawson ES, Clarkson, PM (2000)

The purpose of this study was to examine the effects of short term Cr supplementation in older adults on isokinetic leg extension strength and isometric strength of the elbow flexors.

Seventeen older males (65 years) were given 20 grams day of either Cr monohydrate (n=9) or a placebo (n=8). The supplement was consumed in four equal servings per day for five days. Participants complete all strength testing prior to and following supplementation. Isometric elbow strength was tested on a preacher curl bench with the humerus against the pad and the hand grabbing on to a bar that was attached to a strain gauge. Leg extension peak torque was measured during three sets of 30 maximal concentric leg extensions at 180 $^{o}\cdot s^{-1}$ on a isokinetic dynamometer. The sum of the peak torque values per set were used as a measure of strength. Following the five days of supplementation peak torque increased 25.2Nm for the second set and 72.6Nm for the third set for leg extension strength (P <0.05) in the Cr group, but not differ from the placebo group. There was no significant change in elbow strength (P >0.05) for either group. In conclusion, the authors determined that supplementing with Cr does not increase upper or lower body strength. The authors suggest the Cr kinetics may be reduced in an elderly population which could be one reason why there was not an improvement in strength.

Rossouw F, Kruger PE, and Rossouw J (2000)

The purpose of this study was to evaluate the effects of Cr supplementation on deadlift and isokinetic leg extension performance in well trained power lifters. Thirteen well trained power lifters were administered nine grams of either Cr monohydrate (n=8) or sucrose (n=5) for six days. Isokinetic leg extensions were performed prior to and at day five of supplementation, and a 1RM deadlift was done prior to and at day six of supplementation. The isokinetic testing consisted of three sets of 25 isokinetic leg extensions at 180 $^{o}\cdot s^{-1}$. Peak torque, peak power, work and work from reps 1-5 and 21-25 were calculated for leg extension performance. Deadlift was measured as the maximum amount weight lifted according to competition standards. Both groups experienced an increase in isokinetic strength following the five days of supplementation

(*P*=.01−.05), but the Cr group experienced an increase (*P*=0.01) in power during the third set while the placebo did not. The Cr group deadlifted 7.81±7.95 kg more than they did prior to supplementation (*P*=0.02), the placebo group did not significantly lift more (*P*=0.20). The results of this study indicate that even well trained lifters may benefit from Cr supplementation as it improved some markers of isokinetic strength and deadlift performance.

Smith-Ryan AE, Ryan ED, Fukuda DH, Costa PB, Cramer JT, and Stout JR (2013)

The purpose of this study was to examine the effects of fatigue on neuromuscular function following Cr loading. This study addressed the issue of Cr's ability to prolonged fatigue, but since fatigue is not part of this review, the focus will be on measures pre and post supplementation prior to the fatiguing task. Twelve females consumed 20 grams of fructose with either five grams of Cr monohydrate or a placebo four times a day for five days. Maximal isometric plantar flexion strength was performed prior to and following five days of supplementation on a custom made force plate. During the maximal voluntary contraction (MVC) the twitch interpolation technique was used to determine percent voluntary activation and peak force. From the resting twitches, peak twitch force, peak rate of force development, half relaxation time, and M-wave amplitude of the soleus and medial gastrocnemius. There were no differences between the placebo and the supplement group in any of the neuromuscular measures (*P*=0.805 −0.064) (MVC, peak RFD, relaxation time and RMS). The results of this study indicate that short term Cr supplementation does not influence isometric measures of strength or evoked twitch properties of the lower leg in women. In conclusion, isometric strength may not be an appropriate measure to evaluate the muscles ability to use Cr.

Stevenson, SW and Dudley GA (2001)

The purpose of this study was to address the effects of Cr loading on fatigue, dynamic resistance exercise performance, voluntary and evoked isometric strength. Thirty one resistance trained males (n=29) and females (n=20) consumed either 20g of Cr monohydrate (n=18) or a placebo (n=13) per day for five days. Participants performed all tests prior to and following five days of supplementation. Five maximal isometric contractions were performed with the leg extensors at 45º below the horizontal plane on a dynamometer. Five repetitions of electrically stimulated contractions were performed at 70% of maximal torque. Leg extension one maximal repetition (1-RM) and repetitions to failure were conducted on a leg extension machine. The load for the repetitions to failure test was subjective but remained the same for pre and post testing. From both voluntary and evoked isometric contractions, rate of torque development and relaxation time were calculated. Rate of torque was calculated during 20-80% of the maximal torque. Cr supplementation did not significantly improve rate of torque development (*P*=0.0807 Pre: 801, Post: 753$N \cdot m \cdot s^{-1}$) and was not different from the placebo. There was no significant difference in relaxation time (pre: 30, post: 29 ms), 1 RM strength, or repetitions to failure between groups. The authors concluded that short term Cr supplementation in resistance trained men does not improve leg extension rate of force development, relaxation time or fatigability.

Urbanski RL, Loy SF, Vincent WJ, and Yaspelkis BB (1999)

The purpose of this investigation was to determine if Cr supplementation improves maximal isometric strength and time to fatigue following loading. Ten males (26±6 years) consumed either 20 g of Cr monohydrate or a placebo for five days, then following a 35 days washout period were given the opposite supplement they previously received for five days. The

crossover design of this study allowed for all 10 participants to be given both the placebo and Cr. Isometric strength tests were performed prior to and following both supplementation periods (5 days). Isometric leg extension strength was examined on a dynamometer with the leg flexed at 90º. Isometric grip strength was performed with the arm by their side and flexed to 90º on a hand held dynamometer. Time to fatigue was measured by having the participant perform three trials of isometric holds at 67% of MVC for as long as possible for both the leg extension and grip exercise. Isometric peak strength and time to fatigue were recorded and compared between the placebo and Cr. Cr supplementation increased (~2 Kg·m) isometric leg extension strength ($P<0.05$) but failed to increase hand grip strength. Time to fatigue was significantly increased ($P<0.001$) for both leg extension and grip strength following Cr supplementation when compared to the placebo during all three bouts (~3s). The authors attributed the increase in time to fatigue to the more readily available sources of ATP via the PCr system as a result of Cr supplementation. The authors concluded that Cr supplementation improves performance when ATP is a limiting a factor, and may be more beneficial in larger muscles.

van Leemputte M, Vandenberghe K, and Hespel P (1999)

The purpose of this study was to evaluate the effect of Cr supplementation on voluntary isometric torque and relaxation time. Sixteen males (18-23 years) were asked to consume either 20 grams of Cr monohydrate (n=8) or maltodextrin for five days. Participants performed all tests of the elbow flexors prior to and following the five days of supplementation. Testing began with three isometric MVCs of the elbow flexors at 110º of flexion on a dynamometer and held for three seconds. Following the MVCs, participants performed twelve consecutive MVCs with 10 seconds rest intervals and EMG was recorded to muscle activation. Over the course of the twelve contractions, peak torque and relaxation time were calculated. Relaxation time (RT) was

calculated as the time course of isometric torque decay from 75 to 25% of maximum torque. Muscle activation was the normalized peak EMG amplitudes during the initial contraction (25-75 % of peak torque) and during RT. Cr supplementation had no effect on peak torque during the 12 contractions (pre: 57Nm, post: 58Nm). Cr supplementation did reduce (~20%) RT during the 12 contractions while the placebo did not ($P<0.05$). There was also no significant change in muscle activation between groups. The authors attribute the decrease in RT to an increase in Ca^{2+} kinetics due to an improvement in the PCr system as a result of Cr supplementation. The authors attributed the lack of change in peak torque was a result of the fiber composition of the bicep muscle group being an equal distribution of fiber type, not primary type II which would utilize more Cr. In conclusion, short term Cr supplementation shortens relaxation time during brief isometric contractions.

The influence of Cr supplementation on ROM and muscle stiffness

Sculthorpe N, Grace F, Jones, and Fletcher I (2010)

The purpose of this study was to determine the effects of Cr loading on joint range of motion. Forty healthy young participants (24±3yrs) consumed either 25grams of either Cr (n=20) or a placebo (n=20) for five days then five grams for the remaining three days. Prior to and following supplementation, active range of motion (ROM) assessments were taken with a universal goniometer. The tests consisted of: shoulder flexion, extension, lateral & medial rotation and abduction; elbow flexion; hip: flexion, extension abduction, medial and lateral rotation; ankle plantar and dorsiflexion. The results showed that the control group's ROM did not differ from pre to post ($P < 0.05$). The Cr group had significant decreases in ROM for shoulder extension (15.8%, $P < 0.01$; effect size = 0.371), shoulder abduction (1.7%, $P < 0.05$;

ES = 0.25) and ankle dorsiflexion (14.8%, $P < 0.01$; ES = 0.301). The authors concluded that ROM was affected by Cr supplementation in joints where smaller muscles were working against larger antagonist muscles. There was no change in ROM in joints with larger agonist muscles. The authors determined that altered ROM was not due to an inhibition in the active muscle but an enlargement in size of the antagonist muscle. The authors contributed the increase in size of the antagonist muscle to the fluid shift that occurs with Cr supplementation. The authors speculate that the increase in size of the antagonist muscle would trigger the stretch reflex sooner as it is lengthening during the active ROM causing a contraction of the antagonist muscle leading to a reduction in active ROM in the smaller agonist muscle.

Watsford ML, Musphy, AJ, Warwick, SL and Walshe ED. (2003)

The purpose of this study was to examine the effects of Cr supplementation on musculotendionus active stiffness, isometric force and jump performance. Twenty men consumed either 20 grams of Cr (n=10) or a placebo (n=10) for seven days then consumed 10 grams a day for 21 days. Testing was conducted prior to loading, following loading (day 7) and after the supplementation period (day 28). Active stiffness of the plantar flexors was determined by using the oscillation technique measuring peak to peak distance at 50, 100 and 200% of body weight. Isometric contractions of the plantarflexors were performed with participant in a seated calf raise position with the knees and hips bent to 90° of flexion with a load cell placed between the ground and the movable arm of the calf raise machine. Countermovement and drop jumps from 80 and 100cm were used to determine jump performance. Addressing only the time point following the loading period (day 7) there was no significant influence of Cr supplementation on active stiffness at 50% of body weight (pre: 3,463.7; post: 3,440.1 $N \cdot m^{-1}$), 100% (pre: 5,199.7; post: 5,275.7 $N \cdot m^{-1}$), or 200% (pre: 8,147.7; post: 7,519.3 $N \cdot m^{-1}$), using the oscillation

technique. There was no significant change ($P > 0.05$) in isometric force (pre: 2,908.8; post 2,958.5N), RFD (pre: 11,677; post: 11,356 $N \cdot s^{-1}$), and jump performance following seven days of Cr supplementation. As a result, the authors concluded that Cr supplementation does not influence isometric strength or jump performance. The authors hypothesized that an increase in water retention with Cr, as observed in previous literature, would alter the muscles size and thus affect stiffness. These results would indicate that there is no influence of Cr supplementation on active stiffness. Based on these findings Cr supplementation did not influence any performance measures or an increase in active stiffness after seven days of loading.

The influence of Cr supplementation on fluid distribution

Bembem. MG. Tuttle, TD., Bembem DA. And Knehans AW. (2001)

The purpose of this study was to address the effect of Cr supplementation on the force time curve during an isometric contraction of different muscles. The secondary purpose was to address the possible fluid shifts during supplementation. Nineteen healthy sedentary males consumed either 20 grams of either Cr (n=11) or corn starch (n=8) for five days, then a maintenance dose of five grams for five days. The fluid shift was examined using bioimpedance spectroscopy. Total body water and intracellular and extracellular water were calculated with a Xitron BIS analyzer system and examined pre and post supplementation. There was no significant difference between the pre and post-testing body water measures ($P > 0.05$). There was a trend in the Cr group, as all three body water measures increased while the placebo group remained unchanged. The authors concluded that ten full days of Cr maybe enough time for the body to rebalance water distribution following Cr supplementation. Cr's osmotic properties may

influence water shift during the initial loading period but may return to balance after continued supplementation.

Kern M, Podewils L, Vukovich M, and Buono M (2001)

The purpose of this study was to evaluate body water changes in relation to gains in body weight following Cr supplementation and to determine if Cr benefits thermal regulation capacity in the heat. Twenty healthy males consumed either 21 grams of Cr monohydrate (n=11) or sugar (n=8) for five days then two grams a day for 23 days. Total body water was determined using a bioelectrical impedance analysis and was measured weekly for the 28 days. Body composition was measured using hydrostatic weighing with the Siri equation. VO_2 max was determined on a cycle ergometer prior to supplementation and following the 28 days. A 60 minute of cycle ergometer test was performed at 60% VO_{2max} in the heat (37º C). Core temperature was assessed prior to and during the cycling. There was no difference in any of the pre values between the groups (P=0.484). Subjects that supplemented with Cr demonstrated an increase in lean mass and body mass after the 28 days. There was a corresponding gain in TBW with the Cr group, which was associated with the increase in body mass. The core temperature rose in all conditions but was lower in the Cr group pre and post supplementation but was not significantly different during the cycling test (P=0.057). The authors concluded that the increase in body mass is attributed to the increase in water during Cr supplementation, and thus an increase in TWB would in theory improve heat buffering in the body when exercising in high temperature conditions. Even though the results were insignificant, a decreasing trend in body temperature during the 60 min cycling following Cr supplementation suggests it may be beneficial when exercising in the heat.

Powers ME, Arnold B, Weltman AL, Perrin DH, Mistry D, Kahler D (2003)

The purpose of this study was to investigate the effects of Cr supplementation on Cr concentrations in the muscle and fluid volumes using direct measures. Sixteen males and 16 females who regularly performed resistance training participated in this study. Subjects reported to the clinic the first day and stayed overnight. During their stay they had their resting vitals and fluid volumes, urinary Cr and muscle Cr levels taken. Participants were stratified by gender then randomly given either a placebo or *Phosphagen* supplement containing five grams of Cr monohydrate. The supplement was taken five times a day for seven days then once a day for the next 21 days. They were then post tested at day seven and 28. Urinary Cr levels were taken from the urine collected during the overnight stay. Muscle biopsies from the vastus lateralis were used to address Cr stores in the muscle. Fluid volumes were measured using deuterium oxide (D2O) dilution for TBW and sodium bromide dilution for ECW and ICW volumes. BIA was also used to with the D2O values. There was no difference in baseline urinary Cr levels but there was a significant increase in Cr in the urine in the supplement group (P= 0.023), Cr stores in the muscle also increased during the supplementation in the Cr group (P= 0.023), but not the placebo group. There was not a significant increase in body mass (P<0.05) at day seven but there was an increase in body mass by day 28 (P=0.044). For fluid distribution, there was an increase in TBW with both groups but the Cr group had a significantly higher increase in TBW (P=0.027). There was no significant difference between groups at any time point for ECW or ICW (P=0.366). In conclusion, Cr's osmotic properties increase the body's ability to retain water as measured with an increase in TBW this coincides with an increase in muscular Cr stores increase with supplementation. The primary finding is that Cr does not affect fluid distribution as seen

with no change between ECW and ICW. Thus, the authors conclude that Cr increases overall water carrying capacity but does not affect how it is distributed.

Weiss BA, and Powers ME (2006)

The purpose of this study was to determine if an acute Cr supplementation alters fluid distribution and thus impairs thermoregulation during a bout of exercise in greater thermal temperatures. Twenty-four male participants consumed either 25 grams of Cr monohydrate (n=12) or a placebo (n=12) for five days. Prior to and following the supplementation period, participants had their body mass, heart rate, body water volumes and core temperature measured. Participants then performed a graded exercise test on cycle ergometer in a 37º C temperature control room. During exercise, HR, sweat loss, and core temperature were taken every 10 minutes during the 60 minute test. Water volumes were measured using bioelectrical impedance spectroscopy. There was no significant difference between the Cr and placebo groups in HR (P=0.754), sweat loss (P=0.144), or core temperature (P=0.870). There was a significant increase in TBW (p=0.004), ECW (p=0.005) and ICW (p=0.046), in the Cr groups following five days of supplementation while the placebo group exhibited no change. The results showed that Cr supplementation does influence water retention but does not unequally distribute fluid within the body. Cr increased water content in the body but did not have any adverse effects on the sweat response or core temperature. The concerns that Cr's osmolality properties may cause a fluid shift resulting in less sweat loss were shown to be invalid as a result of this study. The authors concluded that Cr supplementation maybe beneficial in maintaining proper temperature of the body during prolonged exercise.

Ziegenfuss TN, Lowery LM, and Lemon PW. (1998)

The purpose of this study was to estimate the relative fluid volumes during three days of Cr supplementation. Ten healthy males consumed 0.07g·kg·FFM^{-1} of Cr five times a day for three days. Prior to and following supplementation baseline values of FFM, body fat percentage; TBW, ECW, and ICW were measured for three days. Bioelectrical impedance analysis was used to determined fluid distribution. Following three days of supplementation there was an increase in TBW (P= 0.07), and ICW (P<0.05), but there was no significant change in ECW (P= 0.51). The authors suggest that even though there was a small significant change in fluid volumes this change was not large enough to influence other variables associated with increases in cell size like protein or glucose synthesis. The authors calculated effect size for TBW and ICW and they were 0.39 and 0.64, respectively. In conclusion, even though the significant changes in fluid volumes were small, they may have small to moderate effects on fluid distribution.

The physiological role of Cr supplementation

Harris RC, Soderlund K, and Hultman E. (1992)

The purpose of this study was to determine if Cr supplementation would be absorbed by human tissue and whether absorption would increase with continued supplementation. This is the first study that addressed Cr absorption in human muscle. Five females and 12 males between the ages of 20-62 participated in this study. Twelve of the participants consumed 4 × 5 g a day for 4.5 days. At that point two participants stopped consumption, while the remaining participants consumed Cr for either 7, 10 or 21 days total days. This design was to address how long it took for Cr to saturate muscle. Muscle biopsies of the vastus lateralis where taken prior to

supplementing and following day two and at the completion of each of the supplementation periods. Plasma was taken from a subset of the participants for seven hours following one serving of five grams of Cr. Urine samples were taken from most of the subjects to measure Cr excretion. Cr supplementation resulted in an increase in plasma levels, which peak one hour following Cr ingestion and slowly decline until five hours post consumption. Only four subjects completed the full urine analyses. The results of the urine analysis indicated that during the first two days, 32% of Cr ingested was absorbed and the rest was excreted. This number then dropped to 17% from the second to forth day. The result of the muscle biopsies indicated that Cr supplementation increases muscle concentration of Cr but the level of uptake varied between participants. Participants with higher initials level of Cr stores had a smaller increase in Cr stores then someone with lower initial levels. This indicates that there is a saturation level that occurs in most people around ~145mmols·kg^{-1} of wet weight. The participants that had muscle biopsies taken on day two and following the supplementation period showed that the majority of Cr is absorbed in the first two days. During the supplemental period, five subjects performed leg extension exercises of one of their legs and Cr concentration differences were measured between the exercised limb and the non-exercised limb. Results of the exercise groups showed an increase in Cr content in the exercised limb compared to the non-exercised limb. In conclusion, Cr supplementation of 25 grams or more successfully loads the muscle with Cr; this can further be enhanced with exercise. The majority of Cr absorption takes place in the first two days and then continues until the muscle is fully saturated. The authors suggest that with an increase in Cr stores within the muscle could possibly have an impact on sports performance, as an increase in Cr would lead to an increase in work capacity. This the first study to address Cr supplementation

and thus started the research into Cr as an ergogenic aid. This study has been referred to as the fundamental study in Cr research.

Hultman E, Soderlund, K, Timmons JA, Cederblad G and Greenhaff PL. (1996)

The purpose of this study was to determine whether muscle Cr stores could be effectively increased with different amounts of Cr supplementation and whether elevated levels could be maintained with continued low dose Cr supplementation. Thirty one males where randomly allocated into four groups: group one consisted of six participants who consumed 20 grams of Cr monohydrate for six days; group two consisted of nine participants who consumed 20 grams of Cr monohydrate for six days then two grams a day for the next 28 days; group three consisted of nine participants who consumed three grams of Cr for 28 days; and group four consisted of seven participants who consumed 20 grams of a placebo for five days. Muscle biopsies of the vastus lateralis were taken for all groups at pre testing and at day seven, 21 and 35 of the supplement program to determine Cr concentration levels. Urine was collected on days one, three, five, eight, 11, 18, 22, and 25. As a result of the muscle biopsies, Cr supplementation of 20 grams for six days significantly increases total Cr concentration muscle ($P<0.05$, Group 1 & 2). Group one exhibited a decline in Cr levels following cessation (day 6) of Cr supplementation while group two displayed plateau effect in elevated Cr levels following the loading period with the lesser amount of Cr for remaining 28 days. Group three Cr levels did not significantly increase from baseline until day 14 ($P<0.001$) of Cr supplementation. Group four did not experience an increase in Cr levels during any time point. The results of the urine analysis indicated that urinary creatinine peaks at five days of supplementation ($P<0.05$). The authors concluded that with the typical loading period, Cr will saturate the muscle in five days and can be maintained by a lesser amount (~2 g) of Cr for the duration of supplementation. The authors also discovered

that lower amount of Cr (~3g) can increase Cr concentration, but takes longer to saturate the muscle with Cr. This study was fundamental in addressing the implication of a maintenance dose of Cr and the use of a lesser amount of Cr to saturate muscle.

CHAPTER III

METHODS

Participants

Forty healthy young men (age: 20.3 ± 1.9 years, Stature: 176.5 ± 6.4, mass 72.4 ± 6.6 kg) were recruited for this investigation. All participants completed an informed consent document and a health history questionnaire prior to any testing. To be eligible for inclusion into the study all participants met the following requirements: free of any current or ongoing neuromuscular diseases, having not sustained a lower limb injury within the last six months, body contained no ferromagnetic implanted material (i.e. pacemakers and metal implants), and have not supplemented with Cr within the last six months. Recreationally active subjects where recruited and self-reported actively levels where taken (Table 5). Observed power with a sample size of 40 (n=20 in each group) for the dependent variables ranged from 0.761-0.998, with an effect size of 0.10-.18.

Experimental Design

A randomized, matched-pair, double-blind, placebo controlled design was be used to determine the time course effects of Cr supplementation on muscle function. A matched pair design was selected per the recommendations of Atkinson et al. [4], participants were matched by strength following the familiarization trial and then randomly assigned to the placebo (n=20) or Cr (n=20) group. Participants visited the laboratory on seven occasions (including the

familiarization day). The experimental trials began 2-5 days after the familiarization trial and occur at the same time of day (± 2 hrs). Testing was conducted prior to supplementation, during the loading period (2, 4 and 6 days following supplementation) and during the washout period (15 and 35 days post supplementation). A five week washout period was chosen based on the findings of Rawson et al. [88] who indicated that the time period for muscle Cr levels to return to baseline may be longer than the traditional 28 – 30 day period. During each experimental trial, the participants had their body fluid distribution (ICW, ECW and TBW), the size and architecture of their medial and lateral gastrocnemius, and tibialis anterior, measured. They also underwent passive and active range of motion (ROM) assessments, performed the twitch interpolation procedure and isokinetic strength testing. A randomly assigned subset of participants (n=8) underwent proton magnetic resonance spectroscopy (MRS) to detect changes in total creatine levels within the muscle during supplementation.[71] All tests were conducted on the right leg.

Familiarization Day

During the familiarization trial, each participant completed a health history questionnaire and signed an informed consent, practiced the twitch interpolation procedure, isokinetic and isometric strength testing and ROM assessments and were informed of the ultrasound and proton MRS assessments.

Supplementation Procedure

Creatine monohydrate (20g) or a placebo was given to the participants. The creatine and placebo was supplied by Dymatize® nutrition and independently tested for quality from Covance Laboratories Inc (Madison, WI). The placebo (20g dextrose, 410mg Lemonade flavor, 450mg

citric acid, 500mg Malic acid, 25mg Sucralose) and Cr (same as placebo with 5g creatine monohydrate added) products were supplied in identical white packets and the participants were asked to mix each packet with eight ounces of water and ingest four times throughout the day for five consecutive days. Subjects were asked to bring in any unused packets in which no packets where returned.

Body Fluid Distribution

Total body water (TBW), intracellular water (ICW) and extracellular water (ECW) levels were determined using bioimpedance spectroscopy (BIS) (Impedimed SFB7, San Diego, CA). Participants lied supine on a table with their legs separated and arms approximately 30 degrees from their torso for a 5 – 10 min rest period to ensure the measurements accurately reflect fluid distribution (corrects for fluid accumulating in the limb when standing). Electrodes were placed in a tetra-polar configuration using the right foot and right hand to measure total body resistance, this technique has been shown to be both valid and reliable.[75] Fluid distribution (TBW, ICW, and ECW) was calculated using the equations by Deurenberg et al[19]

$$TBW\ (L) = (height^2 / Z100 \times 0.34573) + (weight \times 0.17065) - (age \times 0.11) + 9.35$$

$$ECW\ (L) = (height^2 / Z1 \times 0.19528) + (weight \times 0.06987) - (age \times 0.02) + 2.3$$

$$ICW = TBW\text{-}ECW^{115}$$

From the BIS measurement fat free mass (FFM) and fat mass (FM) were also recorded.

To account for changes in hydration, urine specific gravity was measured from a urine sample collected on each visit. Specific gravity was measured using a clinical handheld refractometer (Model 1.33Ade Advanced Optics, Oregon city, OR).

Muscle Size and Architecture Measurements

Muscle cross-sectional area (CSA) was determined by ultrasonography with a 12 Mhz linear array probe (LOGIQ e, General Electric Company, Fairfield CT) placed along the axial plane at approximately 1/3 the distance between the popliteal crease and the lateral malleolus. Images were collected up to a depth of 3.5 cm using the musculoskeletal setting at a frequency of 10 MHz and a gain of 68 dB. To avoid a diagonal measurement and an overestimation of muscle size, probe position was closely monitored to ensure appropriate skin pressure and the angle of the probe by moving the probe against a high density foam pad in the transverse plan. To determine muscle CSA, the medial (MG) and lateral gastrocnemius (LG) and the tibialis anterior (TA) were scanned using panoramic ultrasound B mode imaging. For measurements of fascicle length (FL) and pennation angle (PA), the angle of the transducer was adjusted visually to optimize the muscle fascicles and the intramuscular tendon contained within the field of view.[47] Fascicle length and PA of the MG, and LG were measured using panoramic ultrasound B mode imaging, with probe displacement being controlled for by maintaining that the center of the image was at the same location as the CSA measurement. All images were digitized and evaluated using Image J 1.37 software (National Institutes of Health, Bethesda, MD). Muscle CSA was calculated as the area of the manually traced outline of the muscle just inside the surrounding fascia using the polygon function in Image-J. Using the segmented-line function, fascicles will be traced continuously from their origin (deep aponeurosis) to insertion (superficial aponeurosis) just inside the surrounding fascia of the MG, & LG. Pennation angle was measured using the angle tool and defined as the angle between muscle fascicle and the deep aponeurosis. Two fascicles were measured per muscle and the average FL and PA were used for subsequent analysis.[69]

Electromyography

Surface electromyography (EMG) was used to monitor muscle activity during the ROM, twitch-interpolation and isokinetic strength assessments. Pre-gelled bipolar surface electrodes (EL503,Biopac systems inc., Goleta, CA, USA) with an inter-electrode distance of 25 mm were be placed on the prominent bulge of MG, and Sol in accordance with the SENIAM guidelines.[46] A disposable electrode was placed over the tibial tuberosity to serve as a reference electrode. To reduce inter impedance and improve the signal to noise ratio, the skin was shaved, abraded and cleaned with isopropyl alcohol prior to electrode placement. Inter-electrode impedance was kept below 5 kΩ as measured by a multimeter.

Range of Motion and Stiffness Assessment

The passive ROM of the plantar flexors was determined for each participant on every testing day using a Humac Norm dynamometer (CSMI Medical Solution, Stoughton MA) programmed in the passive mode. Participants were seated with the leg extended and restraining straps over the pelvis, shoulders, and contralateral thigh and the lateral malleolus aligned with the axis of rotation of the dynamometer. The foot was secured to a foot plate through a thick rubber heel cup and straps over the toes and metatarsals (distal to the malleoli so that they did not impede any passive foot movement). To examine changes in position, a 150 mm twin axis goniometer (BIOPAC Systems, INC., Santa Barbara, CA, USA) was adhered to the skin just proximal to the lateral malleolus on the distal aspect of the fibula and on the fifth metatarsal of the lateral aspect of the foot. The dynamometer lever arm was passively dorsiflexed the foot at an angular velocity of $5°\cdot sec^{-1}$ until the participants maximum tolerable ROM was reached then immediately returned to -20° of plantar flexion. Active ROM was assessed as the maximal

amount of dorsiflexion the participant can achieve by actively contracting their dorsiflexors. Maximal passive and active ROM was calculated as the ROM attained from 0° to the end ROM.

Passive stiffness was quantified using a fourth-order polynomial regression model thatwas fit to the gravity corrected passive angle-torque curves generated during the passive ROM assessment according to the procedures described by Nordez et al.[80] Passive stiffness values ($Nm \cdot deg^{-1}$) were determined at 10° dorsiflexion (common stiffness), and at 80% of maximum ROM (relative stiffness). EMG onset was determined as the point at which EMG amplitude of the MG or Sol exceeds three times the baseline EMG standard deviation for longer than 100 ms.[10] Blazevich et al[10] suggested the onset of EMG activity may indicate an appendicular tonic stretch reflex feedback mechanism which may be altered during supplementation.[92]

Isokinetic Testing

Isokinetic peak torque (PT) was determined during three consecutive maximal isokinetic muscle actions through a spectrum of velocities (30, 90, & $180° \cdot sec^{-1}$).[29] Velocities were randomized and a two minute rest period was given between tests. The footplate was passively moved into dorsiflexion at a velocity at $60° \cdot sec^{-1}$ until a verbal cue was given by the participant at the point of which the participant felt a slight stretch. The participants then immediately performed a rapid and forceful contraction of their plantar flexors until they reached 20^0 of plantar flexion. Peak torque during isokinetic contractions was measured during the load range (zero acceleration)[14] that occurs at $\pm 10^o$ of neutral (0^o).[68]

Twitch Interpolation Procedure

Isometric peak torque (PT), percent voluntary activation (%VA), and peak rate of torque development (RTDpeak), each participant performed three 5-s isometric maximal voluntary contractions (MVCs) of the plantar flexors at a neutral joint angle (90º) with two min of rest between trials. The participants were instructed to push as hard and fast as possible and strong verbal encouragement was provided by the investigators. To determine %VA, transcutaneous electrical stimuli was delivered to the tibial nerve using a high-voltage (maximal voltage= 400 V) constant-current stimulator (Digitimer DS7AH, Herthfordshire, UK). The stimuli was applied via bipolar surface electrodes that were be placed in the popliteal space over the tibial nerve.[15] Single stimuli was used to determine the optimal probe location (50 mA) and the maximal compound muscle action potential (M-wave) with incremental amperage increases (2 -100 mA). Once a plateau in the peak-to-peak M-wave was determined, despite amperage increases, 20% is added to the amperage that yielded the highest peak-to-peak M-wave to assure as supramaximal stimulus. Doublets (2 single 1-ms square wave impulses) were administered with the supramaximal stimulus intensity during the MVC trials to increase the signal-to-noise ratio and minimize the series elastic effects on torque production.[18] In accordance with the twitch interpolation procedure, a supramaximal doublet was administered approximately 500 ms into the MVC plateau (superimposed twitch) and then again 3-5 s after the MVC trial at rest (potentiated twitch). %VA is calculated with the equation provided by Allen et al.[2]

$$\%VA = \left[1 - \left(\frac{Superimposed\ twitch}{Potentiated\ twitch}\right)\right] \times 100$$

PT was obtained from the average of the first 500 data points preceding the superimposed twitch. RTDpeak was determined from the highest slope value (torque/time) for

any 50ms epoch from the onset of torque production. Three resting twitches were administered prior to MVC testing. From the resting twitches, peak twitch torque (PT_{twitch}), peak twitch RTD (RTD_{twitch}), and twitch half relaxation time (HRT) were calculated. PT_{twitch} was calculated as the highest means of 20 consecutive data points that occurs at the apex of the twitch. RTD_{twitch} was the highest slope value for any 20 consecutive data points during the resting twitches. HRT was calculated as the time it takes the twitch to relax to 50% of its PT.

Proton Magnetic Resonance Spectroscopy

A randomly selected subset of 8 participants underwent proton magnetic resonance spectroscopy (MRS) scans to determine the changes in total Cr levels in the muscle during loading and the subsequent washout period. All proton MRS scans were performed on a 3T MR system (Magnetom Trio, Siemens Healthcare) using a flexible phased array limb matrix coil. All MR imaging and proton MRS sequences that were used in this study are FDA approved. MRS consisted of localizer axial, sagittal and coronal images followed by an axial fat-suppressed fast spin-echo T2 weighted sequence (TR/TE, 2886/100 msec; field of view 200 mm, 5 mm slice thickness) of the gastrocnemius of each participant which were used for MRS voxel placement. A single voxel (40 x 40 x 40 mm^3) was positioned so that the voxel was located entirely within the muscle. Adjacent subcutaneous and intermuscular fat along with boney structures were excluded as much as possible.

Proton MRS scans were performed using a point resolved single volume sequence (SV-PRESS). For the measurement of Cr, the SV-PRESS sequence (TR/TE,2000/135) was applied twice over the medial aspect of the lower leg (same placement as ultrasound measurements), once with water suppression (128 averages) and once without water suppression (16 averages).

Automatic field shimming and manual shimming were used to obtain a full-width and half-height of less than 0.1 ppm of the water peak will be performed to optimize the signal intensity. The scanning times ran less than four minutes for the water-suppressed scan and less than one minute for the scan obtained without water suppression.

Signal Processing

The EMG, torque, and position signals were recorded simultaneously with a Biopac data acquisition system (MP150WSW, Biopac Systems, Inc., Santa Barbara, CA). The torque (Nm) and velocity ($°\cdot sec^{-1}$) signals from the isokinetic dynamometer, position signal from the electrogoniometer (°), and EMG (μV) signals were sampled at 2 KHz. All digitized signals were stored on a personal computer (Think Pad T420, Lenovo, Morrisville, NC) and processed off-line with custom-written software (LabVIEW 8.5). For the ROM assessments: torque, position and velocity signals were filtered with a low pass (10 Hz) zero-phase shift 4^{th} order Butterworth filter. For the strength assessments: torque, position and velocity signals were filtered with a low pass (50 Hz) zero-phase shift 4^{th} order Butterworth filter[16]. The EMG signal were bandpass filtered with a zero phase shift 4^{th} order Butterworth filter with cutoff frequencies of 10 – 500 Hz. The torque signal was gravity corrected for the weight of the footplate. During the isokinetic testing, EMG amplitude was calculated as the root mean square (RMS) of same epoch of peak torque and normalized to the maximal M-wave (M_{max}) per the recommendations of Maffiulette et al[67] for all isokinetic velocities. M-waves amplitudes (M_{max}) were calculated as the RMS values as recommended by Arabadzhiev et al.[3]

Proton MRS data was analyzed using AMARES[79] which is an advanced method for accurate, robust, and efficient spectral fitting and is part of the MR user interface (jMRUI, The

training and mobility researchers of the European Union network programmers). Spectra were processed with a zero-order phase correction based on the water peak. The peak integral values of the creatine signal and the non-suppressed water signal were quantified at 3.0 and 4.7 ppm, respectively. The quality of the spectra were visually assessed on the basis of a discrete creatine peak at 3.0 ppm (creatine linewidth of 10 Hz), and clear separation between creatine and choline peaks with no artifacts.

Statistical Analyses

Multiple two-way mixed factorial analysis of variances (ANOVAs) [6 x 2; time (pre, days 2, 4, 6, 20, 35) x group (creatine vs. placebo)] were used to analyze the effects of Cr loading and the washout period on all body fluid compartments (TBW, ICW, and ECW), muscle architecture (CSA, FL, and PA), isometric strength (PT and RTD_{peak}), muscle activation (%VA and normalized EMG amplitude during all isokinetic velocities), resting twitch variables (RTD_{twitch}, PT_{twitch}, HRT, M_{max}), isokinetic PT at (30, 90,180°·sec^{-1}), active and passive ROM, passive stiffness values, and EMG onset during the passive ROM assessment. Bonferroni-corrected pairwise comparisons were used to address the main effects for time. The alpha level was set at $P < 0.05$, and all analyses were performed with SPSS version 19.0 (SPSS, Inc., Chicago, IL).Reliability for main dependent variables where evaluated using model 2,1 to determine intraclass correlation coefficient (ICC), standard error of measurement (SEM) and percent SEM (%SEM).

CHAPTER IV

RESULTS

Demographics

Both groups where similar at day 0 in stature, body mass, age, and activity participation (P>0.055; Table 5). For body mass, there was no interaction (P=0.449) and no main effect for group (P=0.134), however there was a main effect for time (P=0.004). Body mass increased at day 4 and remained higher for the remainder of the time points when compared to day 0 (P=0.003) and day 2 (P=0.012) for all participants (Figure 1). For FFM, there was no interaction (P=0.120), main effect for group (P= 0.057), or main effect for time (P=0.648). For FM, there was no interaction (P=0.872), main effect for group (P= 0.938), or main effect for time (P=0.68). For all dependent variables mean± SD are reported in tables 6 and 7. Test-retest reliability statistics for all the main dependent variables are reported in table 8.

Body Fluid distribution

For TBW, there was no interaction (P=0.348), main effect for group (P= 0.057), or main effect for time (P=0.120) (Figure 2A). For ECW, there was no interaction (P=0.106), and no main effect for group (P= 0.106), or time (P=0.423) (Figure 2B). For ICW, there was no interaction (P=0.508), and no main effect for group (P= 0.590), or time (P=0.172). (Figure 2C) In addition, there was no interaction (P= 0.128), and no main effect for group (P=0.982), or time

(P=0.690) for specific gravity. Cr supplementation did not influence fluid distribution during and following Cr loading and hydration status was consistent for all participants.

Muscle Size and Architecture

For MG CSA, there was no interaction (P=0.452), and no main effect for group (P=0.299), or time (P=0.632) (Figure 3A). For the LG CSA, there was no interaction (P=0.798), and no main effect for group (P=0.540), or time (P=0.682) (Figure 3B). For TA CSA, there was no interaction (P=0.327), and no main effect for group (P=0.360), or time (P=0.052) (Figure 3C). Cr supplementation did not influence muscle CSA during and following Cr loading.

For MG fascicle length, there was no interaction (P=0.201), and no main effect for group (P=0.390), however there was an effect for time (P=0.013). However, follow up pairwise comparisons yielded no significant differences (P>0.075) (Figure 4A). For LG fascicle length, there was no interaction (P=0.356), and no main effect for group (P=0.444), or time (P=0.341). (Figure 5B) For MG pennation angle, there was no interaction (P=0.283), and no main effect for group (P=0.266), or time (P=0.114) (Figure 5A). For LG pennation angle, there was no interaction (P=0.846), and no main effect for group (P=0.289), or time (P=0.341) (Figure 5B). Cr supplementation did not influence muscle architecture during and following Cr loading.

ROM assessments

For passive ROM, there was no interaction (P=0.149), and no main effect for group (P=0.245), however there was an effect for time (P=0.020) with day 20 having greater ROM than day 6 (P=0.014) (Figure 6A). For EMG onset, there was no interaction (P=0.743), and no main effect for group (P=0.522), or time (P=0.224) (Figure 6B). EMG onset was determined

with 32 participants (n=16 for each group) since eight did not exhibit EMG onset during the ROM assessment. For active dorsiflexion ROM, there was no interaction (P=0.785), and no main effect for group (P=0.0362), however there was an effect for time (P=0.035). However, follow up pairwise comparisons yielded no significant differences (P>0.254) (Figure 6C). Cr supplementation did not influence active or passive ROM during and following Cr loading.

For common passive stiffness, there was no interaction (P=0.478), and no main effect for group (P=0.769), or time (P=0.067) (Figure 7A). For relative passive stiffness, there was no interaction (P=0.462), and no main effect for group (P=0.703), however there was an effect for time (P=0.013) with day 20 being stiffer then day 0 (P=0.006) (Figure 7B). Cr supplementation did not influence stiffness during and following Cr loading.

Isokinetic Strength

For isokinetic PT at $30°\cdot sec^{-1}$, there was no interaction (P=0.931), and no main effect for group (P=0.574), however there was an effect for time (P=0.036). However, follow up pairwise comparisons yielded no significant differences (P>0.078) (Figure 8A). For isokinetic PT at $90°\cdot sec^{-1}$, there was no interaction (P=0.971), and no main effect for group (P=0.941), however there was a main effect for time (P<0.001) with day 20 having higher values of torque then day 0 (P=0.001) (Figure 8B). For isokinetic PT at $120°\cdot sec^{-1}$, there was no interaction (P=0.967), and no main effect for group (P=0.629), however there was a main effect for time (P<0.001) with day 20 and 35 having higher PT values then baseline (P=0.001) (Figure 8C). Cr supplementation did not influence isokinetic strength during and following Cr loading.

For the MG EMG amplitude during the isokinetic test at $30°\cdot sec^{-1}$, there was no interaction (P=0.572), and no main effect for group (P=0.284), however there was an effect for

time (P=0.002), with day 35 being more active then day 20 (P=0.030) (Figure 9A). For the MG EMG amplitude during the isokinetic test at 90°·sec^{-1}, there was no interaction (P=0.987), and no main effect for group (P=0.927), or time (P=0.556) (Figure 9B). For the MG EMG amplitude during the isokinetic test at 120°·sec^{-1}, there was no interaction (P=0.133), and no main effect for group (P=0.268), or time (P=0.383) (Figure 9C). Cr supplementation did not influence isokinetic MG activation during and following Cr loading.

For Sol EMG amplitude during the isokinetic test at 30°·sec^{-1}, there was no interaction (P=0.785), and no main effect for group (P=0.726), however there was an effect for time (P= 0.001). However, follow up pairwise comparisons yielded no significant differences (P> 0.128) (Figure 10A). For Sol EMG amplitude during the isokinetic test at 90°·sec^{-1}, there was no interaction (P=0.939), and no main effect for group (P=0.848), or time (P=0.186) (Figure 10B). For Sol EMG amplitude during the isokinetic test at 120°·sec^{-1}, there was no interaction (P=0.307), and no main effect for group (P=0.346), or time (P=0.639) (Figure 10C). Cr supplementation did not influence isokinetic Sol muscle activation during and following Cr loading.

Isometric MVC PT and %VA

For maximal isometric MVC PT, there was no interaction (P=0.364), and no main effect for group (P=0.708), however there was an effect for time (P=0.001) with baseline being stronger then at day 35 (P=0.001) (Figure 11A). For the RTD, there was no interaction (P=0.995), and no main effect for group (P=0.989), or time (P=0.459). (Figure 11B) For % VA there was no interaction (P=0.099), main effect for group (P=0.286), however there was an effect for time (P<0.001), with days 20 and 35 being less active then days 0-6 (P=0.001-0.015)

(Figure 11B). Cr supplementation did not influence maximal or explosive isometric strength and %VA during and following Cr loading.

For MG EMG amplitude during the MVC, there was no interaction (P=0.995), main effect for group (P=0.647), or effect for time (P=0.674) (Figure 12A). For Sol EMG amplitude during the MVC, there was no interaction (P=0.192), main effect for group (P=0.364), or time (P=0.368) (Figure 12B). Cr supplementation did not influence EMG amplitude of the MG and Sol during and following Cr loading.

Twitch Properties

For the twitch PT, there was no interaction (P=0.354), and no main effect for group (P=0.708), however there was a main effect for time (P<0.001) with day 20 having higher PT values then day 0 (P=0.001) (Figure 12A). For twitch RTD, there was no interaction (P=0.698), and no main effect for group (P=0.070), however there was a main effect for time (P=0.011) with day 6 having a higher RTD then day 2 (P<0.001) (Figure 12B). For HRT there was no interaction (P=0.889), and no main effect for group (P=0.675), or time (P=0.707) (Figure 12C). Cr supplementation did not influence resting twitch properties during and following Cr loading.

MRS

There was no change in MRS values either between groups (P=0.053) or within groups (P=0.305; Table 9). (Note: small sample size n= 8)

CHAPTER V

DISCUSSION

Muscular Strength

The results of this study demonstrated that Cr loading does not influence isometric or isokinetic strength (Figures 8 & 11). These findings are in agreement with previous research that reported no improvement in isometric[8,40,52,95,98,104] or isokinetic [21,55,90] PT during Cr loading. However, two previous studies have reported increases in isometric leg extension strength following the loading period. For example, Urbanski et al.[103] and Medieros et al.[72] reported a 4.2 and 6.8% increase in isometric strength , respectively. The discrepancies between these two studies and our findings may be due to differences in muscle size (leg extensors vs. plantar flexors). These authors have speculated that larger muscles have a greater demand for Cr[103], as increases in strength were reported in the leg extensors but not grip strength following Cr loading. However, others who have studied both larger[21,55,90,98] and smaller[39,50,51,100] muscle groups reported that Cr had no effect on isometric strength.

For isokinetic strength, the results of this study (Figure 8) are in agreement with previous literature that have demonstrated Cr loading does not influence isokinetic PT[5,21,55,90] at slower velocities ranging from 15 - 120º•sec⁻¹. Although improvements in isokinetic strength have been observed at higher velocities (180 and 240 º•sec⁻¹)[5], other previous studies at a similar velocity[55,90] (180º•sec⁻¹) have reported no significant increases in leg extension PT. It is possible that improvements in plantar flexion isokinetic PT may be seen at higher velocities than those tested in the current study. However, it is important to note that this study was unable to

examine higher velocities since the load range could not be achieved with the limited ROM (~40º) of the ankle compared to the elbow or knee.

One of the objectives of this current study was to evaluate the effect of Cr loading on the speculated mechanisms that may lead to an increase in strength. These mechanisms include: (1) increases in muscle activation[73], (2) increases in muscle size or pennation angle[52,102], and (3) improvements in Ca^{2+} kinetics[5]. All three possible underlying mechanisms were examined in this current study during and following Cr loading (Figures 3-5, 9-13). Similar to the lack of interaction effects found for all strength measures, we also found no significant changes (P>0.05) when compared to the placebo condition in these variables during and following the Cr loading period.

For muscle activation, our findings (Figures 9-11) are in agreement with previous literature that have reported normalized EMG amplitude values and %VA are not influenced by Cr loading[52,53,95]. There is only one previous study[73] that has reported an increase in muscle activation following Cr loading. The differences between these results may be contributed to the normalization procedures, as the studies that have reported no changes examined normalized EMG amplitude [53,95] or %VA, while Medeiros et al.[73] reported raw RMS EMG amplitudes which have been reported to be influenced by physiological and non-physiological factors.[25] Similarly, there was no influence of Cr loading on muscle CSA, PA and fascicle length (Figures 3-5). These measurements were examined based on the speculation of Jakobi et al.[52] who stated that "it is conceivable that an increase in ICW[102] could affect pennation of the muscle fibers which might affect over all force output"(pg. 458). To our knowledge, is the current study is the only study to examine muscle architecture during and following Cr loading while simultaneously

measuring water distribution. These findings are not surprising given there was no reported changes in ICW.

Lastly, it has been suggested that Cr loading would improve Ca^{2+} kinetics by enhancing ATP availability and thus increase the Ca^{2+}-ATPase pump and subsequently improve cycling rates[5,104]. These variables are often examined indirectly through evoked twitch conditions[44]. However, our findings indicated that there was no change in evoked twitch properties (PT_{twitch}, RTD_{twitch}, & HRT) during and following Cr loading (Figure 13). The majority of papers examining twitch properties following Cr loading have reported no improvements in PT_{twitch}, RTD_{twitch}, HRT, and time to PT[52,53,95]. However, there is one previous study[5] that has reported an increase in PT_{twitch} which may be due to the stimulation procedure utilized. For example, Bazzuchi et al.[5] used a train of stimuli (10 single impulses for 469 μs) compared to single square wave impulses (50-200 μs) that are commonly used in the literature[52,53,95].

Range of Motion

We are aware of only one study that has examined the influence of Cr supplementation on flexibility. Sculthrope et al.[92] examined the influence of Cr loading on a number of upper and lower body active ROM assessments and reported a decrease in shoulder extension (~9º) and flexion (~9º), as well as dorsiflexion (~2º). They speculated that the Cr induced increase in muscle cell volume (i.e. increase in ICW) would either result in an increase in the resistance to stretch thereby increasing overall muscle stiffness, or influence neural outflow from the muscle spindles causing them to be activated earlier in a given ROM. The results of our study demonstrated that both active and passive ROM (Figure 7) were not altered during and following Cr supplementation. In addition, we found there was no change in ICW (Figure 2), passive stiffness at a common and relative joint angles (Figure 8) or EMG onset (Figure 7) which may

indicate muscle spindle activity was not altered. Furthermore, Watsford et al. [111] previously examined the influence of Cr loading on active musculotendinous stiffness of the plantar flexors and reported that Cr supplementation does not alter stiffness. Collectively, these findings may suggest that Cr loading has little influence on altering the passive resistive characteristics of the plantar flexor muscles.

Body Mass

It has often been reported that Cr supplementation increases body mass in men ranging from (1-3 kg)[61,97,107] following a traditional loading period. It is believed that the increase in body mass is a result of the osmotic load caused by Cr retention in the muscle[102]. Our findings indicated that there were no significant changes in body mass when compared to the placebo condition (Figure 1). The Cr group demonstrated changes of (1.1±1.1 kg) following the 5 day loading period which is within the range of commonly reported gains in body mass[62]. While Cr supplementation has been examined extensively[97], very few studies examine changes in muscle Cr stores to verify and often indirectly use changes in body mass to support these changes. Using a subset of participants (n=8) from this study, we examined total muscle Cr content with MRS technology[88]. It is apparent that muscle Cr stores increased during the loading period and declined through day 20 and 35, but where remained above day 0 values (Figure 14). The placebo group demonstrated no increase in Cr levels from day 0. These findings may suggest that Cr stores increased during the traditional loading period and slowly returned to baseline 35 days later which are consistent with the findings of Hultman et al.[48] who showed that Cr levels peak after loading and slowly decline after day 35.

Conclusion

Overall, our findings demonstrate that Cr loading alone does not influence neuromuscular function. These finding support the work of previous authors who reported no changes in isometric [8,40,52,95,98,104] or isokinetic [21,55,90] strength following Cr loading. The few studies[5,73,103] that have reported improvements in strength have only merely speculated the possible underlying mechanisms. Thus, one of the main goals of this study was to address all of these possible mechanisms that may lead to an increase in muscle strength with Cr supplementation. Our results demonstrate that muscle activation, muscle architecture, and Ca^{2+} kinetics measured indirectly through twitch properties remained unchanged during and following Cr loading. Taking into consideration the results of this study and the current body of literature, Cr loading alone has little influence on neuromuscular parameters that may improve muscular strength.

A novel finding of this study was that Cr loading did not influence passive ROM and stiffness. Previous authors have measured active ROM[92] and stiffness[111], separately, and reported a decrease in ROM and no change in active stiffness using the oscillation technique in the plantar flexor muscles. Sculthrope et al.[92] speculated that an increase in muscle size as a result of Cr loading would increase stiffness and/or muscle spindle activity thereby reducing active ROM. However, our results demonstrated that muscle CSA, passive stiffness and EMG onset (an indirect assessment of spindle activity) where not influenced by Cr loading. As a result of this study it appears Cr loading does not influence neuromuscular components that influence flexibility. Previous research has speculated that non-significant results partially could be contributed to "responders" vs "non-responders"[59,101]. It is believed that up to 30% of the population are "non-responders" which have limited Cr absorption by the muscle as defined as

less than 10 mmol·kg^{-1} dw increase in Cr stores[37]. Therefore, it is possible that many of our participants were "non-responders", however, this topic is still not well understood and warrants more investigation using techniques to examine changes in muscle Cr levels.

TABLES

Table 1: The influence of Cr supplementation on neuromuscular function

Authors	Year	Journal	Dosage	Sample	Testing	Outcome variables	Result	Explanation
Bazzucchi et al.	2009	Med Sci Sport Exerc	4 ×5g·day^{-1} x 5d	Cr:8, Pl: 8 moderately active males	Isokinetic Elbow flex w/ Evoked twitch	Evoked/volunteer PT, force velocity relationship, conduction velocity (15,30, 60,90,120,180, &240^{0}·s^{-1})	↑PT with twitch, PT at higher velocity &CV,↔ all others	Increase in neuromuscular function attributed to an increase CA+ cycling rate
Bemben et al.	2001	Med Sci Sport Exerc	4 ×5g·day^{-1} x 5d	Cr: 11, Pl: 8 Healthy non-active males	Elbow flex/ext. knee flex ext	Max force and time to MF,RFD, Impulse and % force decrement	↔ in any variables	Larger muscles may not benefit
Deutekom et al.	2000	Eur J Appl Physiol	4 ×5g·day^{-1} x 6d	Cr: 11, PL: 12 well trained rowers	Leg Cycle Erg and Knee ext	30 s cycle sprints, Isokinetic (180^{0}· s^{-1})and isometric Strength	↔ in any variables	No effect for Cr alone need a training stimulus
Greenhaff et al	1993	Clinical Science	4 ×5g·day^{-1} x 5d	Cr: 6, PL: 6 active 9 males, 3 females	Leg ext	PT (5 × 30 at 180^{0}· s^{-1})	↑ at all reps	Cr phosphate resynthesis
Hamilton et al.	2000	Int J Sport Nutr Exerc Metab	5×5g·day^{-1} x 7d	Cr: 11, PL: 13 active females	Elbow flex. Shoulder Int Rot	PT, Ecc PT, # of reps, 1 RM	↑# of reps by elbow flexors, ↔ Any other measure	more of a lower body effector
Jakobi et al.	2001	Eur J Appl Physiol	4 ×5g·day^{-1} x 5d	Cr: 7, PL: 5 active older men	Elbow flex w/evoked twitch	MVC, EMG, % activation, HR, RT	↔ In any variable	More of a long term change
Jakobi et al.	2000	Exp Physiol	4 ×5g·day-1 x 5d	Cr: 7, PL: 7 active males	Elbow flex w/ evoked twitch	MVC, % activation, peak twitch, TTPT, half Relaxation time, tensions, HRT	↔ in any variable	Cr does not affect contractile properties
Kambis et al.	2003	Int J Sport Nutr Exerc Metab	0.5g/kg·day^{-1} x 5d	Cr: 11, PL: 11 active females	Leg ext/flex	isokinetic leg ext./flex 5 at 60^{0}· s^{-1}and 50 at 180^{0}· s^{-1}	↑power and ↓ in TTPT	Increase in ATP in type II fibers

Table 2: The influence of Cr Supplementation on neuromuscular function cont…

Authors	Year	Journal	Dosage	Sample	Testing	Outcome variables	Result	Explanation
Maganaris et al.	1998	Acta Physiol Scand	2 ×5g·day^{-1} x 5d	Cr: 10 healthy males (1st five days or 2nd)	Leg ext	Isometric MVC, submaximal fatigue bouts	↑ Max PT, PT pre repetition	Neural drive, protein synthesis
Medeiros et al.	2010	Brazil J Sports Med	4 ×5g·day^{-1} x 6d	Cr: 13, PL: 14 active females	Knee ext	MVC, EMG, repeated bouts of isometric tests	↑PT across trials and RMS	resynthesis of ATP
Rousouw et al.	2000	Nutr Res	3 ×3g·day^{-1} x 6d	Cr: 8, PL: 5 trained power lifters	Leg ext, Dead Lift	Isokinetic (180^{o}·s^{-1}), dead lift volume	↑ 1 RM, power and Reps, ↔ PT	More availability of PCr and ATP resynthesis
Smith-Ryan et al.	2014	Med Sci Sport Exerc	4 ×5g·day^{-1} x 5d	Cr: 6, PL: 6 active females	Plantar flex w/ evoked twitch	MVC, % Activation, PTF, RTD, HRT, M wave	↔in any variable	Isometric may not be a good measure
Stevenson et al.	2001	J Strengh Cond Res	4 ×5g·day^{-1} x 7d	Cr: 18, PL: 13 active (29 males, 2 females)	Knee ext	MVC, RTD, T max, reaction time	↔ in any variable	systems tested don't utilize PCr system
Urbanski et al.	1999	Int J Sport Nutr	4 ×5g·day^{-1} x 5d	Cr: 10 healthy males (1st five days or 2nd)	Knee ext	MVC, Time to fatigue	↑PT & TTF	increase in protein synthesis
Van Leemputte et al.	1999	J App Physiol	4 ×5g·day-1 x 5d	Cr: 8, PL: 8 active males	Elbow flex w/ evoked twitch	PT, contraction time, RT	↔ PT, ↓RT	Increase in Ca+ uptake

Table 3: The influence of Cr supplementation on ROM and stiffness

Author	Year	Journal	Dosage	Population	Testing	Outcome variables	Result	Explanation
Scuthrope et al.	2010	Appl Physiol Nutr Metab	5 ×5g·day^{-1} x 5d	Cr: 20, PL: 20 active males	Active ROM	Shoulder ROM, HIP ROM, Knee flex, dorsiflex & platar flex	↓Shoulder flex & ext, dorsiflex ↔ any other ROM	Increase in ICW of asymmetric muscle cause decreased ROM
Watsford et al.	2003	J Strengh Cond Res	4 ×5g·day^{-1} x 7d	Cr: 9, PL: 11 active males	Jump height, plantar flex	Jump Height, PF, RFD, Active stiffness	↔ in any variable	Increase in ICW does not increase stiffness

Table 4: The influence of Cr supplementation on fluid distribution

Author	Year	Journal	Dosage	Population	Testing	Outcome variables	Result	Explanation
Bembem et al.	2001	Med Sci Sport Exerc	4 ×5g·day^{-1} x 5d*	Cr: 11, Pl: 8 Healthy non-active males	BIS	TBW, ICW, ECW	↔ in any variable	*Loaded/maintenance measured day 10
Kern et al.	2001	J Exerc Physiol	4 ×5.25g·day^{-1} x 5d	Cr: 10, PL: 10 healthy males	BIA-Heat related	TBW	↑TBW	Increase in ICW leads to changes
Power et al.	2003	J Athl Train	5 ×5g·day^{-1} x 7d	Cr 16, PL: 16 (8 males & 8 females in each)	D2O & sodium bromide dilution, BIA	TBW, ICW	↑TBW, ↔ ICW	Cr osmotic properties, BIA & D2O had same results
Weiss et al	2006	J Sports Med Phys Fit	5 ×5g·day^{-1} x 5d	Cr: 12, PL: 12 healthy males	BIA	TBW, ICW, ECW	↑ in all variables ~ 1.5L	Osmotic Properties
Ziegenfuss et al.	1998	J Exerc Physiol	0.35g/kg of FFMx 3d	Cr: 10 crosstrained males	BIA	TBW, ICW, ECW	↑TBW,ICW~ 0.8L↔ ECW	Plasma Na osmotic pressure

Table 5: Participant demographics and amount of time per week exercising (mean ± SD)

	Age (yrs)	Stature (cm)	Mass (Kg)	Aerobic training (hrs)	Resistance training (hrs.)
Placebo (n=20)	20.3±2.1	176.9±6.4	74.1±7.2	2.1±1.8	2.8±2.2
Creatine (n=20)	20.4±2.3	176.1±5.8	70.7±5.6	2.8±1.8	2.1±1.8

Table 6: Dependent variables (mean± SD) for all days)

		Day 0	Day 2	Day 4	Day 6	Day 20	Day 35
Mass (Kg)	Pl	74.2 ± 7.2	74.3 ± 7.3	74.4 ± 7.2	74.3 ± 7.1	74.7 ± 8.2	75.1 ± 8.4
	Cr	70.6 ± 5.6	70.6 ± 6.1	71.1 ± 5.9	71.5 ± 5.8	71.6 ± 6.1	71.8 ± 6.3
Specific Gravity ($kg \cdot m^{-3}$)	Pl	1.0 ± 0.4	1.0 ± 0.6	1.0 ± 0.5	1.0 ± 0.4	1.0 ± 0.5	1.0 ± 0.3
	Cr	1.0 ± 0.4	1.0 ± 0.5	1.0 ± 0.4	1.0 ± 0.5	1.0 ± 0.6	1.0 ± 0.5
Fat Free Mass (Kg)	Pl	60.6 ± 6.3	60.8 ± 6.4	60.9 ± 5.9	60.9 ± 6.2	59.6 ± 7.9	61.3 ± 7.2
	Cr	57.3 ± 5.9	56.5 ± 4.2	58.0 ± 4.4	58.9 ± 5.2	56.5 ± 6.1	57.5 ± 4.1
Fat mass (Kg)	Pl	13.3 ± 4.4	13.3 ± 4.9	13.5 ± 5.0	13.7 ± 5.8	14.8 ± 6.1	15.0 ± 5.6
	Cr	13.6 ± 5.3	14.0 ± 4.3	13.0 ± 5.2	13.3 ± 5.0	14.5 ± 6.1	14.4 ± 4.2
Total Body Water (L)	Pl	44.3 ± 4.6	44.5 ± 4.7	44.6 ± 4.3	44.6 ± 4.6	43.7 ± 5.8	44.8 ± 5.3
	Cr	42.0 ± 4.3	41.4 ± 3.1	42.5 ± 3.3	43.1 ± 3.8	41.3 ± 4.5	42.1 ± 3.0
Extracellular Water (L)	Pl	18.3 ± 1.6	18.2 ± 1.9	18.2 ± 1.6	18.3 ± 1.7	17.6 ± 3.0	18.5 ± 2.2
	Cr	17.2 ± 1.5	17.0 ± 1.4	17.5 ± 1.5	17.8 ± 1.6	17.5 ± 1.5	17.3 ± 1.4
Intracellular Water (L)	Pl	26.0 ± 3.5	26.4 ± 3.0	26.5 ± 2.9	26.3 ± 3.1	26.0 ± 3.3	61.3 ± 7.2
	Cr	24.8 ± 3.1	24.3 ± 2.0	25.0 ± 2.1	25.4 ± 2.5	23.8 ± 4.1	24.8 ± 4.1
MG CSA (cm^2)	Pl	13.8 ± 3.3	13.3 ± 3.5	13.3 ± 2.9	14.0 ± 3.2	13.8 ± 3.7	13.3 ± 3.7
	Cr	12.3 ± 1.9	12.6 ± 2.7	12.9 ± 3.0	12.8 ± 3.0	12.8 ± 2.4	12.7 ± 3.0
LG CSA (cm^2)	Pl	5.8 ± 1.8	6.0 ± 1.8	5.7 ± 1.8	5.9 ± 1.9	5.7 ± 1.9	5.8 ± 1.9
	Cr	5.9 ± 1.7	6.3 ± 2.0	6.3 ± 1.9	6.3 ± 1.8	6.2 ± 2.0	6.0 ± 2.2
TA CSA (cm^2)	Pl	4.0 ± 0.9	4.1 ± 0.9	4.1 ± 0.9	4.2 ± 1.0	4.0 ± 0.9	4.2 ± 0.9
	Cr	4.2 ± 1.0	4.2 ± 0.9	4.2 ± 0.9	4.3 ± 0.8	4.4 ± 1.1	4.6 ± 1.0
Fascicle length MG (mm)	Pl	5.8 ± 0.9	6.0 ± 0.9	5.8 ± 0.7	6.0 ± 1.1	5.7 ± 0.8	5.9 ± 0.9
	Cr	5.3 ± 0.7	5.8 ± 0.7	5.5 ± 0.7	5.9 ± 1.1	5.8 ± 0.8	5.7 ± 0.8
Fascicle length MG (mm)	Pl	5.8 ± 0.7	5.8 ± 0.8	5.8 ± 0.6	5.9 ± 1.0	5.6 ± 0.8	5.8 ± 0.5
	Cr	5.3 ± 0.8	5.8 ± 0.9	5.5 ± 0.8	5.9 ± 1.2	5.6 ± 0.9	5.6 ± 1.0
Pennation angle MG (º)	Pl	19.8 ± 3.8	18.3 ± 3.7	20.3 ± 3.6	19.6 ± 4.2	21.5 ± 4.6	18.9 ± 3.2
	Cr	21.0 ± 3.5	19.5 ± 3.7	21.1 ± 3.6	20.7 ± 3.4	19.7 ± 3.6	20.6 ± 3.6
Pennation angle LG (º)	Pl	15.2 ± 3.4	15.5 ± 4.9	15.1 ± 3.6	14.9 ± 3.3	15.6 ± 3.0	15.0 ± 2.2
	Cr	15.6 ± 4.1	16.5 ± 4.2	16.2 ± 3.9	16.2 ± 4.3	17.0 ± 2.9	14.9 ± 3.4
Passive ROM (º)	Pl	18.1 ± 1.1	17.0 ± 1.0	16.7 ± 0.9	19.3 ± 1.6	20.6 ± 1.5*	22.5 ± 1.2
	Cr	20.0 ± 0.2	20.3 ± 0.2	21.3 ± 0.2	19.3 ± 0.2	24.3 ± 0.2	20.7 ± 0.2
Active ROM (º)	Pl	10.1 ± 4.8	9.2 ± 5.4	8.8 ± 5.2	8.3 ± 4.6	8.2 ± 4.4	8.6 ± 4.7
	Cr	13.7 ± 6.1	11.5 ± 4.9	12.1 ± 7.0	12.2 ± 7.4	10.7 ± 7.8	10.7 ± 6.1
EMG onset (º)	Pl	11.9 ± 1.1	7.7 ± 0.6	9.2 ± 0.6	9.6 ± 0.7	10.8 ± 0.9	10.4 ± 1.1
	Cr	11.2 ± 1.2	9.3 ± 0.9	8.4 ± 0.7	10.5 ± 1.1	14.4 ± 2.0	11.6 ± 1.3
Realtive Stiffness ($Nm \cdot ^{o-1}$)	Pl	6.0 ± 2.3	6.8 ± 3.2	6.8 ± 3.6	7.4 ± 2.8	6.9 ± 2.6#	7.6 ± 3.1
	Cr	5.8 ± 3.1	6.3 ± 3.3	5.8 ± 2.7	7.0 ± 2.9	8.1 ± 2.6	7.2 ± 2.2
Common Stiffness ($Nm \cdot ^{o-1}$)	Pl	5.2 ± 3.2	6.8 ± 4.2	6.7 ± 4.6	5.9 ± 4.0	7.6 ± 4.2	7.9 ± 3.6
	Cr	5.8 ± 2.9	5.6 ± 3.2	5.9 ± 4.0	7.2 ± 4.4	7.3 ± 3.9	6.9 ± 4.4

Pl placebo, Cr Creatine, MG medial gastrocnemius, LG lateral gastrocnemius, TA tibialis anterior, CSA cross sectional area, EMG electromyography, ROM range of motion *P≤0.05, significant difference in PT with day 20 > day 6 for both groups, # P≤0.05, significant difference in relative stiffness with day 20 > day 6

Table 7: Dependent variables (mean± SD) for all days cont….

		Day 0	Day 2	Day 4	Day 6	Day 20	Day 35
Isokinetic PT 30°sec·⁻¹ (Nm)	Pl	112.4 ± 29.2	119.7 ± 20.5	119.3 ± 27.8	114.8 ± 20.5	120.5 ± 19.2	123.1 ± 22.2
	Cr	111.5 ± 23.6	112.9 ± 30.9	113.0 ± 21.0	112.0 ± 24.5	118.3 ± 23.0	121.3 ± 21.8
Normalized EMG MG IK 30°sec·⁻¹ (%)	Pl	0.6 ± 0.4	0.6 ± 0.4	0.9 ± 0.9	1.0 ± 0.9	0.6 ± 0.5	1.1 ± 1.1
	Cr	0.9 ± 0.8	0.9 ± 0.8	1.0 ± 0.8	0.9 ± 0.6	0.8 ± 0.7	1.5 ± 1.5
Normalized EMG Sol IK 30°sec·⁻¹ (%)	Pl	2.4 ± 1.8	2.6 ± 2.1	2.2 ± 3.3	2.2 ± 2.4	2.7 ± 2.0	1.9 ± 1.7
	Cr	2.6 ± 2.7	3.2 ± 3.2	2.2 ± 2.3	2.1 ± 2.0	2.8 ± 1.7	3.0 ± 3.3
Isokinetic PT 90°sec·⁻¹ (Nm)	Pl	66.3 ± 17.8	72.0 ± 13.8	71.5 ± 19.4	71.4 ± 16.2	75.6 ± 11.1*	80.7 ± 15.9
	Cr	69.0 ± 18.1	70.1 ± 19.9	70.1 ± 14.9	70.4 ± 18.0	75.4 ± 19.4	80.7 ± 12.5
Normalized EMG MG IK 90°sec·⁻¹ (%)	Pl	1.2 ± 2.9	1.2 ± 2.9	1.0 ± 0.9	1.1 ± 1.0	0.7 ± 0.5	1.2 ± 1.1
	Cr	1.1 ± 1.3	1.1 ± 1.3	1.1 ± 0.9	1.1 ± 0.8	0.9 ± 0.8	1.4 ± 1.4
Normalized EMG Sol IK 90°sec·⁻¹ (%)	Pl	1.5 ± 1.2	1.4 ± 1.2	1.2 ± 2.0	1.2 ± 1.7	2.2 ± 3.8	1.2 ± 1.3
	Cr	1.7 ± 1.7	1.4 ± 1.3	1.1 ± 0.9	1.3 ± 1.5	2.0 ± 1.9	1.7 ± 2.5
Isokinetic PT 120°sec·⁻¹ (Nm)	Pl	53.6 ± 17.2	56.8 ± 17.5	57.3 ± 17.0	56.5 ± 13.1	68.6 ± 9.8	64.3 ± 11.7
	Cr	58.2 ± 12.5	58.1 ± 17.6	57.9 ± 16.6	59.2 ± 20.0	69.5 ± 14.2	65.1 ± 15.9
Normalized EMG MG IK 120°sec·⁻¹ (%)	Pl	1.3 ± 1.0	0.7 ± 0.6	1.0 ± 1.1	1.1 ± 1.0	0.8 ± 0.7	1.2 ± 1.5
	Cr	1.2 ± 1.1	1.4 ± 1.7	1.1 ± 1.0	1.0 ± 0.6	0.9 ± 0.7	1.4 ± 1.5
Normalized EMG Sol IK 120°sec·⁻¹ (%)	Pl	1.3 ± 1.1	1.0 ± 0.9	0.7 ± 0.9	1.3 ± 2.0	1.6 ± 1.9	1.1 ± 1.8
	Cr	1.4 ± 1.7	1.4 ± 1.1	1.4 ± 1.3	1.2 ± 1.5	1.6 ± 1.7	1.6 ± 2.1
Isometric PT (Nm)	Pl	119.3 ± 26.1	114.7 ± 26.9	111.3 ± 27.1	107.4 ± 25.3	111.2 ± 30.3	95.9 ± 21.1
	Cr	116.2 ± 28.0	109.1 ± 34.1	101.1 ± 42.3	105.6 ± 28.3	106.5 ± 32.3	103.8 ± 27.8
% VA (%)	Pl	89.8 ± 10.8	90.0 ± 12.1	85.3 ± 14.3	88.4 ± 12.8	83.7 ± 16.2#	74.7 ± 16.3#
	Cr	95.4 ± 5.6	90.2 ± 12.1	92.1 ± 15.1	87.1 ± 14.2	84.7 ± 17.4	83.5 ± 14.5
RTD (Nm·s⁻¹)	Pl	609.6 ± 179.2	616.7 ± 161.0	560.5 ± 156.2	552.3 ± 123.6	577.7 ± 153.7	623.1 ± 227.4
	Cr	604.0 ± 183.0	605.6 ± 303.0	569.8 ± 138.5	570.1 ± 217.3	589.8 ± 211.1	605.5 ± 205.7
Normalized MG EMG (%)	Pl	1.3 ± 1.0	1.3 ± 1.0	1.4 ± 2.0	1.2 ± 1.8	1.3 ± 1.2	1.1 ± 1.4
	Cr	1.2 ± 1.1	1.2 ± 1.1	1.2 ± 1.0	1.1 ± 0.9	1.3 ± 0.9	0.9 ± 0.8
Normalized Sol EMG (%)	Pl	0.9 ± 0.5	0.9 ± 0.4	0.8 ± 0.6	1.1 ± 0.8	1.2 ± 1.0	0.7 ± 0.6
	Cr	1.2 ± 0.8	1.2 ± 0.8	1.1 ± 0.7	0.9 ± 0.6	1.2 ± 1.0	0.9 ± 0.8
Twitch PT (Nm)	Pl	22.4 ± 6.8	22.4 ± 6.8	23.9 ± 6.6	24.9 ± 6.1	27.3 ± 6.6	26.2 ± 6.8
	Cr	19.5 ± 6.4	19.6 ± 6.4	21.0 ± 5.8	22.5 ± 6.0	24.5 ± 5.7	25.3 ± 6.1
Twitch RTD (Nm·s⁻¹)	Pl	575.9 ± 188.8	575.9 ± 188.8	584.8 ± 206.9	641.4 ± 160.7	693.5 ± 160.1	667.4 ± 198.0
	Cr	481.0 ± 175.5	481.0 ± 175.5	507.0 ± 192.4	584.3 ± 150.5	652.8 ± 177.4	646.2 ± 168.0
HRT (sec)	Pl	83.9 ± 14.6	84.1 ± 14.5	90.2 ± 32.2	92.9 ± 41.7	88.3 ± 29.2	87.1 ± 31.6
	Cr	86.5 ± 12.7	86.5 ± 12.7	83.4 ± 27.1	90.2 ± 22.2	89.2 ± 26.5	80.5 ± 10.3

Pl placebo, Cr Creatine, PT peak torque,IK isokinetic, MG medial gastrocnemius, Sol soleus, EMG electromyography, RTD rate of torque development

*P≤0.05, significant difference in PT with day 20 > day 0 for both groups, # P≤0.05, significant difference in % VA with day 20 and 35 > day 0-6

Table 8: Reliability statistics for the main dependent variables

	ICC2,1	SEM	%SEM	P value
Isometric PT	0.90	7.06	5.93	0.16
Isokinetic PT 30°sec·⁻¹	0.78	15.13	13.51	0.93
Isokinetic PT 90°sec·⁻¹	0.78	7.6	9.50	0.74
Isokinetic PT 120°sec·⁻¹	0.81	9.03	13.28	0.82
Passive ROM (°)	0.84	3.27	18.17	0.78
Active ROM (°)	0.88	1.99	16.58	0.09
Common Stiffness	0.88	1.61	20.38	0.34
% VA	0.83	5.98	6.64	0.71
CSA MG	0.88	1.57	11.38	0.33
Pennation angle MG	0.84	1.92	8.93	0.35
Fascicle length MG	0.76	0.627	10.63	0.46
Total Body Water	0.98	0.99	2.21	0.94
Intracellular Water	0.95	0.87	3.23	0.25
Extracellular Water	0.92	0.66	3.57	0.49
Twitch PT	0.87	3.03	11.10	0.08
HRT	0.94	7.2	7.75	0.06

PT peak torque, CSA crosssection area, ROM range of motion, MG medial gastrocnemius, HRT half relxation time

Table 9: Muscle creatine concentration determined by magnetic resonance spectroscopy (mean ± SD)

	Day 0	Day 2	Day 4	Day 6	Day 20	Day 35
Placebo	67.6± 14.0 (mmols)	64.2± 10.9	64.9± 10.9	60.4± 13.6	56.8.± 9 .1	58.2± 11.8
Creatine	68.9± 11.2	87.6± 18.6	72.6± 11.8	76.6± 10.0	73.5± 9.8	73.3± 3.9

FIGURES

Figure 1:

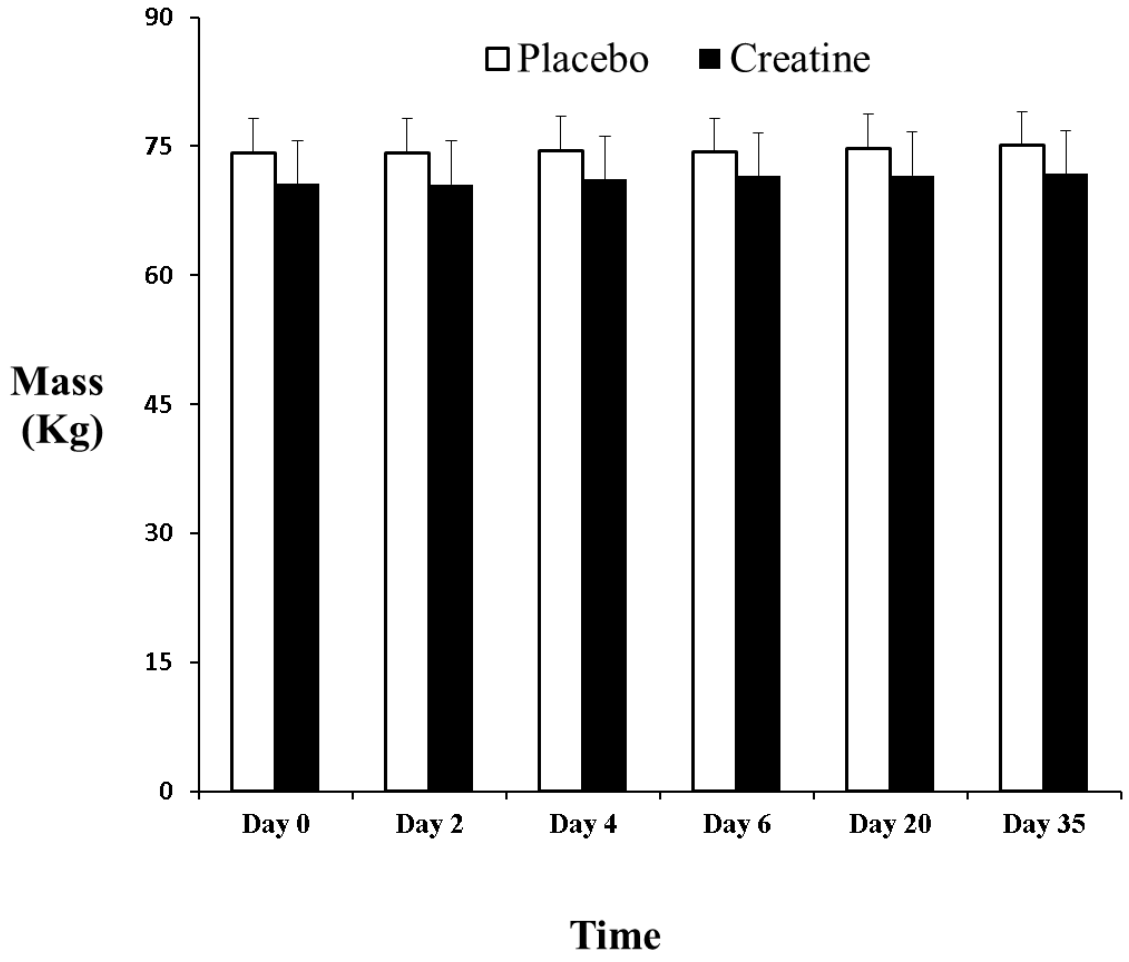


Figure 2:

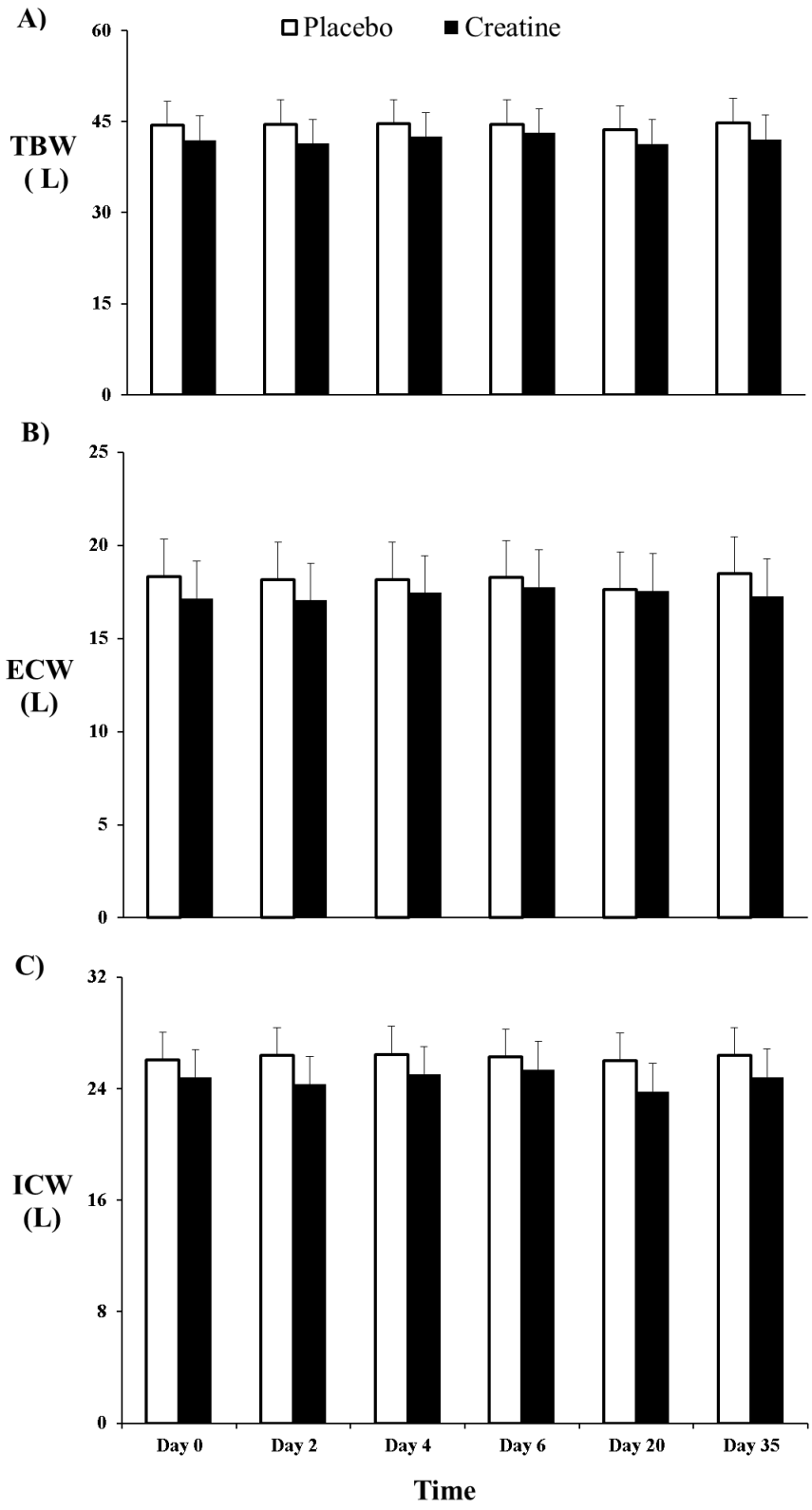

A)
Placebo
Creatine
TBW (L)
60
45
30
15
0
B)
ECW (L)
25
20
15
10
5
0
C)
ICW (L)
32
24
16
8
0
Day 0
Day 2
Day 4
Day 6
Day 20
Day 35
Time

Figure 3:

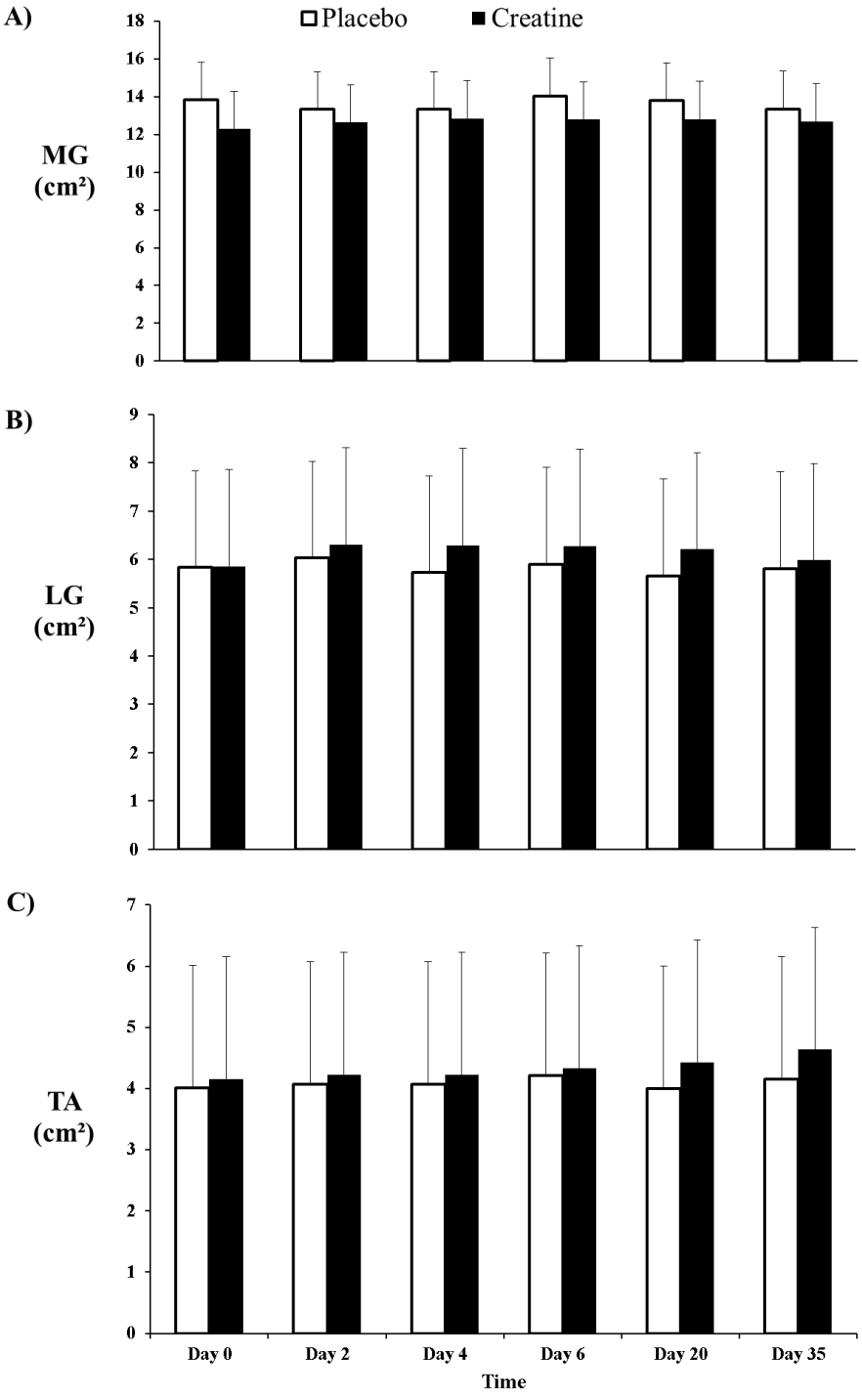

A)
Placebo
Creatine
MG (cm²)
18
16
14
12
10
8
6
4
2
0
B)
LG (cm²)
9
8
7
6
5
4
3
2
1
0
C)
TA (cm²)
7
6
5
4
3
2
1
0
Day 0
Day 2
Day 4
Day 6
Day 20
Day 35
Time

Figure 4:

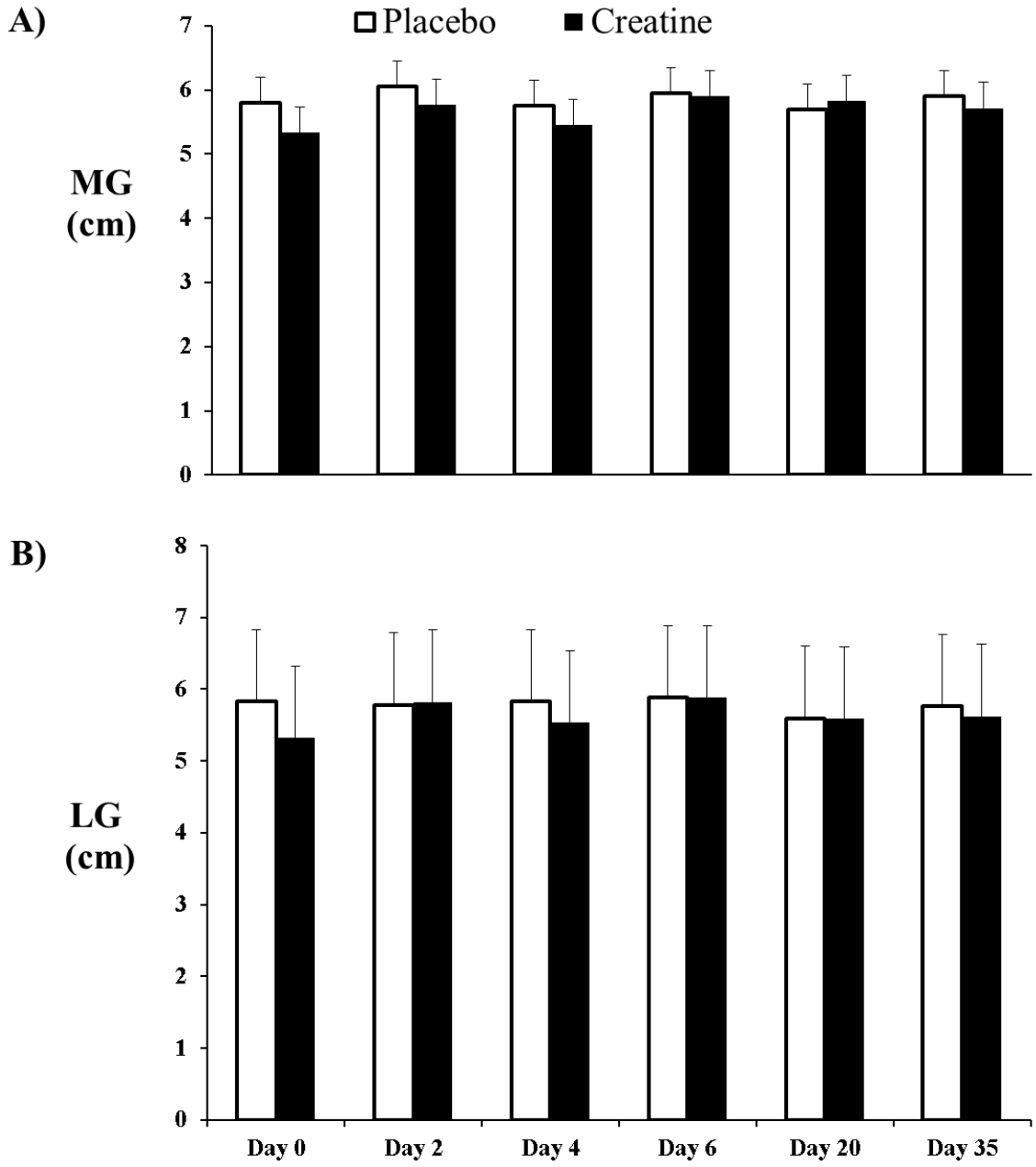

A)
Placebo
Creatine
MG
(cm)
0
1
2
3
4
5
6
7
B)
LG
(cm)
0
1
2
3
4
5
6
7
8
Day 0
Day 2
Day 4
Day 6
Day 20
Day 35

Figure 5:

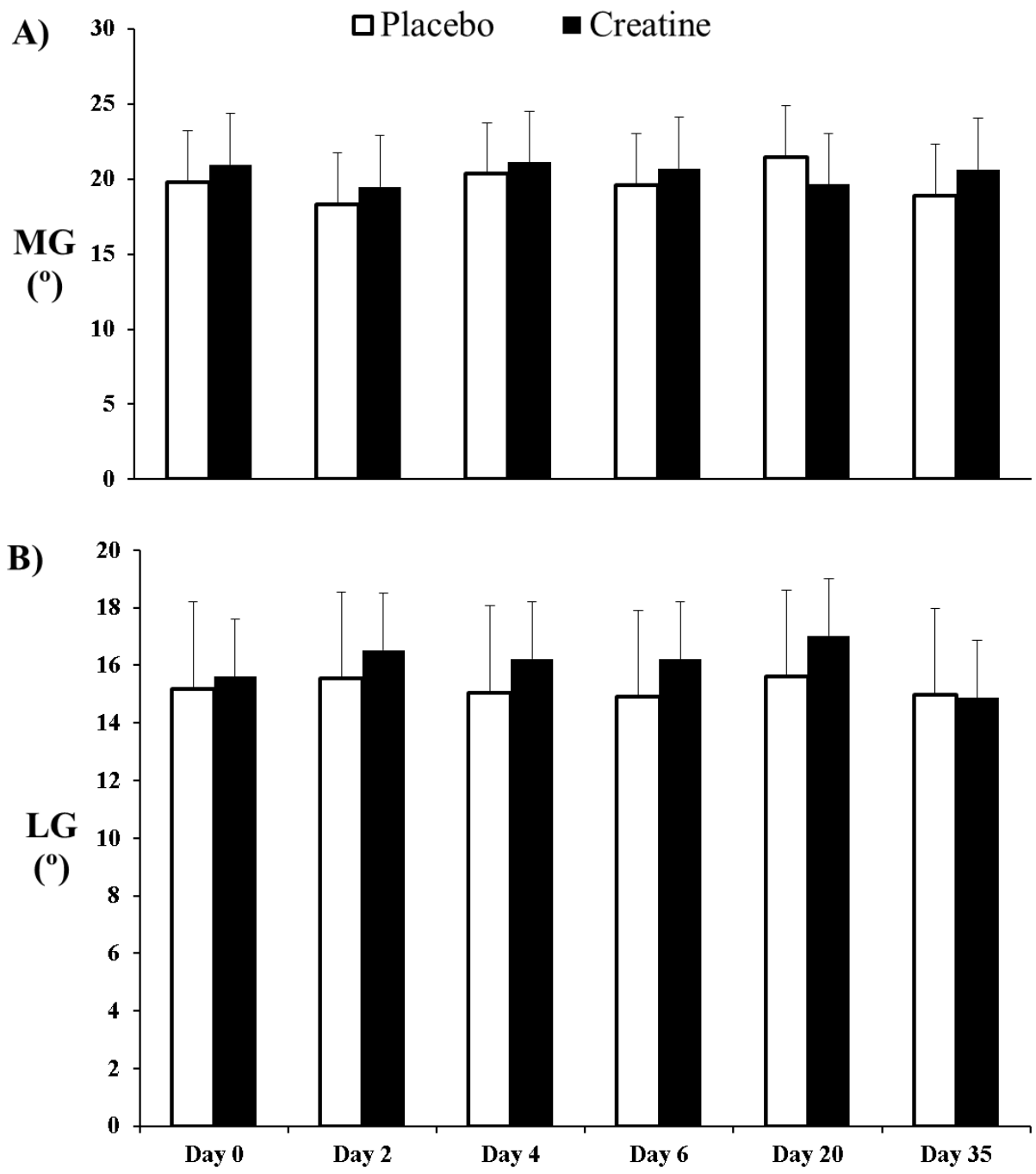

A)
Placebo
Creatine
MG (º)
30
25
20
15
10
5
0
B)
LG (º)
20
18
16
14
12
10
8
6
4
2
0
Day 0
Day 2
Day 4
Day 6
Day 20
Day 35

Figure 6:

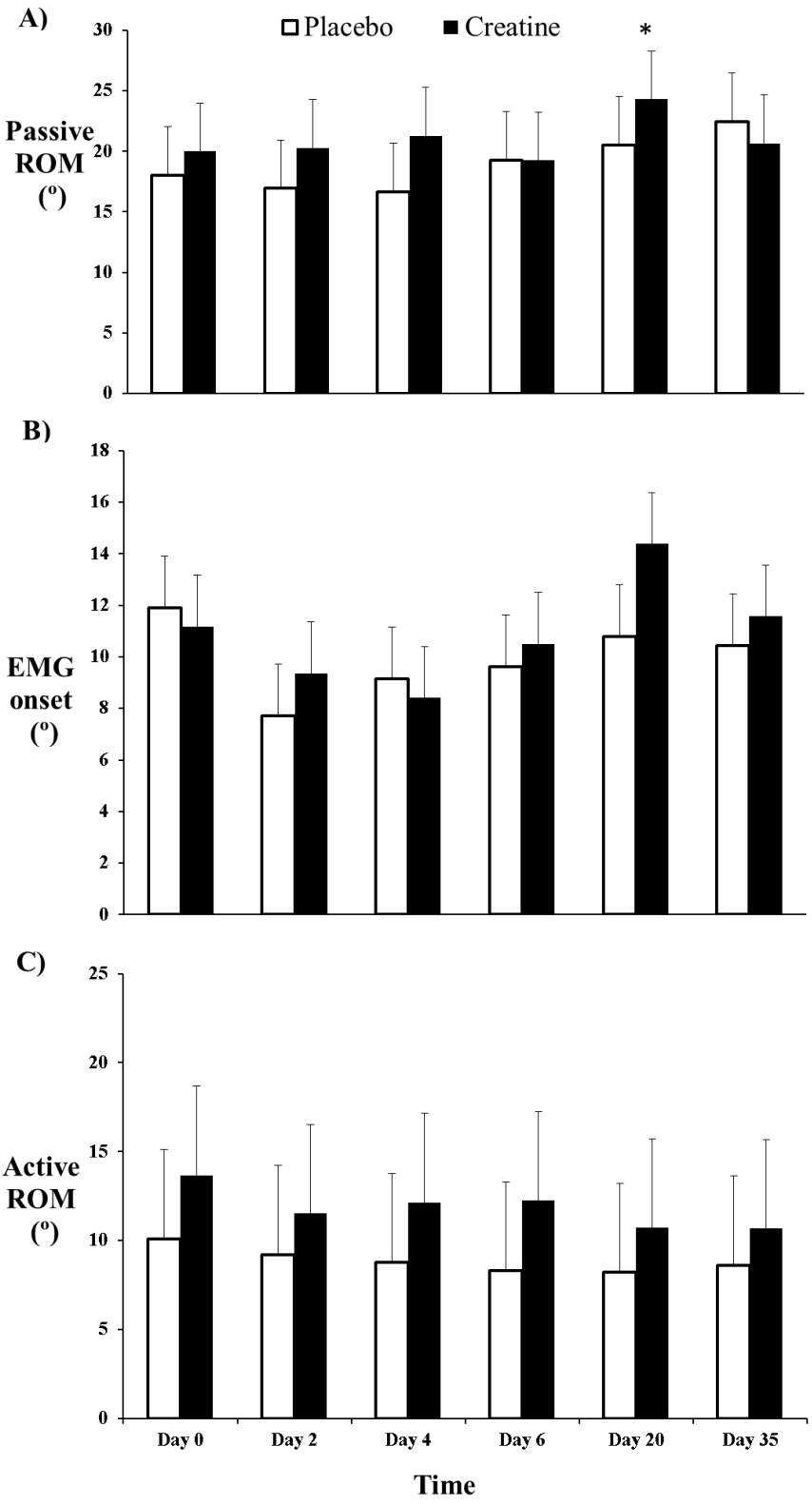

A)
Placebo
Creatine
*
Passive ROM (°)
30
25
20
15
10
5
0
B)
EMG onset (°)
18
16
14
12
10
8
6
4
2
0
C)
Active ROM (°)
25
20
15
10
5
0
Day 0
Day 2
Day 4
Day 6
Day 20
Day 35
Time

Figure 7:

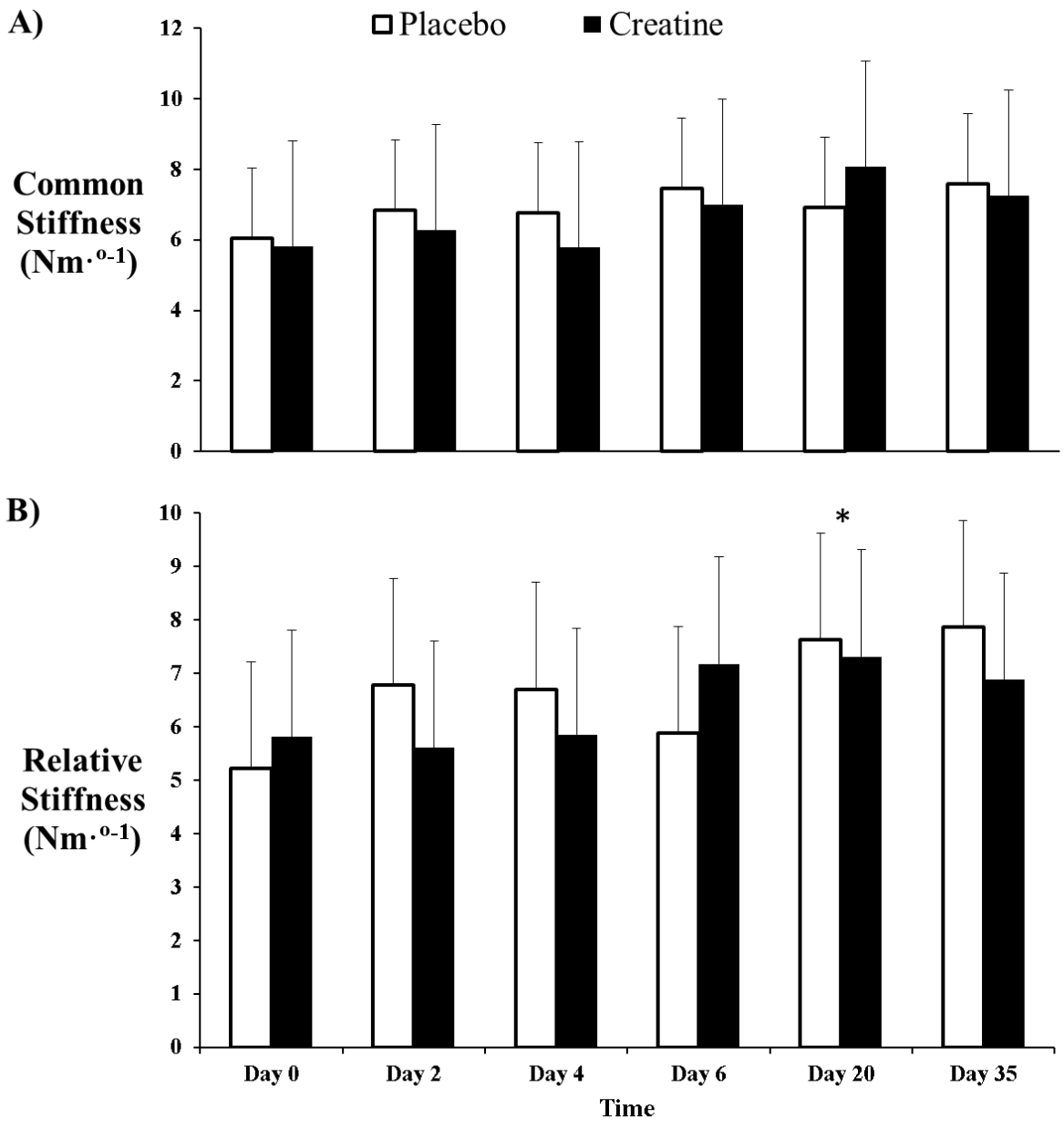

A)
Placebo
Creatine
Common Stiffness (Nm·o-1)
12
10
8
6
4
2
0
B)
Relative Stiffness (Nm·o-1)
10
9
8
7
6
5
4
3
2
1
0
*
Day 0
Day 2
Day 4
Day 6
Day 20
Day 35
Time

Figure 8:

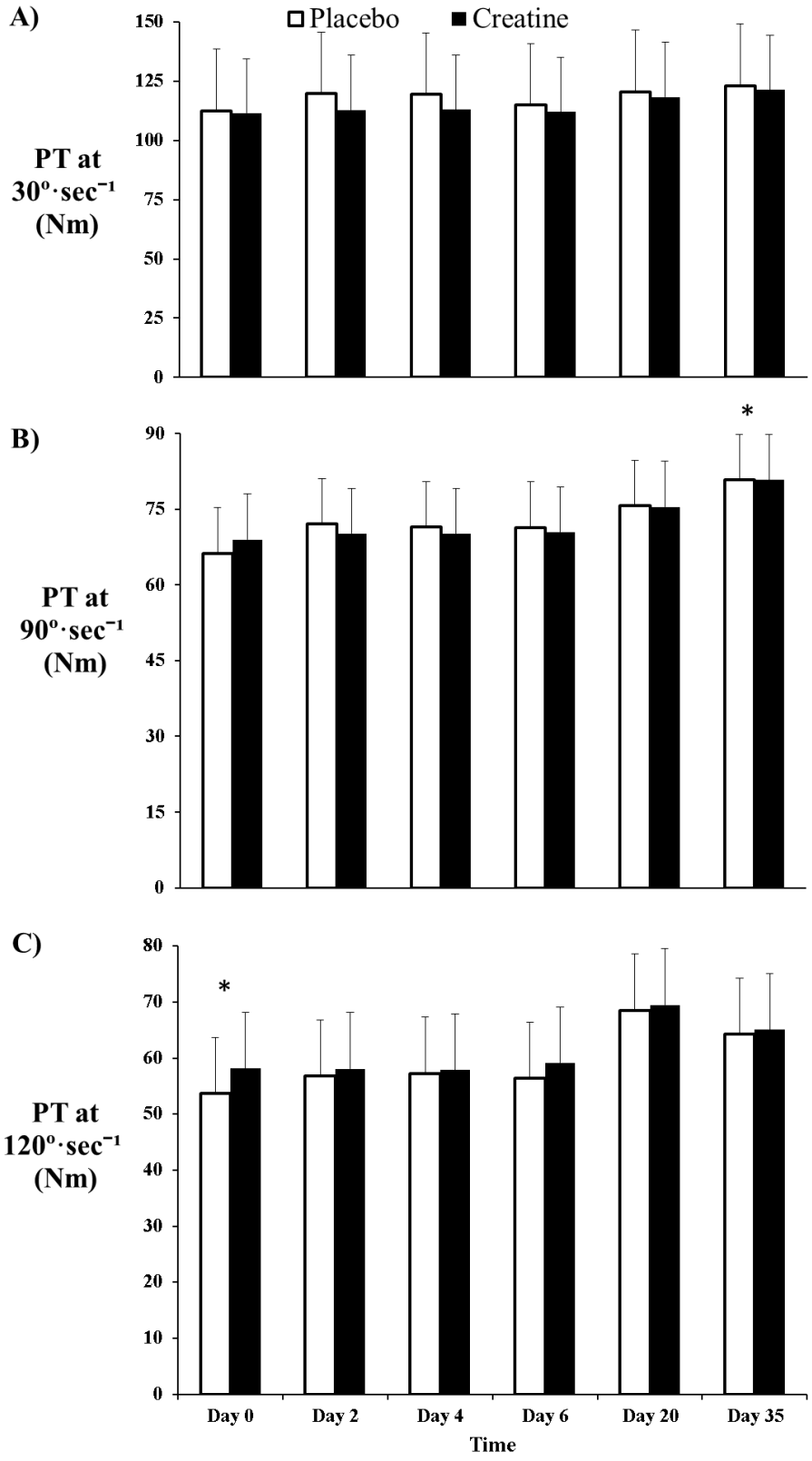

A)
Placebo
Creatine
PT at 30°·sec⁻¹ (Nm)
150
125
100
75
50
25
0
B)
*
PT at 90°·sec⁻¹ (Nm)
90
75
60
45
30
15
0
C)
*
PT at 120°·sec⁻¹ (Nm)
80
70
60
50
40
30
20
10
0
Day 0
Day 2
Day 4
Day 6
Day 20
Day 35
Time

Figure 9:

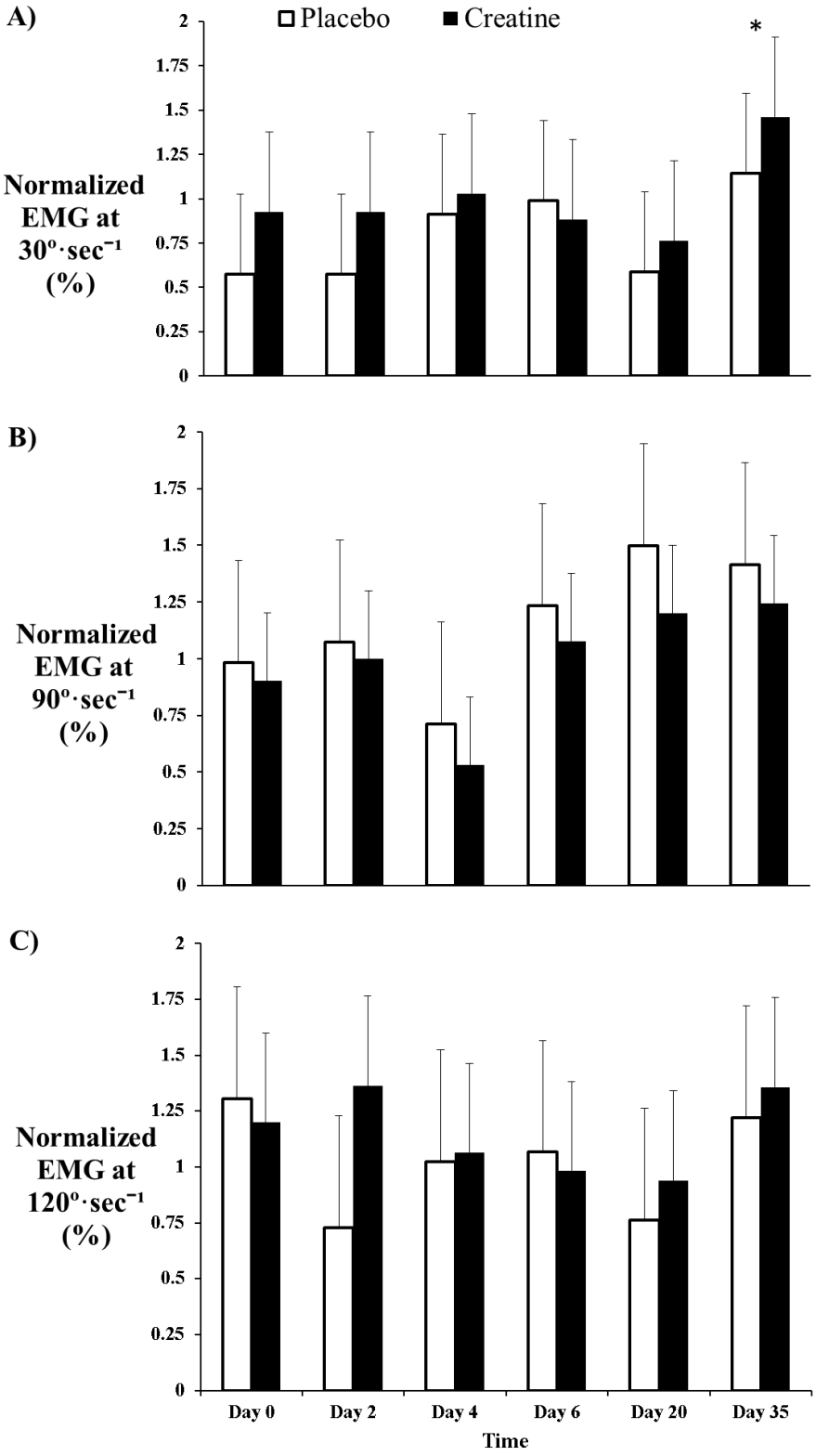

A)
Placebo
Creatine
*
Normalized EMG at 30°·sec⁻¹ (%)
B)
Normalized EMG at 90°·sec⁻¹ (%)
C)
Normalized EMG at 120°·sec⁻¹ (%)
2
1.75
1.5
1.25
1
0.75
0.5
0.25
0
Day 0
Day 2
Day 4
Day 6
Day 20
Day 35
Time

Figure 10:

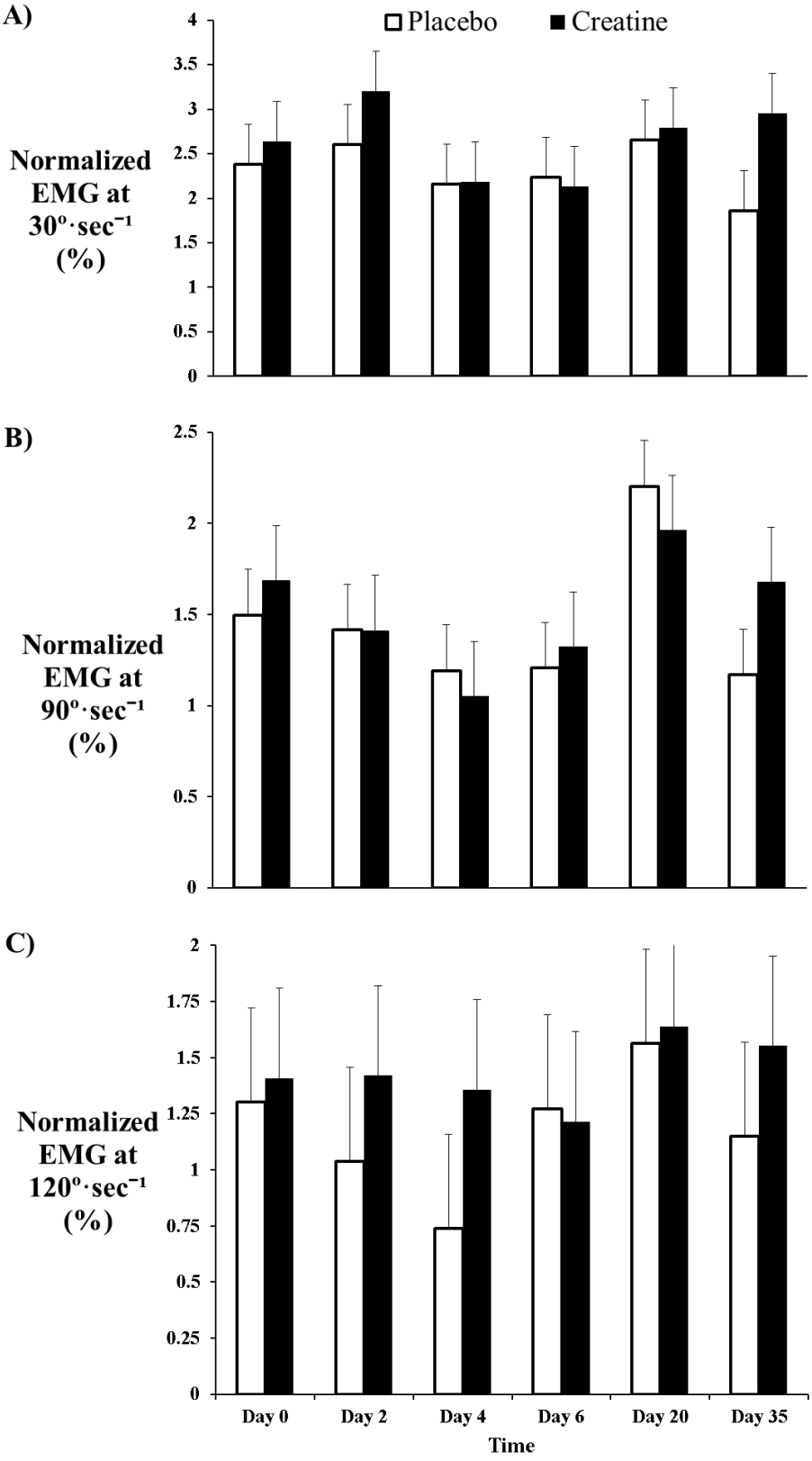

A)
Placebo
Creatine
Normalized EMG at 30°·sec⁻¹ (%)
4
3.5
3
2.5
2
1.5
1
0.5
0
B)
Normalized EMG at 90°·sec⁻¹ (%)
2.5
2
1.5
1
0.5
0
C)
Normalized EMG at 120°·sec⁻¹ (%)
2
1.75
1.5
1.25
1
0.75
0.5
0.25
0
Day 0
Day 2
Day 4
Day 6
Day 20
Day 35
Time

Figure 11:

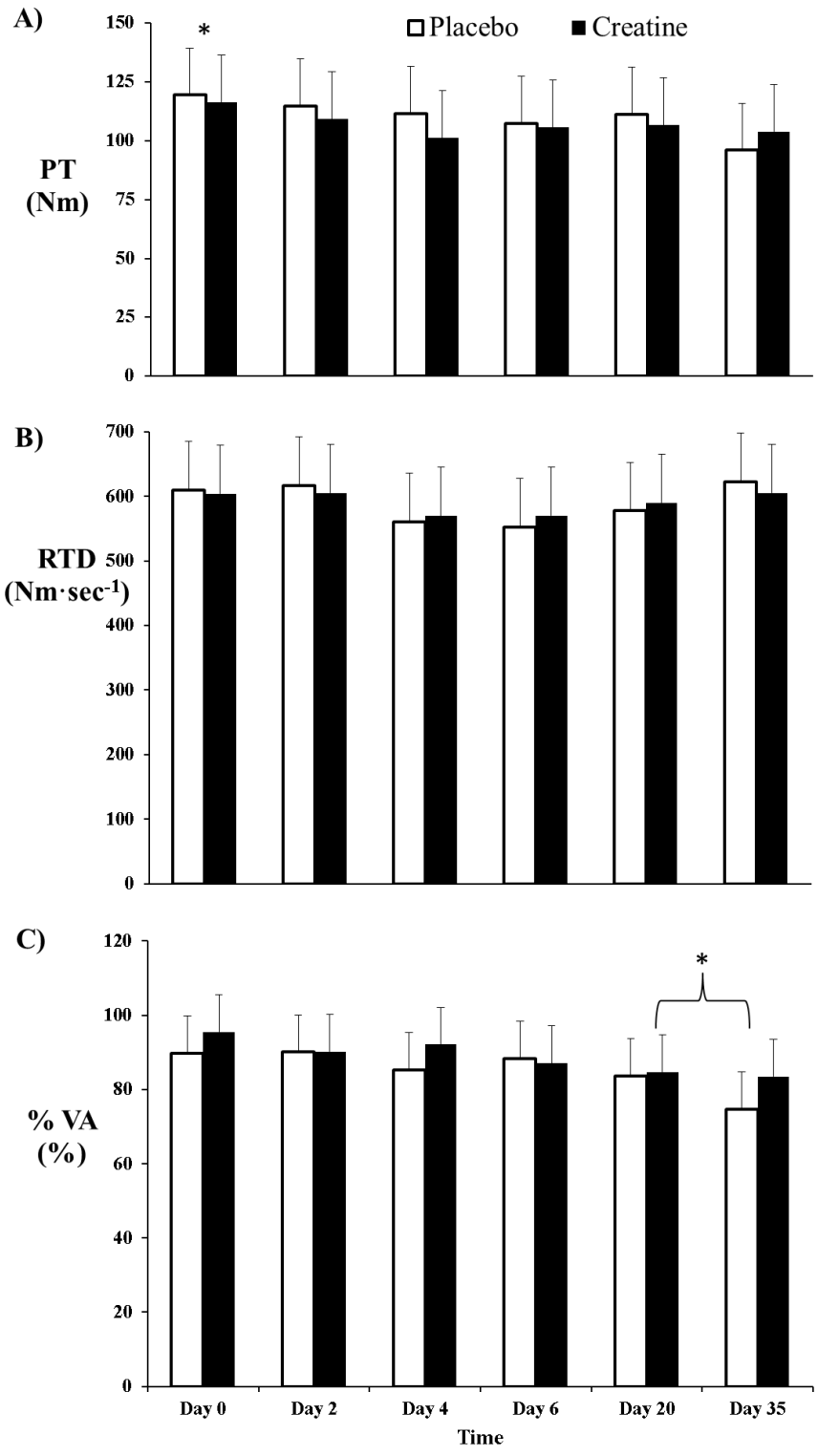

A)
Placebo
Creatine
*
PT (Nm)
150
125
100
75
50
25
0
B)
RTD (Nm·sec-1)
700
600
500
400
300
200
100
0
C)
% VA (%)
120
100
80
60
40
20
0
*
Day 0
Day 2
Day 4
Day 6
Day 20
Day 35
Time

Figure 12:

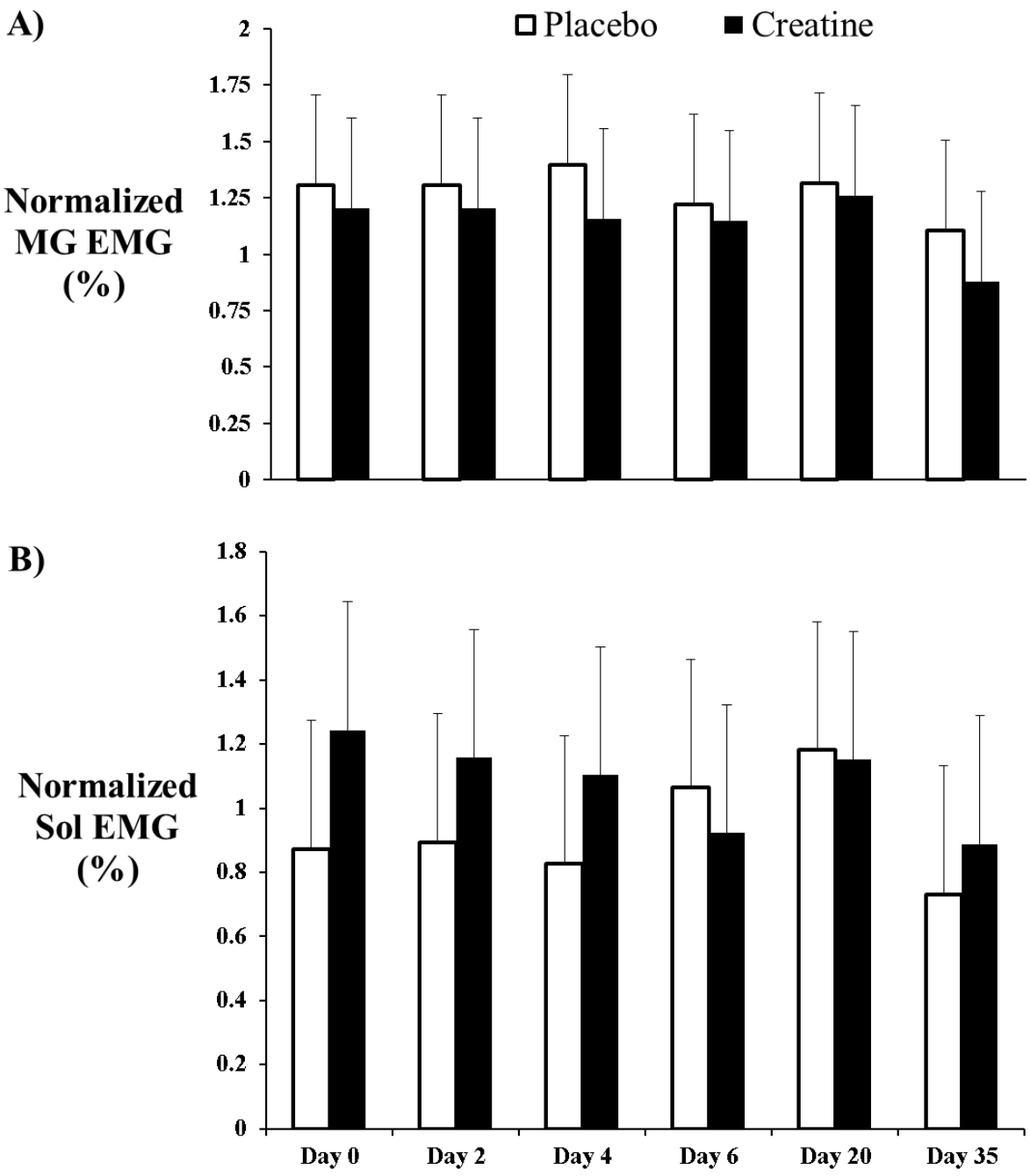

A)
Placebo
Creatine
Normalized MG EMG (%)
2
1.75
1.5
1.25
1
0.75
0.5
0.25
0
B)
Normalized Sol EMG (%)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Day 0
Day 2
Day 4
Day 6
Day 20
Day 35

Figure 13:

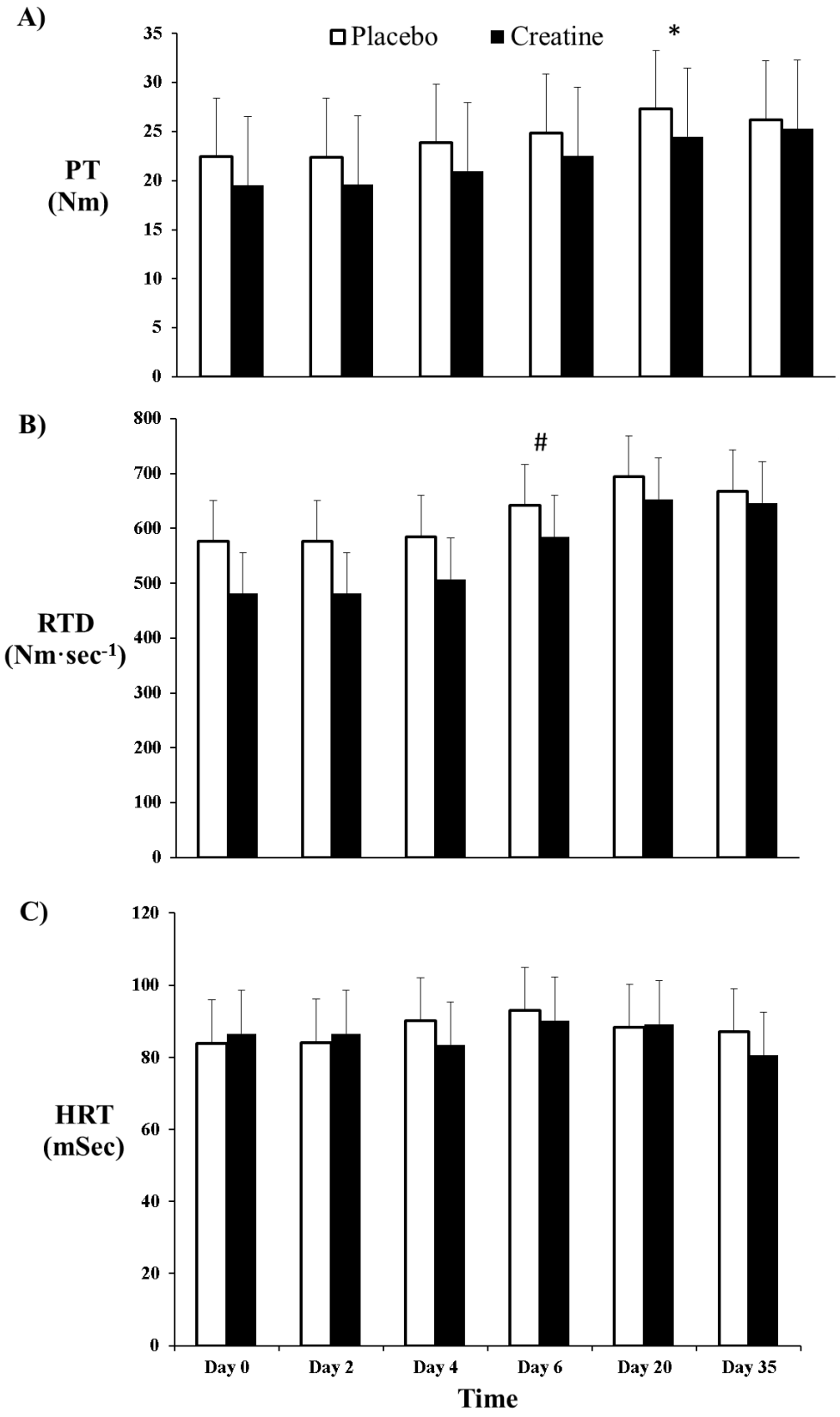
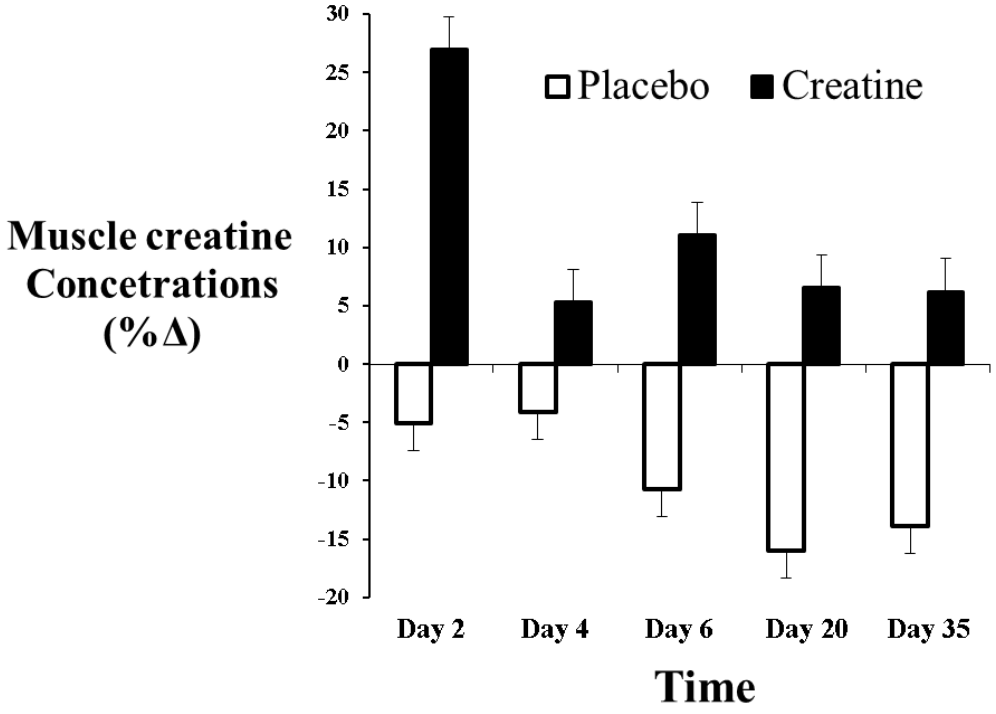

A)
Placebo
Creatine
*
PT
(Nm)
35
30
25
20
15
10
5
0
B)
#
RTD
(Nm·sec-1)
800
700
600
500
400
300
200
100
0
C)
HRT
(mSec)
120
100
80
60
40
20
0
Day 0
Day 2
Day 4
Day 6
Day 20
Day 35
Time

Figure 14:


Muscle creatine Concetrations (%Δ)
30
25
20
15
10
5
0
-5
-10
-15
-20
Placebo
Creatine
Day 2
Day 4
Day 6
Day 20
Day 35
Time

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