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The effect of creatine and magnesium supplementation on delayed onset muscle soreness

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The Effect of Creatine and Magnesium Supplementation on

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MASTER'S THESIS

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The Effect of Creatine Magnesium Supplementation on

Delayed Onset Muscle Soreness

A Thesis
Presented to
The Faculty of
Western Washington University

In Partial Completion
of the Requirements for the Degree
Master of Science

Karla W. Landis

October 2013

Abstract

This purpose of this study was to evaluate the effects of four weeks of magnesium-creatine chelate (MgCr), alkaline creatine (AlkCr), and placebo (P) on delayed onset muscle soreness (DOMS). Subjects were 36 young (18-24 years old), healthy, recreationally active individuals, randomly assigned to groups. Double blind supplementation was dextran (P), 5 g creatine, 400 mg (MgCr), and 5 g creatine plus soda ash (AlkCr) per day. Subjects exercised on an eccentric training machine with foot pedals yielding force that was resisted eccentrically. Peak eccentric force was obtained at 35 cycles per minute. Subjects maintained 65% of the peak eccentric force, for five minutes of continuous work. They reported muscle soreness scores corresponding to a visual analog scale with scores from 1, normal, to 10, very, very sore at 12, 24, 48, 60, and 72 hours post exercise. Repeated measures ANOVA was applied. The creatine groups did have significant main effects of time in DOMS on a visual analog scale after 5 minutes of eccentric exercise ($p<0.05$). There were no significant effects between creatine groups ($p<0.05$). Within group time effects were observed at 12, 24, and 36 hour intervals for AlkCr and at 12 hour intervals up to the 60 hours for MgCr, with DOMS scores lower following intervention. Pre intervention 12- and 72-hour soreness scores were P: 6.0±1.6, 3.6±2.3; MgCr: 5.0±2.9, 3.5±2.3; AlkCr: 4.5±1.9, 3.9±1.9 and post intervention 12- and 72-hour soreness scores were P: 4.7±1.5, 2.7±1.2; MgCr: 3.2±1.6, 2.3±1.6; AlkCr: 3.5±2.0, 2.5±1.9. Creatine supplementation regimens for four weeks ameliorated DOMS perception following 5-minutes of eccentric activity. The mechanism for the findings remains to be elucidated, but may be related to anti-inflammatory effects.

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Chapter 1

The Problem and Its Scope

Introduction

The benefits of eccentric training have been widely researched and include greater

gains in strength, increased hypertrophy, lower metabolic demand and decreased motor unit

recruitment when compared to concentric training programs, thus establishing eccentric

muscle action as more efficient and effective (Babault, Pousson, Ballay, & Van Hoecke,

2001; LaStayo, Woolf, Lewek, Snyder-Mackler, Reich, & Lindstedt, 2003; Tesch, Dudley,

Duvoisin, Hather, & Harris, 2008). Eccentric muscle action is one of the primary causes of

exercise-induced muscle injury as evidenced by decreased maximum voluntary contraction

(MVC) force, increased concentrations of inflammatory markers and blood proteins,

increased passive muscle tension and increased severity of delay onset muscle soreness

(DOMS) post-exercise (Newham, McPhail, Mills, & Edwards, 1983). Creatine and

magnesium are both anti-inflammatory agents and may play a key role in decreasing the level

of DOMS, leading to improved training and performance outcomes for athletes and more

effective, more rapid recovery from exercise and less painful rehabilitation protocols (King

& Duffield, 2009; Rosene, Matthews, Ryan, Belmore, Bergsten, Balisdell, et al. 2009;

Santos, Bassit, Caperuto, & Costa Rosa, 2004; Selsby, DiSilvestro, & Devor, 2004).

Additionally, the lower metabolic cost of eccentric training results in less cardiovascular

stress, making eccentric training appealing to the elderly and individuals with cardiovascular

health issues. The severity of DOMS following high-intensity eccentric training limits many

individuals from reaping the benefits of eccentric training. More research on the attenuation

of DOMS will lead to better informed training and rehabilitation prescription for coaches, trainers and physical therapists (Connolly, Sayers, & McHugh, 2003).

Purpose

The purpose of this study was to assess the effects of magnesium-creatine chelate, alkaline creatine compound, and placebo supplementation on DOMS following eccentrically-induced muscle damage. DOMS was induced using a device that required subjects to resist loads at a rate relative to their maximum value. Study results may help clarify the effects of magnesium-creatine chelate, alkaline creatine and placebo supplementation on exercise-induced muscle soreness as measured using a visual analog scale. The results of this study may also inform future research on nutritional supplementation and exercise prescription.

Null Hypothesis (H_0)

The null hypothesis states that following high-intensity eccentric muscle action, there will be no difference in DOMS as determined with a visual analog scale due to supplementation of magnesium-creatine chelate, alkaline creatine compound, or placebo.

Significance of the Study

The ergogenic potential of creatine has been well-reviewed (Kreider, 2003). It has been established that elevated muscle creatine levels improves muscle strength and augments hypertrophy induced by strength training (Olsen, Aagaard, Kadi, Tufekovic, Verney et al., 2006; Rawson & Volek, 2003). Recent studies show that creatine supplementation may result in reduced inflammation, cell damage and pain following a high-intensity or exhaustive bout of exercise (Bassit, Pineheiro, Vitzel, Spoesser, Silveira et al., 2010; Bassit, Curi, & Costa

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Rosa, 2008; Rawson et al., 2003; Rawson, Conti, & Miles, 2007; Santos, et al. 2004).

Magnesium plays a critical role in muscle function and attenuation of pain (Brilla & Haley,

1992; Dominguez, Barbagallo, Laurentani, Bandinelli, Bos et al., 2006; Manaa and Alhabib,

2012; Neilsen & Lukaski, 2006). Only one study has examined the effects of magnesium-

creatine chelate and alkaline creatine supplementation; thus further research is needed to

better understand the potential synergistic effect of magnesium and creatine on DOMS, an

effect currently not well understood.

Limitations of the Study

1. The participants were college-aged Western Washington University students, enrolled
   in a physical education activity course; thus, conclusions are most relevant to similar
   populations.
2. The measurement of DOMS is subjective and may vary depending on the perception
   of the individual.
3. Previous training of the subjects could influence the results of the data. All subjects
   had no prior experience with eccentric training.
4. Sex of subjects was not taken into account for the results. It is assumed that there is
   no difference due to sex in muscle activation.

Definition of Terms

Adenosine Triphosphate – A high-energy molecule that provides energy for muscle actions (Baechle & Earle, 2008)

Concentric Muscle Action – When the muscle generates tension actively with visible shortening in the length of the muscle (Hamill & Knutzen, 1995)

Delayed Onset Muscle Soreness (DOMS) – Pain or discomfort felt in skeletal muscles following intense exercise with soreness increasing during the first 24 hours and peaking during 24-72 hours post exercise (Armstrong, 1984)

Eccentric Muscle Action – When a muscle is subjected to an external force that is greater than the internal force within the muscle, the muscle lengthens (Hamill & Knutzen, 1995)

Isometric Muscle Action – When a muscle generates force without changing length (Hamill & Knutzen, 1995)

Residual Force Enhancement – Increased force generation as a result of sarcomere stretching (Power, Rice, & Vandervoort, 2012)

Visual Analog Scale (VAS) – A 10-point pain scale, with 1 corresponding to feeling of no soreness and 10 corresponding to feeling very, very sore, commonly used to evaluate the intensity of pain (Scott & Huskisson, 1976)

Chapter II

Review of Literature

Introduction

The purpose of this study was to assess the effect of magnesium-creatine chelate, alkaline creatine and placebo supplementation on DOMS following high-intensity eccentric muscle action. The benefits of eccentric training have been widely studied and, when compared to concentric muscle action, eccentric muscle action has shown to elicit greater force production and fatigue resistance with less muscle activation (Cooke, Rybalka, Williams, Cribb, & Hayes, 2009; Faulkner, Brooks, & Opiteck, 1993). High-intensity or prolonged eccentric muscle action is also the primary cause of exercise-induced muscular injury making the benefits of eccentric training less available (Cooke et al. 2009; Newham et al., 1983; Rawson et al. 2007; Rosene et al., 2009). This literature review will examine the characteristics of eccentric muscle action, existing theories on the mechanism of exercise-induced muscle injury and DOMS, and the role of creatine and magnesium as nutritional supplements and potential aids in the attenuation of DOMS.

Eccentric Muscle Action

Eccentric muscle action is described as muscle lengthening under tension. This muscle action occurs when the opposing force exceeds that of the muscle and causes the muscle to lengthen (Hamill & Knutzen, 2006). Compared to concentric muscle action, eccentric muscle action requires less metabolic energy, elicits less electromyographic (EMG) activity and produces greater force outputs (Babault et al., 2001; LaStayo et al., 2003; Tesch, Dudley, Duvoisin, Hather, & Harris, 2008). Ryschon, Fowler, Wysong, Anthony, and

Balaban (1997) demonstrated the efficiency of eccentric muscle action compared to isometric and concentric muscle action in the tibialis anterior and extensor digitorum longus by measuring work while simultaneously measuring ATP, phosphocreatine, inorganic phosphate, and pH during steady state exercise. During concentric action, phosphocreatine was significantly reduced and ATP hydrolysis was significantly increased when compared to eccentric and isometric action. Despite identical changes in muscle length during concentric and eccentric action, metabolic costs of concentric action remained significantly greater than that of eccentric action. A number of theories have been proposed to explain the underlying mechanism for this observation (Schoenfeld, 2010).

Some theories suggest eccentric force outputs are due in part to changes in actin-myosin bonds (Flitney & Hirst, 1978). When muscle lengthens under tension, sarcomere lengthening occurs as actin-myosin cross-bridges deform and ultimately detach from each other. This mechanical disconnection exposes actin sites, making them available for myosin heads to reattach to a new site. This continues until no more overlap between actin and myosin exists. A classic study by Newham, McPhail, Mills, and Edwards (1983) assessed ultrastructural changes of the quadriceps muscle following a 20-minute step test (step rate 15 steps/minute) intending to elicit one second of concentric muscle action in one leg and one second of eccentric muscle action in the contralateral leg. All subjects reported very little effort or exertion during eccentric muscle action and much more effort during concentric muscle action, especially near the end of the session. Structural changes, including Z-line movement and disorganization of the myofibrils were apparent immediately following eccentric exercise and increased significantly 30 hours post-exercise. Post-exercise pain and

tenderness developed only in the muscles involved in eccentric muscle action (Newham, Mills, Quigley, & Edwards, 1983a).

A study by Power et al. (2012) examined the effect of eccentrically induced muscle damage on residual force enhancement (RFE) of dorsiflexors. RFE protocol included maximal activation of the dorsiflexors at 0° for one second, followed by a one-second stretch at 30°/s, culminating with a three-second isometric contraction at 30° of plantarflexion. Subjects then completed four sets of 25 eccentric isokinetic dorsiflexion contractions at 30°/s through a 30° range of motion with 30 seconds rest in between sets. Following damaging eccentric exercise protocols, significant $30.3 \pm 6.4\%$ and $36.2 \pm 9.7\%$ reductions in eccentric torque and maximal voluntary contraction (MVC) were measured, respectively, compared to baseline measures. However, following eccentric protocols, RFE increased significantly compared to baseline, suggesting stretch prior to contraction attenuates decreased performance outcomes due to muscle damage resulting from eccentric muscle action.

Further, a study by Vaczi et al. (2011) examined the mechanical, biochemical, and electromyographic responses of the quadriceps muscle to high-intensity resistance training in healthy, physically active men. Subjects assigned to the eccentric training group (group E), completed six sets of 15 repetitions of isokinetic knee extension exercise at 60°/s through a range of motion between 20°-80° of knee flexion. Training was completed on a total of seven days (one day of rest was given following day three). Compared to baseline, maximum voluntary isometric torque decreased significantly (-15%,) by day three, but increased significantly (12%,) across all training days. Compared to concentrations of creatine kinase (CK) on day one (202 ± 140 U/L), CK peaked significantly on day three (779 ± 332 U/L,) and remained elevated through day seven, however, there were significant decreases in CK

concentrations from day six (594 ± 321 U/L) and seven (337 ± 257 U/L). Compared to day one measures, perceived soreness, assessed by questionnaire using a scale of 0 (not sore at all) to 10 (very sore), peaked at 24 hours (average perceived soreness rating approximately = 5) and gradually decreased on all subsequent days. Soreness remained significantly elevated on day four (average perceived soreness rating approximately = 2,) before returning to near day one levels. These results suggest early (days one to three) attenuation of maximal voluntary torque may be attributed to muscle damage and acute inflammation, as indicated by significant increases in CK.

Acute inflammation from muscle injury is also known to increase production of inflammatory markers, specifically CK and lactate dehydrogenase (LDH), and is implicated in increased circulation of more global inflammatory markers, C-reactive protein and interleukin-6 leading (IL-6) (Barnes, Trombold, Dhindsa, Lin, & Tanaka, 2010). Prolonged circulation of inflammatory marker has serious health consequences including negatively affecting the elasticity of arteries, resulting in a potentially unhealthy atherosclerotic response (Ross, 1999). Exercise-induced, prolonged increases in concentrations of inflammatory markers may also exacerbate chronic cases of low-grade inflammation, a condition often present in deconditioned individuals.

Exercise-Induced Muscle Injury and DOMS

DOMS develops 24-48 hours after unaccustomed or high-intensity eccentric exercise and is initially attributed to lengthening muscle action whereby sarcomeres are disrupted (Armstrong, 1984; Byrnes et al., 1985; Newham, McPhail, Mills, & Edwards, 1983). Muscle damage leads to inflammation as reflected by increased concentrations of inflammatory

markers such as CK and LDH, which may inhibit injured muscle from repair resulting in attenuated force outputs and reduced range of motion (Clarkson, Byrnes, McCormick, Turcotte, & White, 1986; Cooke et al., 2009; Miles & Clarkson, 1994; Rosene et al., 2009; Váczi et al., 2011). Additionally, muscle damage leads to cellular injury resulting in leakage of inflammatory markers into extracellular fluid and plasma (Barnes et al., 2010).

Attenuation of DOMS post-eccentric exercise has important training implications. To date, investigation of DOMS treatment predominantly includes massage, hydrotherapy, rest and stretching routines (Mancinelli, Davis, Aboulhosn, Brady, Eisenhofer, & Foutty, 2006; Sellwood, Brukner, Williams, Nicol, & Hinman, 2007; Volek, Duncan, Mazzetti, Staron, Putukian, Go'mez et al., 1999). Mancinelli et al. (2006) evaluated the effects of muscle massage on DOMS in the quadriceps muscles of NCAA Division I women's basketball and volleyball players (n=24). DOMS was elicited through unaccustomed, high-intensity exercise as part of each team's normal training plan and measures were made on day four of their normal practice season. Pre- and post-measures in both groups (control and massage, each n=12) included vertical jump, shuttle run and quadriceps femoris length. Additionally, subjects were asked to rate their level of muscular soreness utilizing a pain pressure threshold scale of 1-10. Subjects in the control group rested while subjects in the massage group received the massage protocol. The control group reported no significant change in soreness during pre- and post-measures and performed similarly across all tests. Within the massage group, the average pre-massage rating of perceived soreness was 5 out of 10 and the average post-massage rating of perceived soreness was 3 out of 10 resulting in significantly reduced perceived soreness post-treatment (p=0.0011). Subjects in the massage group also significantly improved performance in the vertical jump test by increasing the average jump

height by 0.04 cm (p=0.0033). No performance differences were seen in any other measures. This study suggests massage may be a reasonable intervention to alleviate DOMS thereby helping athletes and others improve performance during repeated bouts of high-intensity exercise. Other studies evaluating the effects of massage on DOMS suggest massage is effective at alleviating perceived symptoms of DOMS but has no conclusive effect on improved performance during exercise (Best, Hunter, Wilcox, & Haq, 2008).

Hydrotherapy, in the form of cold- or hot-water immersion, is another commonly used treatment for DOMS (Howatson, Goodall, & Van Someren, 2009; Sellwood, Brukner, Williams, Nicol, & Hinman, 2007; Vaile, Halson, Gill, & Dawson, 2008). Some studies show cold-water immersion to be ineffective, eliciting no changes in pain perception, swelling, maximal isometric strength, or concentrations of serum creatine kinase (Howatson et al., 2009; Sellwood et al., 2007) while others show improvements across some performance variables, such as the isometric squat and improved perceptions of pain following cold water immersion therapy (King & Duffield, 2009; Pointon et al., 2011; Vaile et al., 2008). Vaile et al. (2008) examined the effects of three different water therapy interventions on DOMS following high-intensity exercise protocol. In a randomized crossover design, subjects (n=38 strength trained men) completed two experimental trials separated by eight months. One trial involved passive recovery (PAS) for all subjects, 14 minutes of seated, inactivity, and the other utilized one of three water therapy interventions, each for 14 minutes: cold water immersion (CWI: full-body immersion, excluding head and neck in15°C water); hot water immersion (HWI: full-body immersion, excluding head and neck, in 38°C water); or contrast water therapy (CWT: full-body immersion, excluding head and neck, alternating every minute between hot and cold). Exercise protocol consisted of five

sets of 10 eccentric bi-lateral leg press contractions with a load of 120% of 1-RM followed by two sets of 10 repetitions at a load of 100% of 1-RM. Experimental measures included weighted squat jump, isometric squat, perceived pain (visual analog scale of 0 to 10) and thigh girth and concentrations of inflammatory markers creatine kinase, lactate dehydrogenase and interleukin-6, all of which were evaluated immediately after, and at 24, 48 and 72 hours post-exercise. Experimental measures for each hydrotherapy intervention group were compared to measures from the PAS group.

Percent change from baseline in isometric squat performance for subjects in the HWI was reduced significantly at 24, 48 and 72 hours post exercise (-12.8%, -10.1% and -3.2%; $p<0.05$) compared to PAS (-17.0%, -16.0%, -9.8%). A significant change at 24, 48, and 72 hours post exercise was also demonstrated following CWT (-10.3%, -7.4%, -2.8%; $p<0.01$) compared to PAS (-17.3%, -14.0%, -11.5%). Additionally, change in peak performance power (% of change from baseline) was significantly less for CWI at 48 hours (-7.0%; $p=0.01$) and 72 hours (-4.0%; $p=0.03$) post-exercise following as compared to PAS at 48 and 72 hours (-16% and -8%). However, HWI did not positively affect recovery for squat jump performance compared to PAS. Both CWT and CWI interventions elicited significant ($p<0.01$ and $p<0.03$) reductions in swelling at 24, 48 and 72 (CWT: 56.4 ± 4.5, 56.3 ± 4.6, 56.3 ± 4.5; CWI: 57.1 ± 3.8, 56.9 ± 3.8, 56.9 ± 3.8) hours post-exercise compared to PAS (CWT: 56.9 ± 4.7, 56.9 ± 4.7, 56.7 ± 4.7; CWI: 57.1 ± 3.8, 56.9 ± 3.8, 56.9 ± 3.8). Compared to PAS (average VAS at 24, 48 and 72 hours post exercise approximately= 6, 8, 5) perceived pain was significantly ($p<0.01$) reduced at 24, 48 and 72 hours post-exercise following CWT (average VAS at 24, 48 and 72 hours post exercise approximately = 5, 6, 4). Both CWI and HWI were ineffective in pain reduction post exercise. Additionally, Vaile et al. (2008)

observed significant reductions in creatine kinase at 24, 48 and 72 hours post-exercise following CWI and 48 hours following HWI. Pointon et al. (2011) also found cold-water therapy results in lower perceptions of pain, however no changes in concentrations of inflammatory markers (creatine kinase and asparate aminotransferase) were observed post-exercise.

Stretching has also been identified as a possible method for attenuating the effects of DOMS (Chen, Nosaka, Chen, Lin, Tseng, & Chen, 2011; Henschke & Lin, 2011). Chen et al. (2011) studied the effects of eight weeks of static stretching (SS) and proprioceptive neuromuscular facilitation (PNF) on DOMS after six sets of high-intensity eccentric muscle action of knee flexors. After eight weeks of flexibility training, both groups improved range of motion significantly and showed significant increases in isokinetic concentric strength of knee flexors (SS = 11% ± 3%, PNF = 16% ± 4%). The decrease in peak torque across all six sets of eccentric muscle action was significantly greater for the control group (18% ± 4%) when compared to both SS (12% ± 2%) and PNF (10% ± 2%) groups. Both SS and PNF groups performed significantly more work during the eccentric exercise test and demonstrated improved performance following 5-day recovery period when compared to controls. This research supports the use of flexibility training, either SS or PNF, as an effective intervention for DOMS.

Successful management of DOMS has yet to be determined though interventions such as water-therapy, massage, and stretching are being explored. The role of creatine in energy production during muscle action makes creatine supplementation a potential player in decreasing exercised-induced DOMS. Magnesium is another known anti-inflammatory agent and could potentially lead to reduced DOMS when coupled with creatine supplementation.

Role of Creatine

Creatine is one of the most popular and well-researched ergogenic aids available (Cooper, Naclerio, Allgrove, & Jimenez, 2012; Lawler, Barnes, Wu, Song, & Demaree, 2002). Creatine is produced in the kidney, pancreas, and liver from three amino acids (glycine, arginine, and methionine) and 95% of total intracellular creatine (free and phosphorylated) is located in skeletal muscle (Persky & Brazeau, 2001). Phosphorylated creatine, or phosphocreatine (CrP), plays an essential role in ATP synthesis via the Phosphagen System (Bessman & Carpenter, 1985). CrP reacts with ADP and hydrogen ions via the enzyme creatine kinase, resulting in ATP and free creatine. This reaction happens quickly, during initial seconds of high-intensity anaerobic exercise, and newly synthesized energy is then translocated from the site of production to the site of use (Persky & Brazeau, 2001). CrP also serves as an energy reservoir and a buffer of hydrogen ions, delaying the onset of acidosis as a result of increased lactate production during exercise (Walsh, Tonkonogi, Söderlund, Hultman, Saks, & Sahlin, 2001). During recovery, CrP stores are replenished up to 80-90% of their resting values within two minutes (Walsh et al., 2001).

Some research shows creatine supplementation reduces exercise-induced muscle damage as evidenced by significant reductions in concentrations of inflammatory markers such as CK and LDH, and improved performance during recovery time (Bassit et al., 2008; Cooke et al., 2009; Santos et al., 2004). In a double-blind study, Bassit et al. (2010) assessed the effects of short-term creatine supplementation on inflammatory markers for muscular damage. Eight experienced triathletes were randomly assigned to either a control or creatine group. Five days prior to competition, the control group ingested a maltodextrin supplement (50 grams per day) and the creatine group ingested the same amount of maltodextrin

supplement plus creatine (20 grams per day). Blood samples were collected before, 36, and 60 hours after competition and plasma concentrations of CK, lactate dehydrogenase (LDH), aldolase (ALD), glutamic oxaloacetic acid transaminase (GOT), glutamic pyruvic acid transaminase, and C-reactive protein (CRP) were assessed. This study also measured plasma concentrations of CK and LDH and muscle vascular permeability in the gastrocnemius muscle of rats. Plasma levels of CK, LDH, ALD, GOT, and GPT were significantly reduced in the creatine group (CK increased 5-fold, $p < 0.01$; LDH showed no changes; ALD increased 2.9-fold, $p < 0.01$; GOT showed no changes; GPT showed no changes) as compared to the control (CK increased 11-fold, $p < 0.001$; LDH increased 1.7-fold, $p < 0.05$; ALD increased by 3.9-fold, $p < 0.001$; GOT increased 4.2-fold, $p < 0.01$; GPT increased 1.9-fold, $p < 0.05$). In the rat study, CK and LDH levels were reduced and muscle vascular permeability was significantly reduced in the creatine group.

An earlier study by Bassit et al. (2008) examined the effects of creatine supplementation on the change in inflammatory markers following a triathlon competition. Post-competition, Bassit et al. (2008) observed significantly reduced increases in concentrations of tumor necrosis factor-alpha (TNF-α), interferon-alpha (IFN-α), interleukin-1 beta (IL-1β), and plasma prostaglandin E2 (PGE2) in the creatine group when compared to controls. Post-race results for the creatine group show inflammatory marker TNF-α at 24 and 48 hours was reduced by 42 and 64% to 166.64 ± 9.54 pg/ml and 117.22 ± 5.55 pg/ml; IFN-α at 24 and 48 hours was reduced by 50.5 and 80.1% to 147.08 ± 2.46 pg/ml and 58.24 ± 6.10 pg/ml; IL-1β at 24 and 48 hours was reduced by 72 and 71% to 43.86 ± 5.67 pg/ml and 45.82 ± 3.23 pg/ml; and PGE2 at 24 and 48 hours was reduced by 85.5 and 91% to 65.70 ± 5.85 pg/ml and 52.56 ± 7.10 pg/ml. All concentrations of inflammatory markers reported above

are significantly lower than those in the control group at 24- and 48-hours post-race. In this experiment, interleukin-6 (IL-6) levels remained consistent across groups however other research shows changes in IL-6 concentrations in response to exercise (Barnes et al., 2010; Deldicque, Atherton, Patel, Theisen, Nielsens, Rennie, & Francaux, 2008). More research is needed to determine if short-term creatine supplementation significantly lowers concentrations of inflammatory markers post high-intensity exercise (Bassit et al., 2010; R. Bassit et al., 2008; Cooke et al., 2009; Santos et al., 2004).

A study by Rosene et al. (2009) examined the role of creatine supplementation on reducing acute and longer-term exercise-induced muscle damage thus aiding in recovery following eccentric training. Twenty, physically active males were randomly assigned to a creatine or placebo group. Subjects in the creatine group consumed a loading (20grams per day day for seven days) and sustaining (6 grams per day for 29 days) dose of creatine while the placebo group consumed equal amounts of a placebo (exact placebo not specified in research, supplied by AST Sports Science). On day eight, subjects performed knee extension eccentric exercise to induce knee extensor muscle damage. On day 30, subjects repeated the protocol on their non-dominant leg using 150% of one repetition maximum (1-RM) of non-dominant leg. The protocol consisted of a warm-up bout (1 set of 10 at 50%) followed by 7 sets of 10 reps at 150% of concentric 1-RM with 15 seconds between each repetition with three minutes rest between sets. Blood concentrations of inflammatory markers CK and LDH were collected prior to exercise and at 12, 24, and 48-hours post-exercise. Maximum Isometric Force (MIF) was determined for subjects sitting with leg positioned at approximately 45 degrees of knee flexion. Muscle dynamic strength (MDS) of the dominant (acute) and non-dominant (longer-term) thigh was assessed using a seated leg extension

machine after which the eccentric load was set at 150% of the concentric 1-RM. Knee range of motion (KROM) and perceived soreness were also assessed. Perceived soreness was evaluated by each subject placing a mark along a 25.4 cm scale, with 0 indicating no muscle soreness and 25.4 cm indicating very, very sore. MIF, MDS, KROM, and soreness were evaluated 12-hours post-eccentric exercise and every 24 hours for the next five days. Performance and blood tests after eight days of creatine supplementation resulted in no differences between groups indicating that creatine supplementation does not reduce exercise-induced muscle damage after short-term supplementation. This is contrary to a result reported earlier. After 30 days of creatine supplementation, maximum isometric force outputs improved significantly when compared to the performance by subjects in the placebo group (approximately 245Nm vs. 195 Nm for creatine and placebo respectively). Rosene et al. (2009) suggests this may be due to decreased exercise-induced muscle damage after longer periods of supplementation time or could be attributed to hypertrophy of muscle fibers and not to decreased damage at all.

Effects of Creatine Supplementation on Strength

Numerous studies have examined the effect of creatine supplementation on strength and increases fat free mass, both of which are improved with resistance training (Buford, Kreider, Stout, Greenwood, Campbell, Spano et al., 2007; Byrnes et al., 1985; P. Greenhaff, Bodin, Soderlund, & Hultman, 1994; Lawler et al., 2002; Rawson & Volek, 2003; Volek et al.,1999). Creatine supplementation may lead to enhanced strength gains in bench press, squat, elbow flexion, deadlift, and leg press (Kreider, Ferreira, Wilson, Grinstaff, Plisk, et al., 1997; Cribb & Hayes, 2006; Becque, Lochman, & Melrose, 2000; Herda et al., 2009; Saremi, Gharakhanloo, Sharghi, Gharaati, Larijani, et al., 2010). A widely reviewed study by Kreider

et al. (1997) examined the effects of 28-days of creatine supplementation on strength and performance in 25 NCAA division I football players. Players were assigned to either the placebo group (placebo consisted of 99 g/day of glucose, 3 g/day of taurine, 1.1 g/day of disodium phosphate, and 1.2 g/day of potassium phosphate) or the creatine group (placebo plus 15.75 g/day of creatine monohydrate). Pre- and post-supplement strength and power testing included bench press, squat and power cleans. DEXA scan for body mass (placebo 0.77 ± 1.8; creatine 2.22 ± 1.5 kg) and fat free mass (placebo 1.33 ± 1.1; creatine 2.43 ± 1.4 kg) were significantly increased in the creatine group. Bench press lifting volume (placebo -5 ± 134; creatine 225 ± 246 kg) and the sum of bench press, squat, and power clean lifting volume (placebo 105 ± 429; creatine $1,558 \pm 645$ kg) were significantly increased in the creatine group. Thus, elite athletic performance may be significantly enhanced by creatine.

More recently, Herda et al. (2009) examined the effects of 28-days of creatine supplementation (5 g creatine per day) without resistance training on 1RM for leg press and bench press. When compared to the placebo (3.6 g of microcystalline cellulose per day), subjects taking creatine showed significant increases in both 1RM for bench press (approximately 8% increase) and leg press (approximately 10% increase). Saremi et al. (2010) examined the effects of resistance training (three days per week for eight weeks) and creatine supplementation on 1RM for leg press and bench press in healthy, college-age men. Subjects (n=8) in the creatine group completed the resistance training program while supplementing creatine with a loading dose equivalent to 0.3 g/kg body weight per day for one week followed by a maintenance dose equivalent to 0.05 g/kg body weight per day for seven weeks. Subjects in the placebo group consumed placebo (cellulous power) and completed the resistance training protocol. Compared to the placebo group, subjects taking

creatine showed significant increases in 1RM for bench press at week four (placebo 59.5 ±
9.3; creatine 64.87 ± 5.9) and week eight (placebo 63.02 ± 10.1; creatine 71.75 ± 7.7). The
creatine group also showed significant increases in 1RM for leg press at week four (placebo
165.25 ± 13.9; creatine 160.62 ± 14.2) and at week eight (placebo: 172.12 ± 14.5;
creatine166.87 ± 12.5).

Research also shows that lower doses of creatine supplementation ingested before and
after a bout of resistance training may positively affect strength gains (Candow, Chilibeck,
Burke, Mueller & Lewis, 2010; Cribb & Hayes, 2006). In a double blind, repeated measures
study, Candow et al. (2010) examined the effects of creatine supplementation frequency on
muscle size and strength. Subjects (n=38 physically active, non-resistance trained college
students) were randomly assigned to one of four groups: CR2 (0.15 g/kg creatine during 2
days/week of resistance training), CR3 (0.10 g/kg creatine during 3 days/week of resistance
training, PLA2 (rice flour placebo during 2 days/week of resistance training) and PLA3 (rice
flour placebo during 3 days/week of resistance training). All groups completed six weeks of
resistance training protocol which included leg press, chest press, lat pull-down, shoulder
press, knee flexion and extension, triceps extension, biceps curl, and standing calf raise.
Subjects in CR2 and PLA2 performed 3 sets of 10 repetitions for each exercise at 80% of
1RM on 2 days/week. Subjects in CR3 and PLA3 performed 2 sets of 10 repetitions for each
exercise at 80% of 1RM on 3 days/week. Baseline and post-training measurements of elbow
and knee flexor and extensor thickness and leg press and chess press 1-RM strength were
collected. Urinary microalbumin and diet were also assessed. Results show the frequency of
resistance training (2 vs. 3 days/week) had no significant effect on body mass, muscle
thickness, and strength. Post hoc analysis for body mass showed significant increases in the

CR2 (1.8 ± 0.2 kg) and CR3 (1.5 ± 0.4 kg) groups when compared to PLA3 (0.2 ± 0.3 kg). All groups experienced significant increases in muscle thickness with the CR2 (0.6 ± 0.9 cm) and CR3 (0.4 ± 0.6 kg) groups increasing significantly when compared to PLA2 (0.05 ± 0.5 cm) and PLA3 (0.13 ± 0.7 cm). Leg press and chess press strength increased by 63.1 ± 57 kg, 35 ± 25 kg, 40 ± 35 kg, and 40 ± 18 kg for CR2, CR3, PLA2, and PLA3 respectively. Though there was no significant difference in strength gain between groups, men who supplemented creatine did show significantly greater strength gains in leg press when compared to women supplementing creatine (77.3 ± 51.2 kg and 21.3 ± 10 kg for men and women on creatine, respectively). These results show that creatine supplementation pre- and post-resistance training elicits increased muscle thickness and that equal doses of creatine on 2 or 3 days/week elicit similar increases in muscle thickness and strength. In addition, results show that men taking creatine may experience greater strength gains than women taking creatine.

Furthermore, a study by Chilibeck et al. (2004) examined the effects of creatine (0.2g Cr/kg body weight) supplementation and placebo (equal amount of corn starch) immediately post-exercise during six weeks of resistance training on muscle thickness and strength in men and women. Without regard for sex, results showed creatine supplementation led to significant increases in muscle thickness of elbow flexors (creatine 0.32 ± 0.07 cm; placebo 0.24 ± 0.07 cm with and without creatine supplementation, respectively) and trended toward significance for knee extensors (0.22 ± 0.09 cm and 0.14 ± 0.09 cm with and without creatine supplementation, respectively). Bench press strength showed significant increases over time ($p<0.01$) with creatine supplementation eliciting greater strength gains compared to non-supplememementation (29 ± 2.0 kg and 17 ± 3.0 kg) and males eliciting greater strength gains

compared to females (27 ± 3.0 kg and 19 ± 3.0 kg). With regard to elbow flexor thickness, both males and females increased (p<0.05) but males increased more than females (0.3 ± 0.1 and 0.2 ± 0.1 cm, respectively). These results show that creatine supplementation directly following resistance training can lead to increased muscle size in upper limbs and may be more effective in males.

Research on short-term (five to seven days) creatine-loading shows mixed results. Some studies show short-term creatine loading also improves strength performance (Law, Ong, GillianYap, Lim & Chia, 2009; Rossouw, Kruger, & Rossouw, 2000) while other studies show no significant increases in strength as a result of supplementation (Zungia, Housh, Camic, Hendrix, Russell, et al., 2012).

Role of Magnesium

Magnesium is the fourth most abundant mineral in the body and plays a vital role in many reactions essential to healthy functioning, including muscular action, blood pressure, and glycogen breakdown (Weaver & Nieves, 2009). Increasing daily ingestion of magnesium may positively affect many inflammatory conditions such as metabolic syndrome, hypertension, and type-2 diabetes (Hata, Doi, Ninomiya, Mukai, Hirakawa et al., 2013; Al-Delaimy, Rimm, Willett, Stampfer, & Hu, 2004; Chacko, Sul, Song, Li, LeBlanc, You et al., 2011; Rayssiguier, Libako, Nowacki, & Rock, 2010; Song, Manson, Buring, & Liu, 2004). Magnesium supplementation has also been reported to improve performance, due to its role in ATP production, and has recently shown to support down-regulation of genes related to inflammatory pathways (Chacko et al., 2011; Lukaski & Nielsen, 2002). Research shows intra- and extracellular concentrations of magnesium fluctuate with exercise (Laires &

Monteiro, 2007). Some research shows submaximal exercise over a long period of time results in decreased concentrations of plasma magnesium due to loss through perspiration and urination (Stendig-Lindberg, Shapiro, Epstein, Galun, Schonberger, Graff et al., 1987).

Magnesium deficiency leads to activation of immune cells such as macrophages and neutrophils and up-regulation of genes associated with inflammatory pathways. Additionally, magnesium deficiency is reported to up-regulate Vascular Cell Adheasion Molecule-1 (VCAM) and Plasminogen Activator Inhibitor-1 (PAI) thereby increasing thrombosis and atherosclerosis (Maier, Malpuech-Brugère, Zimowska, Rayssiguier, & Mazur, 2004). The inflammatory response resulting from magnesium deficiency relates to cytosolic calcium elevation and results in inhibition of macrophages and promotes stabilization and reduction of plaque (King, 2009). Another way magnesium may contribute to anti-inflammatory pathways is through its role in maintaining low triglyercide levels, which increase with hypomagnesemia (Nassir, Mazur, Giannoni, Gueux, Davidson, & Rayssiguier, 1995). Epidemiologic studies offer even greater insight into the role and importance of magnesium. Guerrero-Romero et al. (2002) examined C-reactive protein levels in obese individuals and found that individuals with the lowest magnesium intakes were twice as likely to have significantly increased C-reactive protein. Song et al. (2006) found magnesium intake was inversely proportional to C-reactive protein levels. It is the anti-inflammatory effects as well as the role of magnesium in hundreds of biological reactions, including ATP production and cell permeability that supports the need for more research on the effects of magnesium supplementation on DOMS.

Selsby et al. (2004) conducted one of the only existing studies to examine the effects of creatine and magnesium-creatine chelate supplementation on exercise performance.

Thirty-one weight-trained subjects were randomly assigned to a placebo, creatine, or magnesium-creatine chelate group and each subject ingested 2.5 grams of their respective supplement or placebo for seven days. At baseline and after 10 days, subjects completed a 1-RM bench press test and a fatigue test. The fatigue test was performed at 70% 1-RM during which subjects performed the lengthening and shortening phase of the bench press each in one second. The fatigue test ended when subjects could no longer complete the full repetition or could not maintain the proper cadence. Work was then calculated. Post-supplementation results showed the creatine and magnesium-creatine chelate groups both performed significantly more work (6,658 ± 306 J and 7,647 ± 524 J, respectively) than the placebo group (6,196 ± 437 J) when compared to baseline measures (6,239 ± 535 J, 5,613 ± 427 J and 6,384 ± 438 J). There was no significant difference in total work for the creatine and magnesium-creatine chelate group (6,658 ± 306 J vs. 7,647 ± 524 J). In this particular study, the first of its kind to demonstrate such low doses of creatine may improve performance in short, high-intensity bouts of exercise, magnesium-creatine chelate did not improve performance more than creatine ingestion. Though this research shows a lack of effect, the researchers feel their theory on a new mechanism for creatine transport is viable and requires further investigation. Due to the side effects of magnesium on the gastrointestinal tract, there was a limit to how much magnesium and creatine supplement could be ingested in this study.

Effect of Magnesium Supplementation on Strength

Magnesium deficiency affects muscle function and results in strength reductions, cramps, and muscle fiber damage during exercise and sport (Benardot, 2012). Athletes participating in sports that categorize individuals by weight are often susceptible to

magnesium deficiency due to dehydration as a result of rapid weight loss (Lakasaki, 2001).

Investigations of the effects of dietary magnesium on muscular strength suggest magnesium

deficiency is correlated with decreased performance in handgrip strength, knee extension

torque, lower-leg muscle power, and ankle extension strength (Dominguez, Barbagallo,

Lauretani, Bandinelli, Bos et al., 2006). Furthermore, a study by Matias, Santos, Moneiro,

Silva, Raposo, et al. (2010), evaluated the effects of magnesium changes on strength in elite

male athletes (n=20) who participate in the sport of judo. Serum magnesium, intracellular

water, fat-free mass, nutrient intake, and maximal strength were measured at baseline

(hydrated state) and pre-competition. Maximal handgrip strength was measured using a

dynamometer and upper body power was measured using a computer-interfaced bench press

machine. Subjects who lost more than 2% intracellular water from baseline to pre-

competition measures also lost a significant amount of red blood cell magnesium (44.8 ±

36.8% loss) and showed decreased performance (-4.6 ± 6.6%) in handgrip strength test. This

result positively correlates magnesium blood loss with decreased handgrip strength.

Another study by Santos et al. (2011) evaluated the effects of magnesium levels on

elite male basketball, handball, and volleyball players. Strength tests included maximal

isometric trunk flexion, extension, and rotation, handgrip, squat and countermovement

Abalakov jump, and maximal isokinetic knee extension and flexion peak torques. Seven-day

nutrition logbooks were assessed to determine individual magnesium levels and correlation

to performance on strength tests. Results showed the average level of magnesium intake in

these athletes (244.7 ± 78.8 mg) to be significantly (p<0.001) lower than the recommended

daily allowance of magnesium (400 mg). Regression analysis indicated that magnesium

intake was directly associated with trunk flexion (R^2=0.193, p<0.025), trunk rotation

(R^2=0.357, p<0.073) and handgrip strength (R^2=0.111, p<0.096). After adjusting for energy intake, these associations remained significant: trunk flexion (p <0.028), trunk rotation (p<0.055) and handgrip strength (p <0.027).

Brilla and Haley (1992) examined the effects of magnesium supplementation on a 7-week strength training program in untrained subjects. Compared to controls, subjects taking the magnesium supplement (total daily intake, including dietary magnesium, was 8g/kg body weight/day) showed significant (p<0.05) increases in absolute quadriceps torque, (211 vs. 174 Nm) relative torque adjusted for body weight (3.07 vs. 2.58 Nm/kg), and relative torque adjusted for lean mass (3.84 vs. 3.36 Nm/kg). The results of this study reflect the significant role of magnesium in torque gains after strength training while the results of the studies presented earlier reflect the impactful effects of magnesium deficiency on strength.

Effects of Creatine and Magnesium on Fatigue

Resistance to exercise-induced fatigue may be enhanced by creatine (Rawson, Stec, Fredrickson, & Miles, 2011; Hoffman, Stout, Falvo, Kang, & Ratamess, 2005) and magnesium (Cheng et al. 2010). Compared to a placebo group (specific placebo not listed), Rawson et al. (2011) observed significant resistance to fatigue in subjects (n=10) who ingested a creatine supplement (0.03 g/kg body weight/day) for six weeks. The fatigue test consisted of 5 sets of 30 repetitions of knee extension at 180°/sec. Each set was assigned a score based on the summation of the 30 peak torques generated during the set. While there were no significant changes in maximum strength (3RM concentric knee extension test at 180°/sec), body mass, fat free mass, or total body water, plasma creatine significantly increased (+182%, range 0-129 mmol/L) in subjects in the creatine group. The creatine group

was also shown to be more fatigue resistant than the placebo group in set 2, 3, 4, and 5 by 7%, 9%, 9%, and 11% respectively.

Hoffman et al. (2005) examined the effects of low dose creatine supplementation (6 g/day) over six days with pre- and post-fatigue tests consisting of three, 15-second Wingate tests. No significant change in peak power, mean power, or total work were observed, however, change in the rate of fatigue of total work was significantly lower in the creatine group as indicated by an elevated linear slope for the creatine group. These results are similar to those reported in other fatigue research (Selsby, 2004) and suggest that larger doses of creatine may not be necessary to realize the benefit of enhanced fatigue resistance.

Magnesium supplementation has been reported to improve performance and resist fatigue due to its role in ATP production (Lukaski & Nielsen, 2002). A study by Chen et al. (2010) examined the effects of magnesium sulfate supplementation on swimming performance in rats. Researchers observed increased glucose and magnesium levels (175% and 302% of basal levels) with pre-treatment of magnesium sulfate in non-swimming rats. In rats forced to swim, pre-treatment of magnesium sulfate reduced lactate levels 150% of basal during swimming. Magnesium levels also increased significantly during forced swimming and recovery (152-144% of basal). Another study of patients with chronic obstructive pulmonary disorder found that 2g of magnesium sulfate positively affects maximal exercise in patients as compared to 2g of placebo (saline) (do Amaral, Rodrigues-Junior, Terra Filho,Vannucchi, & Martinez, 2008). COPD patients receiving magnesium significantly improved performance on the cycle ergometer cardiopulmonary exercise test by pedaling for 53 seconds longer (p = 0.011) and reaching a workload of 8.0 (p =0.018). Thus, magnesium supplementation has been shown to attenuate lactate production and increases glucose and

exercise time thereby making a person's exercise more sustainable and less susceptible to fatigue.

Summary

Eccentric muscle action elicits many benefits but, as indicated in earlier sections, is also the primary cause of muscle-induced injury, DOMS and inflammation. Acute inflammation from muscle injury is also known to increase production of inflammatory markers, specifically CK and lactate dehydrogenase (LDH), and is implicated in increased circulation of more global inflammatory markers. Creatine and magnesium both may play a crucial role in the attenuation of exercise-induced muscle damage, inflammation, and enhancement of normal physiological gains form exercise. As a result, this study's examination of magnesium-creatine chelate, alkaline creatine compound and placebo supplementation on DOMS following high-intensity eccentric muscle action adds to the understanding of supplementation effects on DOMS.

Chapter III

Methods and Procedures

Introduction

The purpose of this study was to assess the effects of magnesium-creatine chelate, alkaline creatine compound and placebo supplementation on DOMS following eccentrically-induced muscle damage. DOMS was induced using a device that required subjects to resist loads at 65% of their relative maximum value.

Description of Study Population

Thirty-six young (18-24 years old), healthy, recreationally active individuals were the subjects for this project. Subjects were chosen randomly based on their volunteer responses and were physically active students from Western Washington University. Prerequisite to participation, each subject completed an informed consent form previously approved by the institution's Human Subjects Committee, in accordance with the National Institute of Health guidelines (Appendix A). Informed consent was obtained from all subjects (Appendix B). Subjects who had supplemented magnesium or creatine in their habitual diet within 60 days prior to orientation or any who were suffering from kidney, liver, or endocrine disease or any disorder that might affect normal cellular levels of creatine or fluid balance (or both) were excluded. The medical history questionnaire filtered any subjects taking any substance classified as a diuretic, other than caffeine, in their habitual diet. Subjects were instructed to keep exercise regimens consistent throughout the study.

Design of Study

The design of the study followed a double blind, three-group format analyzed pre and post a four-week treatment period. Each subject was randomly assigned to one of the following groups: alkaline creatine, magnesium-creatine chelate or placebo group. During the treatment period, subjects in the alkaline creatine group ingested 5 g of creatine plus an alkalizing agent, soda ash, each day. Subjects in the magnesium-creatine chelate group ingested 5 g of creatine plus 400 mg of magnesium, a dose of magnesium that is equivalent to 100% or the recommended daily allowance (RDA) (Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 1997). Subjects in the placebo group ingested 5 g dextran per day. All subjects were instructed to take their respective supplements with 16 oz (0.47 L) of liquid. The treatment period was four weeks to allow for tissue saturation.

Comparisons were made from baseline, between and within groups for DOMS using an eccentric machine and weight using a balance beam scale. Exercise testing was conducted at the same time of day at baseline and after the treatment period. Post-treatment data collection also included height, measured by stadiometer.

Data Collection Procedures

All testing was performed in the Biomechanics Laboratory at Western Washington University. The testing procedure lasted an average of 30 minutes with the exercise protocol lasting five minutes.

Subjects completed an eccentric training session using a prototype eccentric trainer. The device pedals perform a cyclic unilateral motion requiring eccentric muscle action of the

hip and knee extensors. The eccentric trainer includes a performance monitor displaying force production during exercise. This exercise and machine were used for the purpose of eliciting DOMS. The subjects experienced a brief familiarization protocol with the eccentric device. This protocol consisted of a mid-range velocity set at 35 cycles per minute. During the familiarization period, the subjects were asked not to resist the machine to an extent that could cause fatigue. When the subjects became familiar with the motion, peak eccentric force was obtained on the eccentric trainer at the velocity of 35 cycles per minute. The average of three maximal eccentric contractions was used to establish each subject's workload.

After the maximal values were calculated, the subjects were instructed to get off the eccentric machine and take a five-minute rest prior to their testing session. During the rest period, subjects were allowed to walk, stretch or sit-down in order to obtain a sufficient recovery. During the initial moments of the test, subjects were given a few repetitions to become comfortable with the velocity of the movement. After this brief re-familiarization, subjects were instructed to maintain a force of 65% of the peak eccentric force for the full training session. The testing session consisted of five minutes of continuous work, an exercise duration thought to elicit DOMS (Clarston et al., 1988)

DOMS measurements were assessed 12, 24, 48, 60, and 72 hours post-exercise. Subjects were instructed to send an email with a muscle soreness score that corresponded to the visual analog scale (Appendix C) with scores from 1 (normal) to 10 (very, very sore). Subjects were also instructed to refrain from rigorous exercise until the last muscle soreness questionnaire was completed. During the four-week training period, subjects continued their normal activities.

Descriptive statistics were calculated. Data were analyzed using a group by time mixed ANOVA at an alpha level of 0.05. Data analysis was done with Microsoft Excel 2007 and SPSS software version 11 (Chicago, IL).

Chapter IV

Results and Discussion

Introduction

The purpose of this study was to assess the effects of magnesium-creatine chelate, alkaline creatine compound, and placebo supplementation on DOMS following a bout of eccentric muscle action. At baseline and post-treatment period, subjects completed one five-minute bout of eccentric knee and hip extensor exercise. Following both bouts of exercise, subjects reported muscle soreness on a visual analog scale every 12 hours for 72 hours post exercise. Comparisons were made between and within groups for DOMS and weight at baseline and post-treatment period. Pre-treatment data collection also included height.

Subject Characteristics

Thirty-six subjects participated in this study. Twelve subjects were assigned to the alkaline creatine group (M: 6, F: 6), 13 subjects to the magnesium-creatine chelate group (M: 7, F: 6) and 11 subjects to the placebo group (M: 8, F: 3). The number of subjects' data included in the data set (Appendix D) is much smaller than originally expected, due to incomplete reporting from subjects. Despite email reminders, many subjects neglected to submit their pain scale reports as required. This resulted in attrition of two subjects from each creatine group and seven from the placebo group. There were no significant differences in subject physical characteristics ($p>0.05$). Group data are presented in Table 1.

Table 1: Subject Characteristics

Group		Age	Height (cm)	Weight Pre (kg)	Weight Post (kg)
Placebo	Mean	20.45	173.08	70.15	70.93
	SD	2.21	12.92	12.86	13.32
Alkaline Creatine	Mean	21.17	175.03	77.09	77.57
	SD	2.21	9.97	17.26	17.57
Mg Creatine Chelate	Mean	21.85	175.99	76.68	77.32
	SD	1.82	10.84	14.38	14.71

Results

Delayed Onset Muscle Soreness

There were no significant interaction effects ($F[5,1.95] = 0.36$, $p = 0.86$). The creatine groups did have significant main effects pre to post testing in DOMS using a visual analog scale after a 5-minute eccentric bout of exercise ($F[5,26.95] = 5.23$, $p = 0.0002$). The effects were observed at 12, 24, and 36 hour intervals for the alkaline creatine subjects and at 12 hour intervals up to the 60 hour interval for the magnesium-creatine chelate group. The descriptive data are presented in Table 2. A display of group responses from the pre-test are displayed in Figure 1 and the post-test results are shown in Figure 2. The finding that the placebo group scores were higher at all times in the post-test is demonstrated in Figure 3. However, they were not significantly different from the pre-test data ($p > 0.05$).

Table 2. DOMS reports after 5-Minute Bout of Eccentric Exercise

Groups		**DOMS pre**						**DOMS post**					
		12 hrs	24 hrs	36 hrs	48 hrs	60 hrs	72 hrs	12 hrs	24 hrs	36 hrs	48 hrs	60 hrs	72 hrs
Placebo	**Mean**	6.0	5.2	6.4	5.6	5.0	3.6	4.7	4.8	4.7	4.3	3.5	2.7
	SD	1.6	1.9	1.1	1.8	2.2	2.3	1.5	0.8	1.9	2.7	1.9	1.2
Alkaline Creatine	**Mean**	4.5	6.1	6.1	5.3	3.9	2.9	3.5	3.8	4.2	3.8	3.3	2.5
	SD	1.9	1.6	1.9	2.2	1.9	2.0	2.0	1.8	2.2	2.6	2.5	1.9
Mg Creatine Chelate	**Mean**	5.0	5.9	5.8	6.4	5.0	3.5	3.2	3.8	4.2	3.9	2.9	2.3
	SD	2.9	2.6	2.7	2.3	2.6	2.3	1.6	2.5	1.8	1.6	1.5	1.6

Figure 1. Delayed Onset Muscle Soreness Pre-Supplementation

DOMS Pre--- Test

	12 hrs	24 hrs	36 hrs	48 hrs	60 hrs	72hrs
Placebo	6	5.2	6.4	5.6	5	3.6
Alkaline Crea>ne	4.5	6.1	6.1	5.3	3.9	2.9
Mg Crea>ne Chelate	5	5.9	5.8	6.4	5	3.5
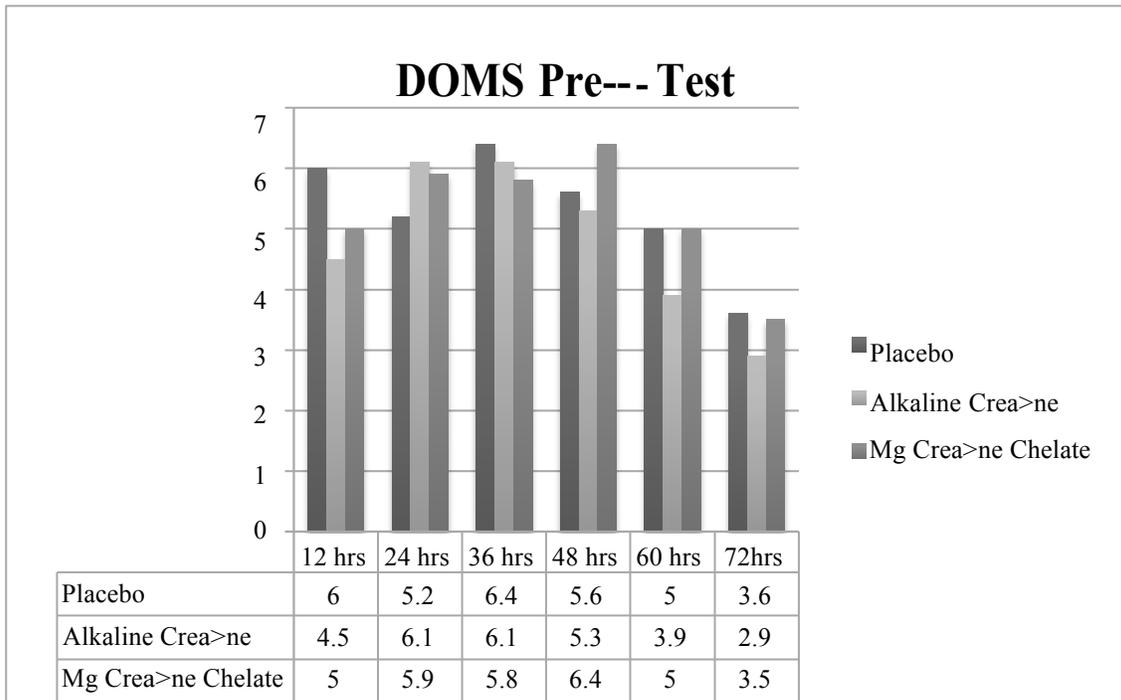
Figure 2. Delayed Onset Muscle Soreness Post-Supplementation

DOMS Post-Test

	12 hrs	24 hrs	36 hrs	48 hrs	60 hrs	72hrs
Placebo	4.7	4.8	4.7	4.3	3.5	2.7
Alkaline Creatine	3.5	3.8	4.2	3.8	3.3	2.5
Mg Creatine Chelate	3.2	3.8	4.2	3.9	2.9	2.3
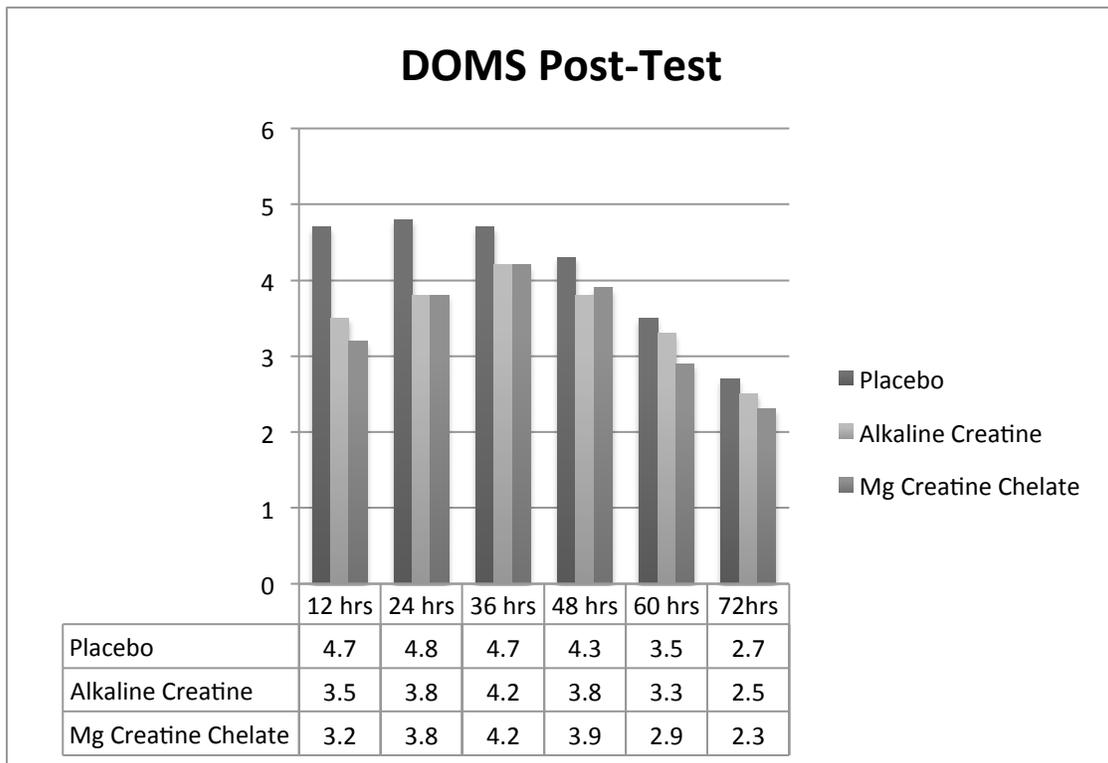
Figure 3. Creatine Effect on Delayed Onset Muscle Soreness

DOMS Post-Test

	12 hrs	24 hrs	36 hrs	48 hrs	60 hrs	72 hrs
Placebo	4.7	4.8	4.7	4.3	3.5	2.7
Alkaline Creatine	3.5	3.8	4.2	3.8	3.3	2.5
Mg Creatine Chelate	3.2	3.8	4.2	3.9	2.9	2.3
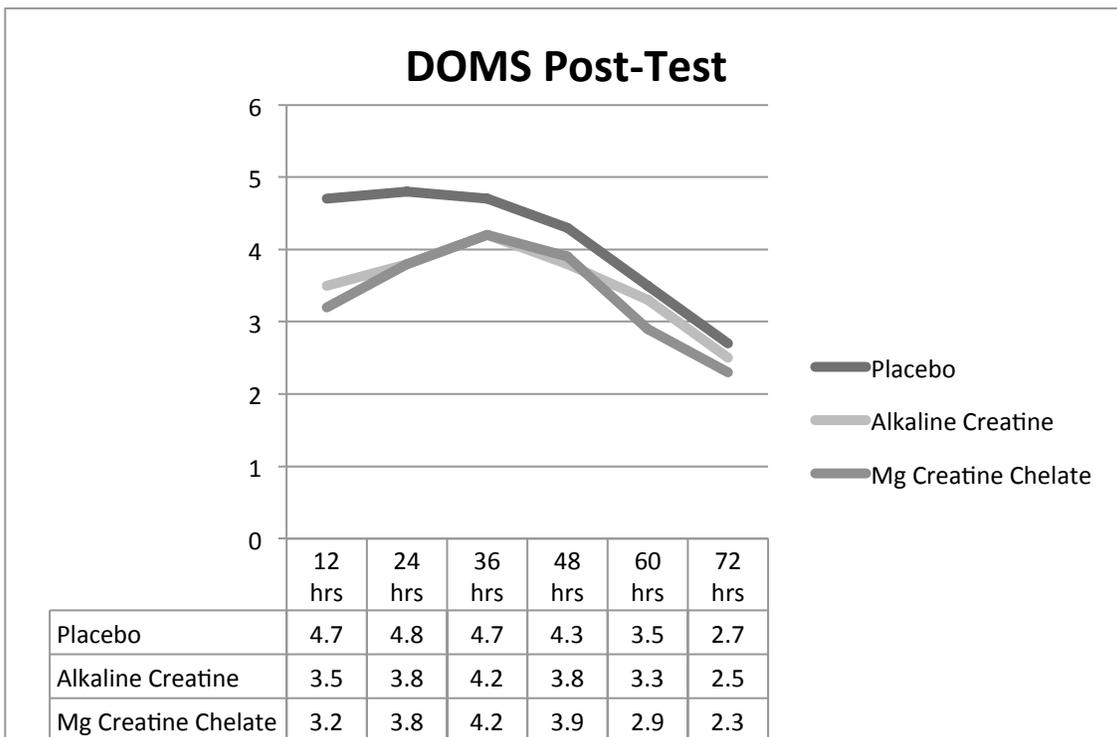
Discussion

This study examined the effects of magnesium-creatine chelate, alkaline creatine compound, and placebo supplementation on DOMS following a bout of eccentric muscle action to better understand the effects of creatine and magnesium supplementation on exercise-induced muscle soreness. DOMS improved in the creatine groups as demonstrated by lower levels of perceived soreness during the three days post exercise after the intervention period.

The perceived lower level of soreness compared to the pretest persisted in the alkaline creatine subjects for 36 hours. The findings presented in this project are consistent with results reported by Santos et al. (2003) who reported that creatine supplementation resulted in significant attenuation of muscle inflammatory markers (CK, LDH, PGE_2, and TNF-α) following exhaustive long distance running when compared to controls. Bassit et al. (2008) reported similar findings regarding marked reductions in TNF-α , INFα, IL-1β, and PGE_2 following half-ironman competition.

Conversely, in 2001 and again in 2007, Rawson et al. found creatine supplementation was ineffective compared to controls, with both treatments resulting in equally significant increases in inflammatory markers (CK and LDH) following high intensity eccentric muscle action of the elbow flexors (2001) and hypoxic exercise via intensive leg squat exercise protocol (2007). Interestingly, compared to the four-week supplementation time utilized in this project, Bassit et al. (2008), Rawson et al. (2007), Santos et al. (2003) and Rawson (2001) each utilized five-day loading doses of creatine, suggesting that a greater duration of supplementation may be a better regime for attenuation of DOMS.

Magnesium-creatine chelate subjects experienced reduced soreness enduring for 60 hours post exercise. While the effects of magnesium-creatine chelate supplementation did not result in significantly improved DOMS compared to that of the alkaline creatine group, research shows magnesium to have a positive effect on many inflammatory markers such as C-reactive protein (Hata, et al., 2013) and TNF-α (Roman, Desai, Rochelson, Gupta, Solanki et al., 2013). Additionally, research has shown magnesium is inversely related to more general systemic inflammation (Song et al., 2005).

Summary

Alkaline creatine and magnesium-creatine chelate supplementation leads to improvements in DOMS following a five-minute bout of high-intensity eccentric muscle action after a four-week training period. The four-week supplementation time utilized in this study was greater than that of many other research studies, which utilized only five-days of creatine loading. Supplementation periods longer than four weeks may be necessary to elicit positive effects on DOMS.

Magnesium and creatine are both known anti-inflammatory agents and active buffers in the human body. These properties combined make creatine and magnesium especially relevant for DOMS intervention considering inflammation is a characteristic of exercise-induced DOMS and energy production may produce acidic conditions. While the role and specific impact of creatine and magnesium supplementation is not clear, the anti-inflammatory properties of both supplements should inspire new research to better understand their impact on DOMS.

Chapter V

Summary, Conclusions, and Reconditions

Summary

Eccentric exercise protocols are superior for generating more efficient gains in strength, power and hypertrophy and improving activities of daily living (Kaminiski et al.; LaStayo et al., 2003; Tesch et al., 2008). While the mechanism of eccentric muscle action remains unclear, it is different than that of concentric muscle action as indicated by greater efficiency of force generation (Ryschon et al., 1997). Delayed onset muscle soreness (DOMS) is a well-known consequence of eccentric muscle action, the significance of which may prevent individuals from reaping the benefits of eccentric training (Clarkson et al., 1986; Newham et al., 1983; Rawson et al., 2001; Rawson et al., 2007).

Researchers have examined many interventions, including massage, hydrotherapy, nutritional supplements and various exercise prescription protocols to ameliorate the effects of DOMS (Best et al., 2008; Henschke et al., 2011; Vaile et al., 2008). None of these interventions have proven reliably effective at DOMS reduction. However, creatine and magnesium supplementation may be the most promising intervention for DOMS due to their role in ATP generation and buffering as well as possible positive effects on inflammatory markers (Bassit et al., 2008; Greenhaff et al., 1994; Parkhouse & McKenzie, 1984; Santos et al., 2004). Through better understanding of the effects of creatine and magnesium supplementation on DOMS, more informed decisions can be made regarding exercise and supplement protocol.

Conclusion

DOMS improved in the alkaline creatine and magnesium-creatine chelate groups as demonstrated by lower levels of perceived soreness during the three days post exercise. Compared to the pretest, the perceived lower level of soreness persisted in the alkaline creatine subjects for 36 hours and endured for 60 hours in the magnesium-creatine chelate group. Therefore, supplementation for 4 weeks may elicit more positive benefits compared to shorter supplementation periods predominantly utilized in other research studies which were inconsistent whether creatine attenuated DOMS.

Recommendations

Based on the findings of this study, the following are recommendations for future research:

1. Conduct the same study with a larger sample population to protect against attrition and outliers. This study experienced a high degree of attrition which may have affected the results.

2. Conduct the same study with a different age group, specifically older adults, to better understand the interaction between supplementation and age.

3. Conduct the same study with a longer supplementation period to further investigate if creatine groups would yield different results. It is common practice by those who supplement with creatine to take it continuously.

4. Conduct the same study utilizing blood analysis of inflammatory markers in addition to DOMS reporting. This would provide additional data to balance the subjective reporting of subjects which is inherently skewed.

5. The results of this study suggest that a period of creatine and magnesium-creatine chelate supplementation may protect against DOMS following a bout of eccentric exercise. The following are recommendations for application of finding:

    1. Creatine and magnesium combined may be more effective than just creatine. This requires further study.

    2. The effects in this study show that four weeks of creatine may be a better regimen than a loading dose in amelioration of DOMS used in other studies (Rawson et al, 2003; Rawson et al., 2007)

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Appendix A

Human Subjects Proposal

Effects of Creatine Magnesium Supplementation on Delayed Onset Muscle Soreness
Karla Landis
Human Subjects Activity Review Form

1. The Null Hypothesis states: Following high-intensity eccentric muscle action, there will be no difference in DOMS due to supplementation of magnesium-creatine chelate, alkaline creatine compound or placebo.

2. The potential benefits of this experiment include adding to a growing body of literature regarding efficacy and nutritional safety of creatine supplementation. Limited experiments focus on testing the effect of combining creatine with magnesium or other alkalizing agents. With comparison between alkaline creatine and magnesium-creatine chelated supplementation on body water and physical performance attributes, the efficacy of this combination is tested with subsequent meaning on the related changes for physical performance.

3. As a benefit for participation, the subjects will be introduced to a new form of resistance training.

4. A) The subject population will be pooled from recreationally active participants who volunteer either based on responses to fliers around Western Washington University campus or from announcements made in Physical Education activity classes. The investigator will then contact the subjects individually to explain the study, its duration or expected time involvement and information regarding performance or nutritive benefits to be expected from performing this experiment.

   B) Thirty six (36) apparently healthy subjects will be chosen from this pool and randomly assigned numerically to one of the three supplementation groups, magmesium-creatine chelate, alkaline creatine, or placebo, maltodextrin, by a third party. No additional compensation will be used.

5. Before testing, the participants will be informed of the testing procedures and will be provided with the informed consent documents (See attached). During the presentation of this study, the participant data will be assigned an identification number in order to ensure confidentiality and anonymity. At no point will an individual's identifiable data be released to public sources.

Methods and Procedures

Description of the Study Population

The study population consisted of thirty-six apparently healthy, recreationally active participants. Subjects were chosen randomly based on their volunteer responses to brochures and flyers posted around Western Washington University (WWU) campus and solicited from beginning weight training and conditioning classes. Prerequisite to participation, each subject received an informed consent form previously approved by the institution's Human Subjects Committee, in accordance with the National Institutes of Health guidelines. Subjects who had supplemented creatine or

magnesium in their habitual diet within the 60 days prior to orientation or any who were suffering from any kidney, liver, or endocrine disease or any disorder that might affect normal cellular levels of Cr or fluid balance (or both) were excluded. The medical history questionnaire filtered any subjects taking any substance classified as a diuretic other than caffeine in their habitual diet. Subjects were instructed to keep exercise regimens consistent throughout the study.

Design of the Study

This study followed a double blind, three group, format analyzed for a 4-week testing period. These three groups consisted of an alkaline creatine group, a magnesium-creatine chelate group, and a placebo group. Comparisons were made from baseline, between and within groups for delayed muscle soreness [DOMS] after eccentric exercise. Testing procedures were conducted at the same time of day to avoid confounding errors.

Procedures and Instrumentation:

During baseline and posttest data collection, DOMS assessed by a 10-point scale after a 5-minute lower body eccentric exercise bout. These measures are repeated at the end of the study.

Supplementation:

Supplementation was given orally and all capsules provided by the manufacturer appeared identical. Subjects were randomly assigned to groups (magnesium-creatine chelate, alkaline creatine, placebo), and these were administered in a double blind fashion. Subjects were given 5-g creatine equivalent per day in four equal doses or placebo, maltodextrin. The magnesium was equivalent to 400 mg per day, a dose that has previously been well tolerated in studies done in our laboratory. The alkalizing agent in the other condition was soda ash. Subjects were instructed to take the supplement with 16 oz (0.47 L) of liquid. The treatment period was four weeks to allow for tissue saturation.

Exercise Protocols:

Subjects completed a 5-minute lower body eccentric exercise bout on a PreCor exercise machine designed for eccentric muscle activity. Subjects were asked to rate any DOMS at 12, 24, 48, and 72 hours after the exercise bout.

Data Analysis:

Descriptive statistics, means and standard deviations were calculated. Data were analyzed using a repeated-measures ANOVA at an alpha level of 0.05. Data analysis was done with Microsoft Excel 2007 and SPSS software version 11 (Chicago, IL).

6. All the testing was performed on a unilateral hip and knee extensor device developed to elicit eccentric muscle actions. A similar device was used by LaStayo et al. (2003) to examine eccentric muscle actions. The device was an eccentric ergometer which was powered by a three horse power motor the drives the pedals, backward. An eccentric muscle action was elicited by the subjects resisting the devices rotation. A

similar device was constructed which consisted of eccentric ergometer was employed by Evans et al. (1986). Rodenburg, Bar, and Boer (1993) used a device that had similar applications but was used for the elbow flexor muscles. The subjects sat in a chair with their chest against a support. The arm was also placed on a support in line with the shoulder. A rope with weight was attached to the wrist via a pulley system. Subjects resisted the weight using their elbow flexor muscle while it was being lowered. This elicited a muscle action while muscle was being lengthened.

7. Control for this experiment consists of a placebo group ingesting a simple sugar, maltodextrin, and given the same body composition and strength assessments as the two creatine magnesium treatment groups. Eccentric muscle action is known to elicit delayed onset muscle soreness therefore the eccentric ergometer is an appropriate instrument.

8. Kurosawa, Hamaoka, Katsumura, Kuwamori, Kimura, Sako, & Chance (2003) examined 25 healthy males using a handgrip dynamometer performed for a single 10 second maximal grip exercise. These subjects were measured pre and post supplementation with either a 30 gram creatine monohydrate or placebo dosage each day for 14 days. The subjects' forearm muscles were evaluated by 31-phosphorus magnetic resonance spectroscopy. The results indicate an increase in total anaerobic ATP synthesis during the 10 second hand grip exercise after creatine supplementation which positively correlated with the increase in ATP synthesis through PCr hydrolysis (Kurosawa, Hamaoka, Katsumura, Kuwamori, Kimura, Sako, & Chance, 2003). Creatine supplementation produced a 15.1 +/- 3.8% increase in mean power output during hand grip exercise. It is strongly indicated that an improvement in performance was associated with the increased PCr availability for the synthesis of ATP (Kurosawa, Hamaoka, Katsumura, Kuwamori, Kimura, Sako, & Chance, 2003). Brilla and Haley (1992) studied the effects of magnesium supplementation on strength training in humans. Their study investigated the effects of dietary magnesium on strength development during a double-blind, 7-week strength training program in 26 untrained subjects aged 18 to 30 years old. Three-day diet records were analyzed and Mg content was calculated. Body composition was assessed using BIA. The BIA results demonstrate that although the Mg group reduced fat percentage while the control group increased in fat percentage, these changes were not statistically significant. Pre and post quadriceps torque was also measured; yet, unlike body fat, the results indicated a significant (p < .05) increase in absolute torque for the Mg supplementing group (Brilla & Haley, 1992).

9. As with any exercise, the possibilities of muscle fatigue or muscle injury can not be entirely avoided. Supplementation with creatine can also cause some mild discomforts that may occur in some subjects, including: dehydration, muscle cramps, flatulence, and gastrointestinal distress. Dehydration and muscle cramping can be typical effects of supplementation with creatine but can be decreased by assuring proper hydration. Magnesium toxicity is rare and mostly affects people with kidney

disease. However, this is not a concern for healthy people. All proper procedures will be taken to reduce the risk of exercise-induced injuries including proper warm-up and cool down activities.

10. In an effort to minimize risks and to limit side effects, a low dose short-term creatine supplementation is used. Subjects who had supplemented creatine or magnesium in their habitual diet within the 60 days prior to orientation or any who were suffering from any kidney, liver, or endocrine disease or any disorder that might affect normal cellular levels of creatine or fluid balance (or both) were excluded. The medical history questionnaire filtered any subjects taking any substance classified as a diuretic other than caffeine in their habitual diet.
All proper procedures, including a five-minute warm up and cool down provided by the investigator, will be taken to reduce the risk of exercise-induced injuries.

11. Subjects identifiable information will be undisclosed during the study. During the presentation of this study, the participant data will be assigned an identification number in order to ensure confidentiality and anonymity. At no point will an individual's identifiable data be released to public sources. Subjects will be given personal composition and strength measures only upon experiment completion or termination. All data will be maintained electronically and hard copy in a cabinet in a locked room.

12. N/A

13. N/A

Appendix B

Informed Consent

Western Washington University
Physical Education, Health and Recreation Department
Title: Effect of Creatine Magnesium Supplementation on Delayed Onset Muscle Soreness

Print Name: Karla Landis
_____

The purpose of this study is to establish whether a low dose creatine compound, in two different forms, supplemented for 28 days would show improved physical performance. This study will contribute to the current literature on the possible benefits of creatine supplementation for sport performance and the time course in which these benefits may be achieved. This study will also investigate changes in body water. The benefit of this research is that the subjects may develop increased strength and power allowing them to see improved performance.

All participants will be required to take a supplement of magnesium-creatine chelate, alkaline creatine, or placebo, a sugar capsule of maltodextrin for 28 days. Each participant will complete tests pre and post supplementation. The tests include: 3-day dietary analysis and 3-day physical activity log; body water and percent fat by bioelectrical impedance analysis (BIA); neuromuscular function including strength and fatigue, anaerobic power, and delayed onset muscle soreness [DOMS] after a 5-minute eccentric activity. They will be collected pre-post each treatment phase. The testing consists of the orientation to equipment, initial baseline testing and supplement distribution. Before beginning the program you will be required to fill out a health history questionnaire. If you have any medical or physical limitations, or have any renal disorders, you may not be eligible to participate in this study.

The baseline data will be collected the first week of the study and again during the last week of the study. A familiarization session will take place prior to the initial testing to establish measurements necessary for the testing procedures. The familiarization session will last approximately 30 minutes and will be conducted in the Physiology Lab (CV 210) and Biomechanics Lab (CV 146). The testing procedures after baseline and familiarization will last approximately 30-45 minutes.

During the familiarization session your weight and height will be measured on a balance beam scale and stadiometer. At this time you are instructed to complete the 3-day diet record and 3-day activity log which will be returned on the day of testing.

Each testing session will consist of:

- A 5-minute eccentric muscle test where a force is resisted at low velocity. Subsequent emails will be sent by the subject to the investigator at 12, 24, 48, and 72 hours after the test about any delayed onset muscle soreness [DOMS].
- Total testing is expected to last approximately 20-30 minutes.

Data will be used to quantify any differences between the results during each of the testing periods.

Mild discomforts associated with exercise include: muscle fatigue, muscle soreness, or muscle strain. Supplementation with creatine can also cause some mild discomforts that may occur in some subjects, including: dehydration, flatulence, and gastrointestinal distress. Dehydration can be typical effects of supplementation with creatine but can be decreased by assuring proper hydration. Magnesium toxicity is rare and mostly affects people with kidney disease. However, this is not a concern for most healthy people. Occasionally, people may experience loose stools if they take a large dose of magnesium at one time. The supplementation in this study separates the doses into morning and evening to avoid this potential side effect. All proper procedures will be taken to reduce the risk of exercise-induced injuries including proper recovery, warm-up, and cool down activities. Possible benefits that some subjects may see resulting from participation in this study include: short term gains in strength, power, muscle torque, increase in total body water and an increase in exercise performance.

All subjects will be assigned a reference number to assure that all records will be kept confidential. Data for each individual from each testing session will not be shared with other subjects. Data will be collected on computer and kept in separate files that will be accessed only by reference number.

Subject questions and concerns will be initially addressed during the orientation session and subjects should feel free to ask questions and voice concerns as the study progresses. Your participation in this study is greatly appreciated. This participation is voluntary and you may withdraw yourself from participation at any time.

If you have any questions or comments during the course of the study please contact Karla Landis directly at 206-849-0521, karla.landis@wwu.edu. I will answer any questions you may have concerning the procedures. If you have any questions about your participation or you rights as a research participant, you can contact Geri Walker, WWU Human Protections Administrator, (360) 650-3220, geri.walker@wwu.edu.

Investigators: Lorrie Brilla, Kathy Knutzen, Brandi Row, and Karla Landis

I have read and understand the procedures for the study described above. I am aware of the potential risks and I agree to participate as a subject in the study described above. I understand that I may withdraw from participation at any time during the course of the investigation.

Subject Signature:_____ Date:_____

Witness Signature:_____ Date:_____

Appendix C

Visual Analog Scale

Perceived Muscle Soreness (Clarkson et al., 1986).

Rate the general soreness of entire knee and hip extensor muscle groups when moving or
using the muscle.
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1 (normal) – 2 – 3 – 4 – 5 – 6 – 7 – 8 – 9 – 10 (very, very sore)

Appendix D

Subject Data

Subject Data

Number	P.O.C.	Creatine Pre-Testing DOMS Results						Creatine Post-Testing DOMS Results					
		12 hrs	24 hrs	36 hrs	48 hrs	60 hrs	72 hrs	12 hrs	24 hrs	36 hrs	48 hrs	60 hrs	72 hrs
2	P	5	7	6	6	4	2	7	5	5	3	2	1
1	P	6	4	7	8	8	6	1	2	2	3	3	1
2	P	4	7	8	6	6	6	3	5	5	4	4	3
2	P	8	2	6	6	5	4	1	6	6	8	4	3
1	P	6	6	5	3	2	1	4	2	1	1	1	1
1	P	7	5	6	5	5	3	4	5	6	7	6	4
2	O	6	7	7	5	4	2		4	6	6	6	5
1	O	3	3	4	2	1	1	2	4	4	4	3	3
2	O	6	8	9	8	7	5	3	6	8	8	7	6
4	O	5	6	7	6	5	5	5	6	6	7	7	5
1	O	4	6	6	5	3	2	1	1	1	1	1	1
1	O	1	6	8	7	4	3	1	1	1	1	1	1
4	O	6	9	7	7	6	3	4	6	6	5	5	2
6	O	2	6	5	5	3	2	2	4	4	5	2	2
4	O	5	5	4	3	2	1	4	3	4	1	1	1
1	O	5	5	3	2	2	1	6	4	3	2	1	1
3	O	7	6	7	8	6	7	4	3	3	2	2	1
3	C	7	8	9	8	7	6						
4	C	9	10	10	10	9	9	4	6	6	5	4	3
9	C	3	5	7	7	6	4	5	7	6	5	2	1
3	C	10	8	9	9	8	4	6	8	10	10	7	6
2	C	3	6	5	5	5	3	3	2	4	5	4	4
1	C	2	4	4	5	2	1	3	2	3	6	3	2
3	C	3	4	4	4	3	2	1	1	2	2	1	1
4	C	4	3	2	2	1	1	1	1	1	1	1	1
5	C	3	6	4	7	4	3	3	3	4	4	3	2
4	C	8	9	7	7.5	6.5	4	4	5	6	5	3	2
4	C	3	2	3	6	3	2	2	3	4	3	2	1