COMPARISON OF DIFFERENT FORMS OF CREATINE ON CREATINE AVAILABILITY, RETENTION, AND TRAINING ADAPTATIONS

A Dissertation

by

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ABSTRACT

The purpose of this study was to determine if a buffered creatine monohydrate (KA) that has been purported to promote greater creatine retention and training adaptations with fewer side effects at lower doses is more efficacious than creatine monohydrate (CrM) supplementation in resistance-trained individuals.

In a double-blind manner, 36 resistance-trained participants (20.2±2 years, 181±7 cm, 82.1±12 kg, and 14.7±5 % body fat) were randomly assigned to supplement their diet with CrM or KA at two different dosages. Muscle biopsies from the vastus lateralis, fasting blood samples, body weight, DEXA determined body composition, and Wingate Anaerobic Capacity (WAC) tests were performed at 0, 7, and 28-days while 1RM strength tests were performed at 0 and 28-days. Data were analyzed by a repeated measures multivariate analysis of variance (MANOVA) and are presented as mean ± SD changes from baseline after 7 and 28-days, respectively.

Muscle free creatine content obtained in a subgroup of 25 participants increased in all groups over time ($p=0.03$) after 7 and 28-days, respectively, with no significant differences among groups ($p=0.46$). Although some significant time effects were observed, no significant group x time interactions ($p>0.05$) were observed in changes in body mass, fat free mass, fat mass, percent body fat, or total body water; bench press and leg press 1RM strength; WAC mean power, peak power, or total work; serum blood lipids, markers of catabolism and bone status, and serum electrolyte status; or, whole blood markers of lymphocytes and red cells. Neither manufacturers recommended

doses (1.5 g/d) or KA with equivalent loading (20 g/d for 7-days) and maintenance doses (5 g/d for 21-days) of CrM promoted greater changes in muscle creatine content, body composition, strength, or anaerobic capacity than CrM (20 g/d for 7-days, 5 g/d for 21-days). There was no evidence that supplementing the diet with a buffered form of creatine resulted in fewer side effects than CrM. These findings do not support claims that consuming a buffered form of creatine is a more efficacious and/or safer form of creatine to consume than creatine monohydrate.

DEDICATION

I would like to dedicate this work to my parents and wife.

Mom and Dad, thank you for all of your love and support throughout my educational journey. I could not have done this without all of your help emotionally, spiritually and financially. The two of you have been an inspiration and an example of what hard work and determination can get you.

Vanessa, you have been with me along my side since the beginning. Thank you for putting up with me during the times of struggle and being there to support me. I could not have done any of this without you. Your love and support have been a staple throughout this process and you deserve this as much as I do.

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NOMENCLATURE

CrM	Creatine Monohydrate
KA	Kre-Alkalyn
PCr	Phosphocreatine
Cr	Creatine
MP	Mean Power
PP	Peak Power
TBW	Total Body Water
1RM	1 Repetition Maximum
g/d	Grams per Day

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CHAPTER I

INTRODUCTION AND RATIONALE

Background

Creatine is a amino acid-like compound that is involved in the transfer of high energy phosphates within the body[1]. There is strong evidence to support the use of creatine as an ergogenic aid, especially for those involved in high-intensity exercise such as sprinting and weight lifting [2-4]. Creatine supplementation is becoming more and more popular not only in athletes but in the recreationally active as well. Recent studies estimate that 28-41% of NCAA athletes and 29-57% of recreationally active individuals supplement with creatine [5, 6]. The form of creatine that has been most extensively studied has been creatine monohydrate (CrM) [2]. There are numerous different formulations and combinations of creatine on the market but several studies have indicated that creatine monohydrate seems to be the most effective in terms of increasing the amount of creatine stored within the body and improving performance [2, 7-11]. Research has indicated that creatine monohydrate supplementation can increase creatine and phosphocreatine concentrations by approximately 15-40% [8, 12-14]. These increases in creatine concentrations often times correlate to improvements in performance, particularly anaerobic exercise capacity, and increases in training volume which can lead to greater gains in strength, power, and muscle mass [2-4, 14-20]. Creatine supplementation has repeatedly been shown to be safe with minimal side effects even following long-term supplementation protocols of 3-5 years[21-27]. A number of potential therapeutic benefits have also been suggested in various clinical

populations [28-34]. Again, research has continuously shown creatine monohydrate to be the most efficacious form of creatine because of its high bioavailability. For example, research suggests that 99% of ingested creatine monohydrate is either taken up by tissues or extracted in urine despite allegations of creatine monohydrate being degraded to creatinine in the stomach [35-37]. Regardless, supplement manufacturers continue to create new formulations of creatine with the intent of creating a more efficacious form of creatine. These newer forms have been purported to have better physical and chemical properties, bioavailability, efficacy, and/or safety profiles than creatine monohydrate [2]. For example, a company out of Montana has recently created a buffered form of creatine *(Kre-Alkalyn® [KA], All American Pharmaceutical, Billings, MT, USA)* that is marketed as a more potent form of creatine and purported to be more chemically stable when compared to creatine monohydrate [38]. The company claims their product doesn't degrade to creatinine in the stomach as much as creatine monohydrate because it's buffered and therefore a lower dosage is required to yield similar results [38, 39]. As mentioned previously, research indicates that 99% of ingested creatine monohydrate is either taken up by the tissues or excreted in the urine. Therefore it is unlikely that Kre-Alkalyn is a "*more potent*" form of creatine. In fact, researchers have shown the conversion of creatine to creatinine at a pH of 1.0 was less than 1% after 5, 30 and 120 minutes while KA had a 35% higher conversion to creatinine under similar conditions [40].

Creatine monohydrate has continually been shown to be effective with little to no side-effects and no detrimental effects on markers of clinical health. Regardless, KA

manufacturers again make claims that their product has less negative side-effects and is safer than creatine monohydrate[39]. We therefore designed a study to compare this new formulation of creatine to the well-researched, creatine monohydrate. There were two different dosages of KA used in the study, a high (creatine monohydrate equivalent) and low (manufacturer's recommended) dose. The study utilized a double blind, randomized design with 3 groups: 1) A creatine monohydrate group, 2) A recommended dose of KA group, and 3) A creatine monohydrate equivalent KA group. This design allowed us to determine the safety and efficacy of this new formulation of creatine. The supplementation period consisted of 28-days with the creatine monohydrate and KA equivalent groups consuming loading doses (20 g/d) during the first 7-days, followed by maintenance doses of 5 g/d for the remaining 21-days and the KA recommended dosage group (1.5 g/d) maintained that dose throughout the duration of the study. This design allowed us to assess the acute and long-term effects of the two forms of creatine on muscle creatine retention, body composition, strength, anaerobic capacity and markers of health status. If KA was more efficacious than CrM, the recommended dose of 1.5 g/d should have been just as, if not more, effective than the creatine monohydrate dosage. Additionally, if in fact KA was a more potent form of creatine, ingesting the creatine equivalent loading and maintenance doses of KA would've theoretically promoted greater effects with fewer side effects than those ingesting standard loading and maintenance doses of CrM. It is possible that new formulations of creatine may be more efficacious than creatine monohydrate but they must first be investigated in a controlled setting to monitor its effectiveness and safety before these types of claims can be made.

Specific Aim

Was supplementation with Kre-Alkalyn (a buffered form of creatine) more efficacious than creatine monohydrate in terms of increasing creatine availability, retention and/or training adaptations compared to creatine monohydrate?

Purpose of the Study

The purpose of this study was to compare the effects of different forms of creatine on creatine availability, retention and training adaptations.

General Study Overview

This study was a double-blind 28-day supplementation study that compared the effects Kre-Alkalyn ® and creatine monohydrate on creatine availability, retention and training adaptations. To contrast the effects of a new form of creatine, Kre-Alkalyn, creatine monohydrate was used as a control. Creatine Monohydrate (CM) is well accepted in the literature to be the gold standard of creatine and a successful ergogenic aid. Subjects were randomly assigned to one of 3 groups: 1) Creatine Monohydrate; 2) Kre-Alkalyn: equivalent dose to CM; or 3) Kre-Alkalyn: manufacturer's recommended dose. All three groups returned to the lab for a total of three testing sessions on Days 0, 7 and 28. Assessment of muscle phosphagen levels, markers of clinical health, body composition, body water and anaerobic capacity were measured during each testing session while upper and lower body maximal strength were only assessed on Days 0 and 28.

Hypotheses

The central hypotheses were:

Ho1: There will be a significant difference between groups in phosphagen levels as measured by muscle biopsies after 7 and 28 days of supplementation.

Ho2: There will be no significant difference between groups in markers of clinical health and safety after 7 and 28 days of supplementation.

Ho3: There will be no significant difference between groups in body composition as measured by dual x-ray absorptiometry (DEXA) after 7 and 28 days of supplementation.

Ho4: There will be a significant difference between groups in anaerobic capacity as measured by the 30 second Wingate test on a Lode cycle ergometer after 7 and 28 days of supplementation.

Ho5: There will be no significant difference between groups in strength as measured by 1RM on the leg press and bench press exercises after 28 days of supplementation.

Delimitations

This study was conducted under the following guidelines:

1. Thirty-six (n = 36) resistance trained males age 18-40 were recruited for this study.
2. Subjects refrained from the consumption of dietary supplements, anabolic steroids, and/or ergogenic aids (excluding daily vitamins and protein supplements) for at least three months prior to initiating testing.

3. Eligible participants took part in a familiarization session during which time they were informed of the study protocol, filled out necessary paperwork including an informed consent form, performed a trial anaerobic capacity test and were scheduled for testing.
4. Subjects completed a 4 day dietary record prior to each testing session and were advised to maintain a consistent diet throughout the duration of the study.
5. Subjects were advised to maintain a consistent workout regimen throughout the duration of the study and recorded all workouts in a workout log.
6. Muscle biopsies were obtained from the vastus lateralis at each testing session which were analyzed for muscle creatine content.
7. Subjects refrained from strenuous exercise at least 48 hours prior to each testing session.
8. Subjects were fasted for at least 8 hours prior to each testing session.
9. Subjects performed to their maximal ability on all strength and anaerobic capacity tests.
10. Subjects were instructed to consume all supplements, record times of consumption and report any side effects in a daily supplement log.

Limitations

1. The participants were individuals from the Texas A & M community and surrounding fitness facilities that responded to recruitment fliers and emails; therefore the selection process was not truly random.

2. While there were some variations in testing times and dietary intake, all efforts were made to conduct testing sessions at the same approximate time to account for diurnal variations and subjects were instructed to maintain a consistent diet throughout the duration of the study.
3. Motivations and effort during performance testing may not have been 100% at each testing session.
4. Subjects may not have followed the supplement instructions.
5. All participants were instructed to maintain a consistent training program and keep a workout log. However, exercise habits during the duration of the study may have changed and therefore changes in performance measures may have been influenced by the training program rather than the assigned supplement.
6. All equipment was calibrated according to manufacturer guidelines and all samples were run in duplicate to reduce likelihood of error. However, there are innate limitations of the laboratory equipment that were used for data collection and analysis.

Assumptions

1. Participants followed the protocol that was explained to them during the familiarization session.
2. Participants answered the entrance questionnaires accurately and honestly prior to being accepted into the study.
3. Participants adhered to the supplementation protocol and testing schedule.

4. All laboratory equipment was calibrated and functioning properly prior to all testing sessions.
5. The population, which the sample was drawn from, was normally distributed.
6. The variance among the population sample was approximately equal.
7. The sample was randomly assigned to the different supplement groups.
8. Participants maintained a consistent dietary intake and exercise regimen throughout the duration of the study.

CHAPTER II

REVIEW OF LITERATURE

Introduction

Creatine (N-(aminoiminomethyl)-N-methyl glycine) has become one of the most popular nutritional supplements on the market [2]. It's estimated that nearly 15 to 30% of high school athletes and 48% of male division I college athletes supplement with creatine [41-43]. Creatine, also known as methyl guanidine-acetic, is classified as a nitrogen containing organic acid and has a molecular formula of $C_4H_9N_3O_2$ [1]. It is produced naturally within the body and is located in brain, liver, kidney and muscle tissue with the majority (95%) being located within skeletal muscle [1]. Roughly 60% of muscle creatine is in the phosphorylated form, phosphocreatine (PCr) with the remaining 40% being free creatine [21]. An adult male weighing 70 kg has an average of 100-150 g of total body creatine [1]. The human body is able to synthesize roughly 2 g/day with the remainder of the creatine coming from food sources, specifically meats and fish.

Creatine is essential to life as it plays a vital role in the transfer of energy within cells [44]. Energy is provided to the cell through the hydrolysis of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) and inorganic phosphate (Pi). Through a reversible action involving phosphocreatine (PCr), ADP, hydrogen ions (H+) and the enzyme creatine kinase (CK), ATP can be rapidly resynthesized when the phosphate group from PCr is added to ADP resulting in ATP and creatine. This reaction helps to maintain intracellular ATP availability and acts as a buffer for H+ [1]. Creatine

may also assist with the transfer of high energy phosphates from the mitochondria to areas of ATP utilization via the creatine phosphate shuttle[45].

ATP is rapidly degraded during high intensity exercise in order to provide energy for the contractile proteins within skeletal muscle. Muscle phosphagens are involved in the replenishment of ATP during exercise. Phosphocreatine has been proposed as one of the limiting factors to muscle performance; especially during short bouts of high intensity exercise [1]. There is evidence that suggests PCr is the first and most prominent fuel source used to resynthesize ATP during high-intensity exercise[45]. Previous research has shown that the rate of PCr resynthesis after high intensity exercise is positively correlated with exercise performance during a subsequent bout of high intensity exercise [46, 47]. Therefore by increasing pre-exercise levels of PCr as well as post-exercise rates of PCr resynthesis, improvements in muscle contractile capacity would likely follow due to an enhancement in the shuttling of high-energy phosphates via the creatine phosphate shuttle. A way to increase PCr stores is to supplement the diet with exogenous creatine with the intention of increasing the potential for creatine transport into the musculature.

Effects of Creatine Supplementation on Phosphagen Levels

The human body is able to synthesize roughly 2 g of creatine per day from the amino acids glycine, arginine, and methionine[48]. Also, 1-2% of the creatine pool is broken down in to creatinine and excreted through the urine each day as part of normal daily turnover [21]. Therefore, the creatine content within the body at any given time is a reflection of the balance between endogenous creatine production, the amount of

creatine consumed in the diet and the amount of creatine degraded each day. If enough creatine is not taken in through the diet, creatine supplementation may be necessary to maintain adequate levels. Several supplementation strategies have been implemented with the intent of increasing phosphagen levels within the muscle and ultimately improving performance. The increases seen in muscle creatine stores following supplementation strategies appear to be dependent on the amount of creatine ingested, duration of the supplementation period and the initial levels of creatine stores prior to supplementation [21]. Several researchers have also suggested that there is likely a maximal amount of total creatine that can be stored within the body, somewhere in the range of 150-160 mmol/kg dm [7, 13, 49].

Creatine research began to gain popularity during the late 1980's and early 1990's. One of the earlier creatine supplementation studies done by Harris et al. found that 20 g of creatine per day, ingested for 5 days, led to a 20% increase in total muscle creatine concentration, of which approximately 20% was in the form of PCr [13]. The authors also found that creatine uptake was greatest during the first 2 days of supplementation, suggesting that rapid increases in muscle creatine content can be seen when supplementing with high doses of creatine. Hultman et al. utilized a similar dosing strategy which had subjects following a protocol that involved 20 g/d for 6 days or a lower-dose protocol consisting of 3 g/day for 28 days [14]. Similar to the study mentioned above, authors observed increases in muscle creatine of approximately20% in both groups. However, the 20 g/day group experienced increases in muscle creatine at a much faster rate compared to the lower dose of 3 g/d. These early studies led to the

development a supplementation strategy that is more commonly known as a "loading phase." A loading phase is characterized by the consumption of .3g/kg/day (~20g) of creatine for a 3-5 day period in order to achieve rapid increases in muscle phosphagen levels. Research has shown loading protocols to increase muscle creatine and PCr stores by ~10-40% [21] in as little as 3-5 days[50, 51]. There is also some evidence which seems to suggest there is little to no additional benefit of consuming more than 20 g of creatine per day [1]. Also during a loading phase, researchers recommend ingesting 5g every 4-5 hours (~20 g total) in order to maintain peak creatine plasma levels [13] throughout the day.

Other creatine supplementation strategies, such as "cycling" have been used as well. Cycling protocols consists of multiple "loading" phases of 3-5 days every 3 to 4 weeks [48, 51]. More modest strategies have also been employed which don't include any loading phase and rather consist of maintenance dosages (3-6 g/d [52]) for extended periods of time [53, 54]. These protocols seem to be just as effective as loading strategies but increases in creatine stores appear to be more gradual [21]. There does seem to be a minimal dose of at least 2 g/d in order to elicit significant increases in muscle phosphagen levels [55]. Although the majority of research has shown creatine supplementation to be an effective strategy for increasing muscle phosphagen levels, some studies have failed to show a significant effect. For example, Odland et al. failed to observe any significant increases in muscle phosphagen levels following 20 g of creatine for 3 days [56]. However, the study utilized a crossover design consisting of a 14 day washout period which may not have been enough time for creatine levels to

return back to baseline. Research suggests that increased creatine levels will likely return to baseline around 30 days following the cessation of supplementation [1, 14]

Effects of Creatine on Measures of Performance

Creatine supplementation has been shown to be an effective strategy to improve performance in a variety of ways. There are over a 1,000 published research articles in peer-reviewed journals examining the efficacy of creatine supplementation [57]. The vast majority of them (~70%) have shown creatine to have some sort of ergogenic value, whether it be improved strength, power, muscular endurance or improvements in training adaptations. The average improvement seen in performance typically ranges between 10-15% following creatine supplementation [48]. These improvements can occur following short-term and long-term supplementation periods and seem to be augmented when combined with a structured training program.

Anaerobic Performance

The majority of improvements seen in performance are during those activities consisting of short-duration, high-intensity bouts of exercise. The average effect size (ES) of creatine supplementation on activities lasting less than 30s is 0.24 ± 0.02[58]. The average percent change from baseline is 7.5 ± 0.7% for creatine supplementation groups compared to 4.3 ± 0.6% for placebo. According to a meta-analysis that was done in 2002, the average increases seen in bench press 1 RM was 6.85 kg and 9.76 kg for back squat 1RM in creatine groups when compared to placebo [59].

Improvements in performance have been observed in as little as 3-5 days following the initiation of creatine supplementation when loading doses are used. For

example, Volek and associates observed greater increases in the amount of work completed during 5 sets of bench press and jump squats in a creatine supplementation group (25 g/d) compared to placebo after only 7 days of supplementation [60]. Wright et al. observed significant improvements in peak power and mean power during 6 sets of 10 sec sprints on a cycle ergometer following creatine supplementation (20 g/d for 6 days)[61]. In a similar study, Tarnopalsky et al. reported increases in cycling power following creatine supplementation (20 g/d for 4 days)[62]. Furthermore, Urbanski et al. observed a significant increase in knee extension strength and time to fatigue following creatine supplementation (20 g/d for 5 days). There have been hundreds of other studies done examining the effects of short-term loading strategies on measures of anaerobic performance with the majority of them seeing improvements in strength, total work done, time to fatigue, and power [17, 21, 48, 51, 57, 63-66] . Furthermore, loading protocols typically lead to increases in body mass of about 1 to 2 kg after only 1 week of supplementation[57]. Therefore it appears as though loading protocols consisting of ~20 g/d for 5-7 days are an effective way to improve performance during bouts of short, high-intensity exercise.

Similar improvements in anaerobic performance are seen when longer duration supplementation periods are used. For example, Kreider et al. found that 28 days of creatine supplementation resulted in a significant increases in total work performed during 6 s sprints compared to placebo [19]. In a similar study, Kelly et al. found a significant improvement in upper body 3RM and total work completed during 5 sets of bench press in power lifters following 28-days of creatine supplementation [67]. Similar

improvements were seen in body mass and lean body mass in those supplementing with creatine. Similarly, Grindstaff et al. found that creatine supplementation (21 g/d for 9 days) significantly improved high-intensity swim performance during 3 x 100m freestyle swims with 60 s rest in between sets as well as ergometer performance during 3 x 20s sprints with 60 s recovery in between sprints [68].

However, not all evidence suggests improvements in performance following creatine supplementation. For example, Deutekom et al. reported that creatine supplementation (20 g/day for 5 days) did not improve maximal exercise performance during sprint cycling or improve muscle recovery from electrical stimulation in well-trained rowers [69]. In a similar study, Febbraio et al. failed to observe increases in supramaximal cycling performance following 5 days of creatine loading despite increases in creatine content [70]. Similarly, Green et al failed to see improvements in mean and peak power during consecutive upper and lower body Wingate tests after six days of supplementation (20 g/d) [71]. Barnett et al. failed to observe any improvements in cycling power during consecutive bouts of high-intensity cycling sprints following creatine loading [72]. Several other studies have failed to show any significant improvements in high-intensity exercise performance following creatine supplementation [73][74, 75] [76]. Even when supplementation periods are extended over time, studies still have failed to show any benefits from creatine. For example, Stout et al. failed to observe improvements in vertical jump performance or 100 yd sprint times following 56 days of creatine supplementation (21 g/d for 5 days and 10.5 g /d for 51 days) during an off-season football training program. However, it appears as though

studies that fail to observe improvements in performance, generally still see a 1-7% improvement; albeit not statistically significant [57].

Evidence suggests that when creatine is supplemented with a structured training program, greater improvements can be seen in muscle creatine content, sprint performance, strength, power, lean body mass and rate of force development versus those not taking creatine [48]. In a recent review, researchers summarized the latest research that investigated the effects of creatine supplementation on muscle strength and weightlifting performance following when combined with a resistance training program [77]. Researchers observed that the average increase in muscle strength following creatine supplementation and resistance training was a 20% compared to only 12% in placebo groups. They also concluded that the average increase in muscular endurance or weightlifting performance following a resistance training program was 26% for creatine and 12% for placebo groups. There are several other studies that support these findings. For example, Noonan et al. found a significant improvement in upper body strength when creatine supplementation was combined with an 8 week resistance training program [78]. Similarly, Vandenberghe et al. found significant improvements (20-25%) in 1RM for leg press, leg extension, squat and arm flexion torque following when creatine supplementation was combined with 10 weeks of resistance training in previously sedentary females [79]. Furthermore, the creatine group experienced significantly greater gains in lean body mass +5.8% (creatine) vs. +3.7% (placebo) or +2.6 kgs compared to +1.6 kgs, respectively. Other studies have indicated similar improvements in fat-free mass from groups following a regular resistance training

program when combined with creatine supplementation and typically experience increases of 2-4 lbs. more of lean body mass compared to placebo groups [21, 66, 78].

The majority of research suggests that improvements in anaerobic performance are likely attributable to a higher short-term energy supply before the initiation of exercise as well as improvements in PCr resynthesis rates [1, 7, 58, 80]. This will ultimately delay PCr depletion, helping to sustain force production during not only a single bout but during subsequent bouts of high intensity exercise as well. For example, Birch et al. found lower plasma ammonia levels following 3 successive 30 sec bouts of maximal effort isokinetic cycling following creatine loading (20 g/d for 5 days) [63]. The authors suggested these results indicate an improvement in ATP resynthesis rates and less reliance on the adenlyate kinsase and adenylate deaminase reactions which lead to increases in adenosine monophosphate (AMP) and ultimately ammonia levels if the reactions occur at a high rate. Similar studies have found lower levels of hypoxanthine during subsequent bouts of high intensity exercise following creatine supplementation [17, 81]. Hypoxantine is well accepted as a marker of skeletal muscle adenine nucleotide loss during intense exercise [82].

At this time it is unknown whether improvements in training adaptations are a result of indirect effects, such as an improved ability to resynthesize PCr during exercise and ultimately complete more work during a training bout or a more direct effect by stimulating certain anabolic pathways. After 5 days of creatine loading (21 g/d), Deldicque et al. found increases in collagen mRNA, glucose transporter 4 and myosin heavy chain IIa expression [83]. The authors suggested that the observed increases in

gene expression following creatine supplementation may provide an anabolic environment which could be further augmented when combined with resistance training. Furthermore, Willoughby and colleagues observed increases in creatine kinase, myogenin and myogenic regulatory factor 4 (MRF-4) expression when creatine supplementation (6 g/d for 12 weeks) was combined with a resistance training program [54]. This again would provide an anabolic-like environment for muscle hypertrophy to occur and could lead to increases in fat-free mass over time if combined with a structured resistance training program. Similarly, Burke et al. observed a greater increase in IGF-1 concentration following an 8-week resistance training program in a creatine supplementation group compared to placebo[84]. It has also been proposed that because creatine is a large substance, it may lead to an osmotic gradient and pull water in to the muscle cell thus increasing intracellular water and body mass [60]. Some researchers suggest that this increase in cellular hydration may stimulate protein synthesis and explain some of the anabolic effects of creatine supplementation [20, 49, 60].

Increases in training adaptations following creatine supplementation can also be further enhanced when creatine is combined with other nutrients. For example, Cribb et al. observed further increases in 1RM, lean body mass and muscle cross sectional area when subjects ingested a supplement containing creatine (0.1 g/kg/d) as well as protein and carbohydrates (1.5 g/kg/d) during a resistance training program [85]. Burke et al. observed significantly greater improvements in lean body mass, bench press 1RM and isokinetic strength following 6 weeks of resistance training and creatine + whey protein

supplementation compared to just whey protein alone and/or placebo[86]. In a similar study, Noonan et al. found significant improvement s in upper body strength, 100 yd dash times, and fat-free mass when carbohydrates were added to creatine during 8 weeks of resistance and sprint training [78]

Aerobic Performance

Although the rationale behind creatine supplementation is to enhance short-term energy stores by increasing phosphagen levels within skeletal muscle, creatine supplementation may also improve longer duration aerobic-like performance as well. It's been proposed that creatine supplementation may improve the shuttling of high-energy phosphates between the cytosol and mitochondria [11]. In a study done by Chwalbinska-Monteta et al., investigators observed an increase in lactate threshold in elite male endurance rowers following a short-term loading protocol (20 g/d for 5 days) [87]. In a similar study done by Preen et al., creatine loading (20 g/day for 5-d) was shown to improve exercise performance during 80 min of repeated-sprint exercise in active males [50]. The authors suggested that an improved ability to resynthesize PCr as a result of creatine loading likely led to the increases in performance. Nelson et al. reported decreases in submaximal heart rate and oxygen uptake as well as an increase in ventilatory threshold and time to exhaustion during a maximal exercise test following 7 days of creatine supplementation (20 g/d) [88]. Researchers also observed lower exercise and recovery lactate values during and after a graded exercise test on a cycle ergometer in those supplementing with creatine compared to baseline. Lower lactate

levels during exercise could assist athletes involved in high-intensity interval training and improve the anaerobic threshold.

Conversely, not all evidence suggests that creatine supplementation will improve aerobic performance. Hickner et al. examined the effects of a 28-d creatine supplementation protocol (3g/d) on performance during a 2 hour simulated cycling road race in male triathletes [89]. Subjects completed a 2 hour simulated cycling road race with intermittent sprints before and after supplementation. Results indicated no improvements in sprint time to exhaustion or power output during a post-race sprint following supplementation.

Clinical Safety and Applications of Creatine Supplementation

One of the biggest misconceptions regarding creatine is that it is a potentially dangerous supplement with various side-effects. It is well accepted that the only significant side effect of creatine supplementation is weight gain [21]. Nevertheless, there still seems to be several anecdotal claims in the media and sports world that creatine is a dangerous supplement that can lead to cramping, gastrointestinal distress, and kidney failure. These claims continue to persist even though the majority of research has demonstrated that creatine supplementation is safe with minimal side effects. Even long-term creatine supplementation has been shown to be safe. Poortman et al. has shown no effect of creatine monohydrate supplementation on renal function after 5 years of supplementation [90]. Similarly, Kreider et al. monitored markers of clinical health in college football players who were supplementing with creatine for a 3 year period and failed to observe any changes and/or side effects following

supplementation [91]. Creatine has also been used in clinical settings as well in order to treat certain diseased populations. For example, Vannas-Sulonen and colleagues supplemented gyrate atrophy patients with a low dose of creatine (1.5 g/d) for 5 years and did not observe any side effects [92]. Similarly, infants with creatine synthesis deficiencies were given a creatine supplement (4 to 8 g/d) for two years without any evidence of clinical side-effects [93].

There is some evidence that suggests creatine may actually be useful as therapeutic agent. Research suggests that creatine supplementation may lower total cholesterol levels and/or increase high-density lipoprotein levels [19, 80]. Also, due to creatine's role in proper brain development and functioning, creatine supplementation may be an effective treatment for brain-related disorders linked with deficiencies in energy metabolism such as Huntington's Disease and Parkinson's Disease [94]. Current research is focusing on the possibility of creatine supplementation reducing rates of atrophy and muscle wasting in certain neuromuscular disorders as well as spinal cord injuries [51, 95-98].

New Formulations and Combinations of Creatine Supplementation

The form of creatine that is the most popular and well researched is creatine monohydrate [2]. However, there seems to be a constant influx of new products and formulations of creatine in hopes to surpass the efficacy of creatine monohydrate. For example, some manufacturers will combine creatine with other substances such as salts and phosphates with the intent of augmenting the effects of creatine or enhancing is bioavailability. For example, some of the creatine products on the market include

creatine + B-hydroxy-Bmethylbutyrate (HMB), creatine + sodium bicarbonate, creatine magnesium-chelate, creatine serum or liquid creatine, creatine ethyl ester, and creatine phosphate [21]. There have been several comparative studies in which creatine monohydrate have been compared to new formulations of creatine in order to examine their efficacy, however creatine monohydrate continues to appears to be the most efficacious [21]. Some of the research with creatine + HMB shows promise as well as those products which include additional macronutrients supplements as mentioned above [85, 99-101].

Some new formulations have been shown to be as effective when compared to creatine monohydrate. For example, Peeters and colleagues compared the effectiveness of creatine phosphate supplementation to creatine monohydrate [102]. Results indicated that the groups ingesting creatine monohydrate and creatine phosphate had similar significant increases in body weight, lean body mass, and bench press 1RM following 6 weeks of resistance training and supplementation compared to a placebo control.

In a related study, researchers examined the effects of 3 different forms of creatine on whole body creatine retention, measured by the amount of creatine excreted in the urine subtracted from the amount ingested [8]. Subjects ingested 5 g of dextrose, 5 g of CrM, 5 g of CrM + 18 g of dextrose, or 5 g of an effervescent creatine supplement + 18 g dextrose, four times a day for 3 days. Following the loading period, creatine retention was greatest in the CrM + carbohydrate group however there were no significant differences between the CrM and effervescent groups. These results suggest that the addition of carbohydrates to creatine may augment creatine retention. Also, one

can take away that an effervescent creatine supplement is just as effective as creatine monohydrate in terms of creatine retention. In a similar study, Kreider et al. investigated the effects of consuming a liquid form of CrM compared to powdered form. Subjects consumed either 20 g of CrM, 2.5 g of liquid CrM (recommended dosage) or 20 g of liquid CrM (equivalent dose to CrM and 7 times the manufacturer's recommendations) per day for 5 days [11]. Results indicated that creatine monohydrate supplementation increased muscle free creatine content by approximately 31% whereas no increases in muscle free creatine content were observed following liquid creatine supplementation even though 7 times the recommended dose was used.

Jager et al. examined the effects of three different forms of creatine on plasma creatine concentrations [10]. Subjects ingested either creatine monohydrate (CrM), tricreatine citrate (TCC), or creatine pyruvate (CPY). The CPY group actually had a higher mean peak concentration and area under the curve compared to the other 2 groups. These results suggest that creatine pyruvate may have a higher bioavailability compared to creatine monohydrate. The authors did mention that muscle biopsies are needed in order to conclude that the increases in plasma creatine concentrations observed directly correlate to an increase in creatine uptake at the muscle and thus increasing skeletal muscle creatine content over time.

Adding certain macronutrients with creatine has also been shown to improve creatine retention. Researchers have suggested a synergistic effect when carbohydrates are ingested with creatine [8, 101]. Green et. al observed a 60% increase in muscle creatine stores when 96g of carbohydrates were combined with 5 g of creatine

monohydrate compared to those ingesting solely creatine [101]. The authors suggested that the ingested carbohydrates yielded a rise in insulin levels which likely increased the transport of creatine into the muscles. Furthermore, the authors observed a 50% reduction in urinary creatine levels when carbohydrates were combined with creatine, suggesting an increase in creatine retention. In a similar study, Steenge and associates found that 47g of carbohydrate and 50g of protein was just as effective in improving creatine retention as 96g of carbohydrates [103]. The addition of D-pinitol (a plant extract with insulin like properties) has also been shown to augment creatine retention [104]. This research suggests that creatine bioavailability and retention can be improved through the addition of certain macronutrients to a creatine supplement.

One research group has recently proposed that a buffered form of creatine may have a higher bioavailability compared to creatine monohydrate [39]. This group suggests that a buffered form of creatine won't degrade to creatinine in the acidic environment of the stomach as rapidly as creatine monohydrate [38, 105]. However research has shown that creatine monohydrate is not degraded during normal digestion and nearly 99% of orally ingested creatine is either taken up by tissues or excreted in the urine [35-37]. In fact, in a recent review, authors described that the degradation of creatine can actually be reduced by either lowering the pH to under 2.5, as is seen in the stomach, or increasing the pH [2]. Therefore in a very acidic environment, creatine may in fact be more stable.

Practical Applications

From a practical standpoint, creatine supplementation has been shown to be an effective method of increasing phosphagen levels within the body. This increase in intramuscular phosphagen levels may improve the ability to resynthesize phosphocreatine and sustain a high level of exercise intensity before the onset of fatigue. The majority of research has shown creatine to be an effective ergogenic aid with significant improvements seen during high-intensity exercise. Creatine supplementation can also augment training adaptations when used together with a structured training program. Creatine supplementation has repeatedly been shown to be safe with minimal, if any, negative side-effects. Creatine monohydrate seems to be the most effective form of creatine however it is still important to investigate new formulations as they are developed to ensure the efficacy and safety of these new products.

CHAPTER III

METHODS

Experimental Design

Table 1 presents the experimental design of the study and what was required by participants during each testing session throughout the 28-days. The study was conducted in a double-blind, match controlled manner utilizing three treatment groups. Subjects who qualified for the study participated in a familiarization session during which the study protocol and design was explained to the participants. During the familiarization session, informed consent was obtained. After the familiarization session, subjects were matched for bodyweight, years of training experience, and age and randomly assigned to one of three groups: 1.) KA at manufacturer's recommended doses (KA-L); 2.) KA at creatine monohydrate equivalent dose (KA-H); or 3) CrM). Dietary intake was not controlled but participant's dietary intake was recorded prior to each testing session and analyzed for energy intake and macronutrient content. Participants were instructed to maintain their normal resistance-training program and maintain training logs so training volume could be compared.

Table 1. Experimental Design

Familiarization and Entry	Baseline Day 0	Loading Phase Day 7	Maintenance Phase Day 28
Familiarization session Informed Consent Form Demographic Form Health History Form Exercise History Form 4-day Dietary History General Exam to Determine Qualifications to Participate in Study Height and Body Weight Practice Wingate Anaerobic Capacity Test Randomization into one of three groups (CrM, KA-L, KA-H) Instructions for Supplementation	4-Day Diet History Muscle Biopsy Fasting Blood Sample Body Weight Body Water (BIA) DEXA Body Composition 1 RM Leg Press 1 RM Bench Press Wingate Anaerobic Capacity Test Loading Phase of Supplementation Begins Maintain Training Log	4-Day Diet History Submit Training Log Muscle Biopsy Fasting Blood Sample Body Weight Body Water (BIA) DEXA Body Composition Wingate Anaerobic Capacity Test Low-Dose Maintenance Phase of Supplementation Begins	4-Day Diet History Submit Training Log Muscle Biopsy Fasting Blood Sample Body Weight Body Water (BIA) DEXA Body Composition 1 RM Leg Press 1 RM Bench Press Wingate Anaerobic Capacity Test

Independent and Dependent Variables

The independent variable of interest was the type of creatine ingested. Dependent variables included muscle creatine content, body composition, one repetition maximum (1RM) for bench press and leg press, anaerobic sprint performance capacity, serum and whole blood clinical markers of health, and self-reported side effects.

Study Site

All laboratory testing was conducted in the Exercise & Sports Nutrition Laboratory with the exception of the muscle biopsies procedures which were performed in the Human Countermeasures Laboratory. Both laboratories were located in the Department of Health and Kinesiology at Texas A&M University in College Station, TX.

Subjects

Thirty-six apparently healthy college aged resistance-trained males were recruited to participate in this study. Participants were not allowed to participate in this study if they had any metabolic disorder including known electrolyte abnormalities; heart disease, arrhythmias, diabetes, thyroid disease, or hypogonadism; a history of hypertension, hepatorenal, musculoskeletal, autoimmune, or neurologic disease; if they were taking thyroid, anti-hyperlipidemic, hypoglycemic, anti-hypertensive, anti-inflammatory, or androgenic medications. Participants were not allowed to participate if they had taken any nutritional supplements or ergogenic aids (i.e. creatine, beta-alanine, HMB, DHEA, thermogenics etc) other than a daily multivitamin and protein powder within the previous three months. Participants were recruited from the student population and from area fitness facilities. Recruitment fliers were posted around the Health & Kinesiology building as well as emailed to students enrolled in physical education activity classes. Participants meeting entrance criteria were asked to attend a familiarization session during which they signed an informed consent form in compliance with the Human Subjects Guidelines of the Texas A&M University and the

American College of Sports Medicine.

Familiarization

Prior to the initiation of the study, subjects attended a familiarization session during which details of their participation were explained. This included details of the supplementation and exercise protocols, an explanation of how to record dietary and exercise information, and a thorough description of the muscle biopsy procedures. During familiarization participants completed demographic, health history and exercise history forms to ensure they qualified for the study. Those who met eligibility criteria were again informed of the requirements of the study and signed informed consent statements in compliance with the Human Subjects Guidelines of Texas A&M University and the American College of Sports Medicine. Participants were also weighed using a standard scale and familiarized themselves with the Wingate anaerobic capacity test in order to minimize any learning effects. They were also given further instruction on the importance of continuing their regular dietary and exercise routines in order to maintain consistency throughout the study.

Testing Sessions

Participants were asked to fast for 8 hours and abstain from exercise 48 hours prior to all testing sessions. Subjects handed in their dietary and exercise logs as well as their supplement bags and supplement check sheet (on Days 7 and 28) upon arrival for their testing session. Once reporting to the ESNL lab, subjects were shuttled to the Human Countermeasures Lab and donated a muscle biopsy. Immediately following the biopsy, subjects were transferred back to the ESNL and donated a fasting blood sample

using standard clinical procedures. Subjects were then weighed, and had body water assessed using a bioelectrical impedance analyzer (BIA). Following this test, subjects had their body composition assessed using a Dual-Energy X-Ray Absorptiometer (DEXA).

Following the resting tests, subjects completed 1RM tests on the bench press and hip sled/leg press (on Days 0 and 28 only) and performed a 30-second Wingate anaerobic capacity sprint test on a cycle ergometer at each testing session. Following baseline testing, subjects began a 7-day initial supplementation phase. After 7 days, subjects repeated all tests with the exception of the1RM strength measures. The subjects then followed the maintenance supplementation schedules for 21-days and then returned to the lab and completed all tests. This allowed for the assessment of acute and chronic supplementation protocols on muscle creatine levels, body composition, exercise performance, as well as markers of clinical health and safety. Participants were asked to maintain their current training programs and record all workouts throughout the 28-day period. Participants were asked to report side effects on a daily basis and record the ingestion of all supplements.

Supplementation Protocol

Following baseline testing, participants were matched according to body weight, training status/experience, and age and randomly assigned to one of three groups. Depending on which group they were in, subjects ingested, in a double blind manner, capsules containing CrM (*Creapure® AlzChem AG, Trostberg, Germany, Lot #108631*) or KA *(Kre-Alkalyn® All American Pharmaceutical, Billings, MT, USA, Lot #1067000)*

at two different dosages for a 28-day period. The supplementation protocol that was implemented was one that had been shown to be successful in previous creatine supplementation studies [19, 89, 106]. Supplements were provided by the supporting sponsor in red 0.75 gram (00 sized) capsules and were counted and pre-packaged by an individual outside of the research team. The creatine content of the capsules was independently verified by Covance Laboratories Inc. (*Madison, WI*) and certificate of analysis results are presented in Table 2. Capsules were pre-counted and placed into single serving bags which were then placed in another bag to separate the supplements by day. Each daily bag consisted of the single serving bags in order to insure proper supplementation throughout the week. The supplements were then placed in a non-clear bag labeled with the participants' study identification number in a double-blind manner. Participants in the CrM group ingested 8 capsules per serving, containing approximately 5 g of CrM four times daily (20 g/d total) for the first 7-days of the study. On Day 8 the CrM group began their maintenance dose and consumed 8 pills once per day (5 g/d) for the remaining 21-days. A small amount of dextrose (~60 mg pre capsule) was added to the CrM supplement to enhance flowability during encapsulation. Participants in the KA creatine monohydrate equivalent group (KA-H) ingested 8 capsules per serving with each serving containing approximately 5 g of KA. The participants ingested this dose four times daily (20 g/d) for the first 7-days and then once per day (5 g/d) for the remaining 21-days. Participants assigned to ingest the manufacturers recommended doses of KA (KA-L) ingested 8 capsules containing a total of approximately 1.5 g of KA mixed with 3.5 g of dextrose once per day and 8 capsules containing 5 g of dextrose

three times per day during the initial 7-day loading period. For the remaining 21-days, participants in the KA-L group ingested 8 capsules per day which contained 1.5 g/d of KA mixed with 3.5 g of dextrose. Participants were instructed to ingest supplements at 8:00 am, 12:00 pm, 4:00 pm, and 8:00 pm during the initial 7-day supplementation period and at 8:00 am during the maintenance phase. Supplementation compliance was monitored by having the subjects return empty containers of the supplements at the end of each week. In addition, subjects' compliance was verified by administering and collecting weekly questionnaires. After completing the compliance procedures, the subjects were given the required supplements for the subsequent week.

Table 2. Supplement Certificate of Analysis Results

Group	Entity Weight (g)	Fill Weight (g)	Moisture (%)	Creatine Monohydrate (%)	Total Creatine Monohydrate (g/per 8 capsules)	Creatinine (ppm)
KA-L	0.7609	0.6375	8.2	30.6	1.56	<5,000
KA-H	0.7566	0.6358	8.8	102.0	5.19	<5,000
CrM	0.8171	0.6975	9.4	92.4	5.16	<5,000

Samples analyzed by Covance Laboratory Inc. (*Madison, WI*). Sample size was eight capsules.

Procedures

Dietary and Training Analysis

Participants were instructed to maintain their current dietary habits and to keep detailed dietary records throughout the 28-day supplementation period. Prior to each testing session, subjects completed a dietary record that included 3 weekdays and 1 weekend day. Dietary inventories were analyzed for average energy and macronutrient

intake using the Food Processor Nutrition Analysis Software Version 9.1.0 (*ESHA Nutrition Research, Salem, OR*). After the dietary forms were entered, a registered dietician reviewed and signed off on all dietary inventories. Participants were also instructed to maintain their current training regimen and record the number of sets and repetitions per exercise performed throughout the study on training logs. Training volume was calculated by multiplying the amount of weight lifted times the number of repetitions performed for each set performed per exercise. Total training volume during the study was analyzed by summing all lifts (upper and lower body) to determine if there were any differences among groups.

Body Composition Assessment

Body composition testing occurred on day 0, 7 and 28 during the study. Height and weight were recorded to the nearest 0.02 kg and 0.01 cm, respectively, using a self-calibrating digital scale (*Cardinal Detecto Scale Model 8430, Webb City, Missouri*). Body composition was determined using a Hologic Discovery W QDR series DEXA system (*Hologic Inc., Waltham, MA*) equipped with APEX software (*APEX Corporation Software version 12.1, Pittsburgh, PA*). During this test subjects laid in a supine position for approximately six minutes while a low dose x-ray scanned the entire length of their body to determine fat mass and lean mass. Quality control calibration procedures were performed on a spine phantom (Hologic-X-CLAIBER Model DPA/QDR-1 anthropometric spine phantom) prior to each testing session. DEXA has been validated as an accurate method for body composition assessment with a high test to test reliability[107]. Previous research in our lab, with a similar subject demographic,

has yielded mean coefficients of variation for total bone mineral content and total fat free/soft tissue mass of 0.31% to 0.45% with a mean intra-class correlation of 0.985 [108]. Total body water was estimated using an ImpediMed DF50 bioelectrical impedance analyzer (*ImpediMed, San Diego, CA*). During this test, the subject laid in a supine position while four electrodes were placed on the wrist and ankle and the total body water was measured.

Blood and Muscle Biopsies

Muscle biopsies were obtained on days 0,7and 28 using a modified Bergstrom needle biopsy technique following standard procedures [109] in order to assess muscle creatine content. Biopsy areas were sterilized with alcohol prep pads and an iodine solution. Prior to the incision, the subjects received a subcutaneous lidocaine injection followed by an intra-muscular lidocaine injection to numb the area. The subject then lay still for a ten minute period to allow for the lidocaine to take effect. Incisions were then made on the middle portion of the vastus lateralis of the dominant leg at the midpoint between the patella and the greater trochanter of the femur at a depth of 1-2 cm into the muscle. A biopsy needle was then inserted into the muscle and a small portion of the muscle (50-70 mg) was suctioned into the needle and sliced prior to extraction. Similar procedures were followed for the subsequent biopsies and attempts were made to obtain samples from approximately the same location (2 cm superior) using the previous incision sites. After removal, adipose tissue was trimmed from the muscle specimens which were then immediately frozen in liquid nitrogen and stored at -80°C for later analysis. A total of three muscle samples were obtained (Day 0, 7, & 28).

Subjects donated approximately 10 ml of fasting blood using venipuncture techniques from an antecubital vein in the forearm according to standard sterile procedures. Whole blood samples were analyzed for complete blood counts with platelet differentials using an Abbott Cell Dyn 3500 automated hematology analyzer (*Abbott Laboratories, Abbott Park, IL*). The remaining whole blood was centrifuged at 1,500 rpm for 15 minutes in order to separate out the blood components. Serum blood samples were sent to Quest Diagnostics (*Houston, TX*) for comprehensive metabolic panel analysis using an Olympus AAU 5400 Chemistry Immuno Analyzer (*Olympus America Inc., Center Valley, PA*). If analysis indicated values outside the range for clinical norms, samples were run in duplicate. Previous assays performed in our laboratory have yielded a test to test reliability of 2 to 6%.

Biochemical Analysis for Muscle Creatine

The muscle tissue samples were analyzed using a mass spectrophotometer for muscle creatine (Cr) content. Samples were analyzed in duplicate for Cr based on methods developed by Harris and colleagues [14, 18, 110]. The previously frozen muscle tissue samples were placed in a vacuum centrifuge (*Savant ISS110 SpeedVac Concentrator, Thermo Scientific, Milford, MA*) and centrifuged for approximately24 hours. Following the dehydration process, the samples were grinded into a power using a porcelain mortar and pestle and then placed into pre-weighed microfuge tubes. The first assay used a 0.5 M perchloric acid/ 1mM EDTA solution to extract the muscle metabolites. The solution was added to the microfuge tubes and then placed on ice for 15 minutes while periodically vortexing. Samples were then centrifuged at 7,000 rpm

for 5 minutes. The supernatant was transferred into a pre-weighed microfuge tube. A base solution consisting of 2.1 M KHCO3/0.3 M MOPS was used to neutralize the samples. The samples were then centrifuged again at 7,000 rpm for 5 minutes and the supernatant was removed and placed into microfuge tubes and frozen at -80°C.

The frozen samples were allowed to thaw at room temperature while periodically vortexing. Extracts were then assayed for Cr in the presence of 50 mM imidazole buffer, pH 4.7; 5 mM magnesium chloride; 20 mM potassium chloride; 25 μM phosphoenolpyruvate; 200 μM ATP; 45 μM NADH; 1250 U/mL lactate dehydrogenase; 2000 U/mL pyruvate kinase. The reagents were premixed and combined prior to pipetting. The assay was then carried out in a standard fluorescence microplate reader using 10 μL of sample to 1 mL of reagent. The reactant solution was vortexed and read using a fluorometer (*Shimadzu RFMini 150, Japan*) with an excitation wavelength of 340 nm and an emission wavelength of 460 nm for baseline absorbance values. Five μL of CK (25 μ/mg) were added to 1 mL of the above buffer and stabilized using 1 mL of reagent. After 10 minutes the plate was read again for post-reaction absorbance values.

Performance Tests

One-repetition maximum (1RM) tests were completed for the bench press and leg press. Strength tests were performed using a standard isotonic Olympic bench press and hip sled/leg press (*Nebula Fitness, Versailles, OH*) according to standardized procedures [111]. Participants followed a standard warm up consisting of 10 repetitions using 50% of their estimated 1RM for the leg press. Following warm-up, subjects attempted a single repetition using a weight near their estimated 1RM. Subjects

rested for 2-minutes in between 1RM attempts and more weight was added if they felt they could lift more. Subjects attempted to reach their 1RM within 3-5 attempts. Foot positioning and sled height was recorded and standardized between testing sessions. After a 4-minute recovery period, bench press 1RM was determined following similar procedures as the hip sled/leg press 1RM test. Hand positioning on the bench press was measured and standardized between testing sessions. Previous research in our lab on resistance-trained participants have yielded low day to day mean coefficients of variation and high reliability for the bench press (1.1%, intra-class r=0.99) and hip sled/leg press (0.7%, intra-class r=0.91). Following the maximal strength procedures, subjects rested for roughly 20-minutes and then warmed up on a bicycle ergometer for 3-minutes (70 rpm @ 1 kg resistance). Participants then performed a 30-second Wingate sprint anaerobic capacity test on a Lode Excalibur Sport 925900 cycle ergometer (*Lode BV, Groningen, The Netherlands*) at a standardized work rate of 7.5 J/kg/rev. The participant was asked to pedal as fast as possible prior to application of the workload and sprint at an all-out maximal capacity throughout the 30-second test. Test-to-test variability in performing repeated Wingate anaerobic capacity tests in our laboratory have yielded correlation coefficients of r=0.98 ±15% for mean power. Participants practiced the anaerobic capacity test during the familiarization session to minimize learning effects during which time seat height and pedal position was assessed and recorded.

Side Effect Assessment

Participants were given weekly questionnaires to determine how well they

tolerated the supplement, how well they followed the supplement protocol, and if they experienced any medical problems/symptoms as a result of the supplement. Compliance to the supplementation protocol was monitored by having the subjects turn in empty weekly supplement containers, supplement logs and verbal confirmation. After completing the compliance procedures, subjects were given the required supplements and dosages for the following supplementation period.

Data Analysis

All data were analyzed using the statistical software SPSS 16.0. Study data were analyzed using a 3 x 3 (group x time) repeated measures Multivariate Analysis of Variance (MANOVA). Delta values were calculated and used to determine percent changes from baseline which were analyzed by repeated measures ANOVAs to determine changes from baseline. Participant baseline demographic data were analyzed using a one-way Analysis of Variance (ANOVA). Overall MANOVA effects were examined as well as MANOVA univariate group effects for certain variables if significant interactions were seen. Greenhouse-Geisser univariate tests of within-subjects time and group x time effects and between-subjects univariate group effects were reported for each variable analyzed within the MANOVA model. Data were considered statistically significant when the probability of type I error was 0.05 or less and statistical trends were considered when the probability of error ranged between $p>0.05$ to $p<0.10$. If a significant group, treatment and/or interaction alpha level was observed, Tukey's least significant differences (LSD) post-hoc analysis was performed to determine where significance was obtained. Prior to initiation of the study, we ran a

priori power analysis which indicated a design with an n-size of 12 per group would provide sufficient power to identify previously reported changes in muscle creatine content and training adaptations in responses to creatine supplementation (>0.70).

CHAPTER IV

RESULTS

Introduction

Creatine is one of the most popular and effective dietary supplements for athletes, specifically those engaged in resistance training [2, 3]. A recent study estimated that 28-41% of NCAA athletes and roughly 29-57% of recreationally active individuals supplement with creatine [5, 6]. Creatine is an amino acid-like compound that is involved in the transfer of high energy phosphates within the body and can yield energy at a high rate [2]. The form of creatine that has been most extensively studied is creatine monohydrate (CrM) [2]. Research has consistently indicated that supplementation with CrM can increase muscle creatine and phosphocreatine stores by as much as 10-40%, enhance anaerobic capacity, and enhance training adaptations when combined with a structured training program [2, 7, 13, 14, 17, 19]. Furthermore, creatine supplementation has repeatedly been shown to be safe with minimal negative side-effects, despite several cases of anecdotal evidence in the media reporting cramping, dehydration, compartment syndrome etc.

Research has shown that CrM has a high bioavailability and that the majority of CrM is not degraded during normal digestion, with nearly 99% of orally ingested creatine either taken up by the tissues or excreted through the urine [35-37]. Nevertheless, manufacturers attempt to create new forms of creatine and promote them to be more efficacious than CrM. Recently, manufacturers have created a buffered form of creatine *(Kre-Alkalyn® [KA], All American Pharmaceutical, Billings, MT, USA)*and

it has been marketed as a more "*potent form of creatine*" with "*less side-effects than creatine monohydrate*" [39]. The company claims their product is more chemically stable than creatine monohydrate and doesn't degrade to creatinine in the stomach as much as creatine monohydrate because it's buffered [38]. According to patent filings this is possible by the addition of an alkaline powder (e.g., soda ash, magnesium glycerol phosphate and bicarbonate) to creatine (e.g., creatine monohydrate, creatine phosphate, creatine pyruvate and creatine citrate) [105]. They also claim that because KA does not degrade to creatinine in the stomach, that a lower dose is required to yield similar increases in creatine content as those seen from CrM supplementation [38]. Even though research investigating creatine monohydrate supplementation has continually shown CrM to be safe with no negative side-effects, KA manufacturers also claim their product is safer.

The manufacturer cites several clinical studies on their website performed in Bulgaria to support their claims however we could not find any peer-review published articles on the National Library of Medicine's PubMed related to "Kre-Alkalyn" or "buffered creatine" [38]. We therefore designed a study to compare this new formulation of creatine to the well-researched, creatine monohydrate. Two different dosages of KA were used in the study, a high (creatine monohydrate equivalent) and low (manufacturer's recommended) dose. The study was designed as a double blind match-controlled design with 3 groups: 1) A recommended dose of KA, 2) A creatine equivalent dose of KA group and 3) A creatine monohydrate group. This design allowed us to determine the safety and efficacy of this new formulation of creatine and its effects

on creatine retention, training adaptations and markers of clinical health. The supplementation period consisted of 28-days with the creatine monohydrate and KA equivalent groups consuming loading doses (20 g/d) during the first 7-days, followed by maintenance doses of 5 g/d for the remaining 21-days and the KA recommended dosage group (1.5 g/d) will maintain that dose throughout the duration of the study. This design also allowed us to assess the acute and long-term effects of the two forms of creatine.

Methods

The study was conducted in a double-blind, match controlled design. Table 3 presents the experimental design of the study and the schedule for testing sessions. The independent variable of interest was the type of creatine ingested. Dependent variables included muscle creatine content, body composition, one repetition maximum (1RM) for bench press and leg press, anaerobic sprint performance capacity, serum and whole blood clinical markers of health, and self-reported side effects. Subjects who qualified for the study participated in a familiarization session during which the study protocol and design was explained to the participants and informed consent was obtained. After the familiarization session, subjects were matched for bodyweight, years of training experience, and age and randomly assigned to one of three groups: 1.) KA at manufacturer's recommended doses (KA-L); 2.) KA at creatine monohydrate equivalent dose (KA-H); or 3) CrM). Dietary intake was not controlled but participant's dietary intake was recorded prior to each testing session and analyzed for energy intake and macronutrient content. Participants were instructed to maintain their normal resistance-training program and maintain training logs so training volume could be compared. All

laboratory testing was conducted in the Exercise & Sports Nutrition Laboratory with the exception of the muscle biopsies procedures which will be performed in the Human Countermeasures Laboratory at Texas A&M University in College Station, TX.

Table 3. Experimental Design and Schedule of Testing Sessions

Familiarization and Entry	Baseline Day 0	Loading Phase Day 7	Maintenance Phase Day 28
Familiarization session Informed Consent Form Demographic Form Health History Form Exercise History Form 4-day Dietary History General Exam to Determine Qualifications to Participate in Study Height and Body Weight Practice Wingate Anaerobic Capacity Test Randomization into one of three groups (CrM, KA-L, KA-H) Instructions for Supplementation	4-Day Diet History Muscle Biopsy Fasting Blood Sample Body Weight Body Water (BIA) DEXA Body Composition 1 RM Leg Press 1 RM Bench Press Wingate Anaerobic Capacity Test Loading Phase of Supplementation Begins Maintain Training Log	4-Day Diet History Submit Training Log Muscle Biopsy Fasting Blood Sample Body Weight Body Water (BIA) DEXA Body Composition Wingate Anaerobic Capacity Test Low-Dose Maintenance Phase of Supplementation Begins	4-Day Diet History Submit Training Log Muscle Biopsy Fasting Blood Sample Body Weight Body Water (BIA) DEXA Body Composition 1 RM Leg Press 1 RM Bench Press Wingate Anaerobic Capacity Test

Subjects

Thirty-six apparently healthy college aged resistance-trained males were recruited to participate in this study. Participants were not allowed to participate in this study if they had any metabolic disorder including known electrolyte abnormalities; heart disease, arrhythmias, diabetes, thyroid disease, or hypogonadism; a history of hypertension, hepatorenal, musculoskeletal, autoimmune, or neurologic disease; if they are taking thyroid, anti-hyperlipidemic, hypoglycemic, anti-hypertensive, anti-inflammatory, or androgenic medications. Participants were not allowed to participate if they had taken any nutritional supplements or ergogenic aids (i.e. creatine, beta-alanine, HMB, DHEA, thermogenics etc) other than a daily multivitamin and protein powder (protein or amino acids only) within the past three months. Participants were recruited from the student population and from area fitness facilities. Participants meeting entrance criteria were asked to attend a familiarization session during which details of their participation were explained and they signed an informed consent form in compliance with the Human Subjects Guidelines of the Texas A&M University and the American College of Sports Medicine. Participants were also weighed using a standard scale and familiarized themselves with the Wingate anaerobic capacity test in order to minimize any learning effects. Figure 1 represents a consort diagram for participation throughout the study.

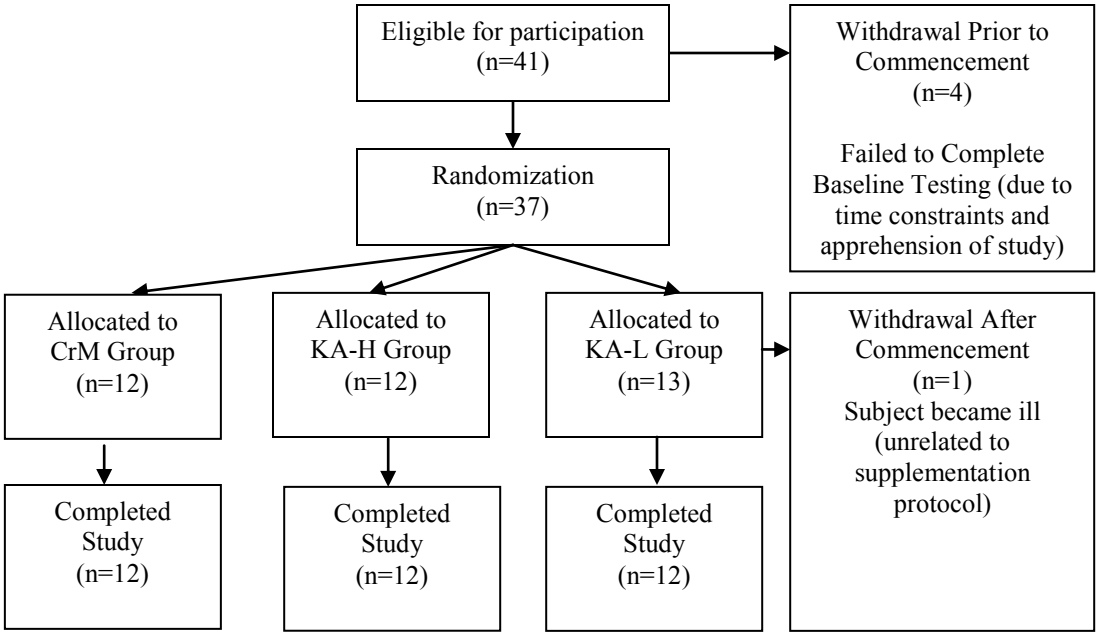

Figure 1. Consort diagram for participation.

Testing Sessions

Participants were asked to fast for 8 hours and abstain from exercise for 48 hours prior to all testing sessions. Subjects handed in their dietary and exercise logs as well as their supplement bags and supplement check sheet (on Days 7 and 28) upon arrival. Once reporting to the ESNL lab, subjects donated a muscle biopsy. Following the muscle biopsy, subjects donated a fasting blood sample using standard clinical procedures. Subjects were then weighed, had body water assessed and body composition assessed.

Following the resting tests, subjects completed 1RM tests on the bench press and

hip sled/leg press (on Days 0 and 28 only) and performed a 30-second Wingate anaerobic capacity sprint test on a cycle ergometer at each testing session. Following baseline testing, subjects began a 7-day initial supplementation phase. After 7 days, subjects repeated all tests with the exception of the1RM strength measures. The subjects then followed a maintenance supplementation schedule for 21-days and then returned to undergo all tests. This allowed for the assessment of acute and chronic supplementation protocols on muscle creatine levels, body composition, exercise performance, as well as markers of clinical health and safety. Participants were asked to maintain their current training programs and record all workouts throughout the 28-day period. Participants were also asked to report side effects on a daily basis and record the ingestion of all supplements.

Supplementation Protocol

Following baseline testing, participants were matched according to body weight, training status/experience, and age and randomly assigned to one of three groups. In a double blind manner, subjects ingested capsules containing CrM (*Creapure® AlzChem AG, Trostberg, Germany, Lot #108631*) or KA *(Kre-Alkalyn® All American Pharmaceutical, Billings, MT, USA, Lot #1067000)* at two different dosages for a 28-day period. The supplementation protocol that was utilized is one that has been shown to be successful in previous creatine supplementation studies [19, 89, 106]. Supplements were provided by the supporting sponsor in red 0.75 gram (00 sized) capsules and were pre-packaged by an individual outside of the research team. The creatine content of the capsules was independently verified by Covance Laboratories Inc. (*Madison, WI*) and

certificate of analysis results are presented in Table 4. Each participant received daily bags which consisted of single serving bags in order to insure proper supplementation throughout the week. Participants in the CrM group ingested 8 capsules per serving, containing approximately 5 g of CrM four times daily (20 g/d total) for the first 7-days of the study. On Day 8 the CrM group then began their maintenance dose and consumed 8 pills once per day (5 g/d) for the remaining 21-days. A small amount of dextrose (~60 mg pre capsule) was added to the CrM supplement to enhance flowability during encapsulation. Participants in the KA creatine monohydrate equivalent group (KA-H) ingested 8 capsules per serving with each serving containing approximately 5 g of KA. The participant ingested this dose four times daily (20 g/d) for the first 7-days and then once per day (5 g/d) for the remaining 21-days. Participants assigned to ingest the manufacturers recommended doses of KA (KA-L) ingested 8 capsules containing a total of approximately 1.5 g of KA mixed with 3.5 g of dextrose once per day and 8 capsules containing 5 g of dextrose three times per day during the initial 7-day loading period. For the remaining 21-days, participants in the KA-L group ingested 8 capsules per day which contained 1.5 g/d of KA mixed with 3.5 g of dextrose. Participants were instructed to ingest supplements at 8:00 am, 12:00 pm, 4:00 pm, and 8:00 pm during the initial 7-day supplementation period and at 8:00 am during the maintenance phase. Supplementation compliance was monitored by having the subjects return empty containers of the supplements at the end of each week. In addition, subjects' compliance was verified by administering and collecting weekly questionnaires. After completing the compliance procedures, the subjects were given the required supplements for the

next week.

Table 4. Supplement Certificate of Analysis

Group	Entity Weight (g)	Fill Weight (g)	Moisture (%)	Creatine Monohydrate (%)	Total Creatine Monohydrate (g/per 8 capsules)	Creatinine (ppm)
KA-L	0.7609	0.6375	8.2	30.6	1.56	<5,000
KA-H	0.7566	0.6358	8.8	102.0	5.19	<5,000
CrM	0.8171	0.6975	9.4	92.4	5.16	<5,000

Samples analyzed by Covance Laboratory Inc. (*Madison, WI*). Sample size was eight capsules.

Procedures

Dietary and Training Analysis

Participants were instructed to maintain their current dietary habits and to keep detailed dietary records. Prior to each testing session, subjects completed a dietary record that included 3 weekdays and 1 weekend day. Dietary inventories were analyzed for average energy and macronutrient intake using the Food Processor Nutrition Analysis Software Version 9.1.0 (*ESHA Nutrition Research, Salem, OR*). After the dietary forms were entered, a registered dietician reviewed all dietary inventories. Participants were instructed to maintain their current training regimen and record the number of sets and repetitions per exercise performed throughout the study on training logs. Training volume was calculated by multiplying the amount of weight lifted times the number of repetitions performed for each set performed per exercise. Total training volume during the study was analyzed by summing all lifts (upper and lower body) to determine if there are any differences among groups.

Body Composition Assessment

Body composition testing occurred on days 0, 7 and 28 during the study. Height and weight was recorded to the nearest 0.02 kg and 0.01 cm, respectively, using a self-calibrating digital scale (*Cardinal Detecto Scale Model 8430, Webb City, Missouri*). Body composition was determined using a Hologic Discovery W QDR series DEXA system (*Hologic Inc., Waltham, MA*) equipped with APEX software (*APEX Corporation Software version 12.1, Pittsburgh, PA*). DEXA has been validated as an accurate method for body composition assessment with a high test to test reliability[107]. Previous research in our lab, with a similar subject demographic, has yielded mean coefficients of variation for total bone mineral content and total fat free/soft tissue mass of 0.31% to 0.45% with a mean intra-class correlation of 0.985 [108]. Total body water was estimated using an ImpediMed DF50 bioelectrical impedance analyzer (*ImpediMed, San Diego, CA*).

Blood and Muscle Biopsies

Muscle biopsies were obtained on days 0,7and 28 using a modified Bergstrom needle biopsy technique following standard procedures [109] in order to assess muscle creatine content. Incisions were made on the middle portion of the vastus lateralis of the dominant leg at the midpoint between the patella and the greater trochanter of the femur at a depth of 1-2 cm into the muscle. A biopsy needle was inserted into the muscle and a small portion of the muscle (50-70 mg) was suctioned into the needle and sliced prior to extraction. Similar procedures were made for the subsequent biopsies and attempts

were made to obtain samples from approximately the same location (2 cm superior) using the previous incision sites. After removal, adipose tissue was trimmed from the muscle specimens which were then immediately frozen in liquid nitrogen and then stored at -80°C for later analysis. A total of three muscle samples were obtained (Day 0, 7, & 28).

Subjects then donated approximately 10 ml of fasting blood using venipuncture techniques from an antecubital vein in the forearm according to standard sterile procedures. Whole blood samples were analyzed for complete blood counts with platelet differentials using an Abbott Cell Dyn 3500 automated hematology analyzer (*Abbott Laboratories, Abbott Park, IL*). The remaining whole blood was centrifuged at 1,500 rpm for 15 minutes in order to separate out the blood components. Serum blood samples were sent to Quest Diagnostics (*Houston, TX*) for comprehensive metabolic panel analysis using an Olympus AAU 5400 Chemistry Immuno Analyzer (*Olympus America Inc., Center Valley, PA*). If analysis indicated values outside the range for clinical norms, samples were run in duplicate. Previous assays performed in our laboratory have yielded a test to test reliability of 2 to 6%.

Biochemical Analysis for Muscle Creatine

The muscle tissue samples were analyzed using a mass spectrophotometer for muscle creatine (Cr) content. Samples were analyzed in duplicate for Cr based on methods developed by Harris and colleagues [14, 18, 110]. The previously frozen muscle tissue samples were placed in a vacuum centrifuge (*Savant ISS110 SpeedVac Concentrator, Thermo Scientific, Milford, MA*) and centrifuged for approximately24

hours. Following the dehydration process, the samples were grinded into a power using a porcelain mortar and pestle and the placed into pre-weighed microfuge tubes. The first assay used a 0.5 M perchloric acid/ 1mM EDTA solution to extract the muscle metabolites. The solution was added to the microfuge tubes and then placed on ice for 15 minutes while periodically vortexing. Samples were then centrifuged at 7,000 rpm for 5 minutes. The supernatant will be transferred into a pre-weighed microfuge tube. A base solution consisting of 2.1 M KHCO3/0.3 M MOPS was used to neutralize the samples. The samples were then centrifuged again at 7,000 rpm for 5 minutes and the supernatant was removed and placed into microfuge tubes and frozen at -80°C.

The frozen samples were allowed to thaw at room temperature while periodically vortexing. Extracts were assayed for Cr in the presence of 50 mM imidazole buffer, pH 4.7; 5 mM magnesium chloride; 20 mM potassium chloride; 25 µM phosphoenolpyruvate; 200 µM ATP; 45 µM NADH; 1250 U/mL lactate dehydrogenase; 2000 U/mL pyruvate kinase. The reagents were premixed and combined prior to pipetting. The assay was carried out in a standard fluorescence microplate reader using 10 µL of sample to 1 mL of reagent. The reactant solution was vortexed and read using a fluorometer (*Shimadzu RFMini 150, Japan*) with an excitation wavelength of 340 nm and an emission wavelength of 460 nm for baseline absorbance values. Five µL of CK (25 µ/mg) was added to 1 mL of the above buffer and stabilized using 1 mL of reagent. After 10 minutes the plate were read again for post-reaction absorbance values.

Performance Tests

One-repetition maximum (1RM) tests were completed for the bench press and

leg press. Strength tests were performed using a standard isotonic Olympic bench press and hip sled/leg press (*Nebula Fitness, Versailles, OH*) according to standardized procedures [111]. Participants followed a standard warm up consisting of 10 repetitions using 50% of their estimated 1RM for the leg press. Following warm-up, subjects attempted a repetition using a weight equal to their estimated 1RM. Subjects rested for 2-minutes in between 1RM attempts and added more weight if they felt they could lift more weight. Subjects attempted to reach their 1RM within 3-5 attempts. Foot positioning and sled height were recorded and standardized between testing sessions. After a 4-minute recovery period, bench press 1RM was determined following similar procedures as the hip sled/leg press 1RM test. Hand positioning on the bench press was measured and standardized between testing sessions. Previous research in our lab on resistance-trained participants have yielded low day to day mean coefficients of variation and high reliability for the bench press (1.1%, intra-class r=0.99) and hip sled/leg press (0.7%, intra-class r=0.91). Following the maximal strength procedures, subjects rested for approximately 20-minutes and then completed a warm up on a bicycle ergometer for 3-minutes (70 rpm @ 1 kg resistance). Participants then performed a 30-second Wingate sprint anaerobic capacity test on a Lode Excalibur Sport 925900 cycle ergometer (*Lode BV, Groningen, The Netherlands*) at a standardized work rate of 7.5 J/kg/rev. The participant was asked to pedal as fast as possible prior to application of the workload and sprint at an all-out maximal capacity throughout the 30-second test. Test-to-test variability in performing repeated Wingate anaerobic capacity tests in our laboratory have yielded correlation coefficients of r=0.98 ±15% for mean power.

Participants practiced the anaerobic capacity test during the familiarization session to minimize learning effects during which time seat height and pedal position were assessed and recorded.

Side Effect Assessment

Participants were given weekly questionnaires to determine how well they tolerated the supplement, how well they followed the supplement protocol, and if they experienced any medical problems/symptoms as a result of the supplement. Compliance to the supplementation protocol was monitored by having the subjects turn in empty weekly supplement containers, supplement logs and verbal confirmation. After completing the compliance procedures, subjects were given the required supplements and dosages for the following supplementation period.

Data Analysis

All data were analyzed using the statistical software SPSS 16.0. Study data were analyzed using a 3 x 3 (group x time) repeated measures A multivariate Analysis of Variance (MANOVA). Delta and percent change values were calculated and used to determine changes from baseline which were analyzed by repeated measures ANOVAs. Participant baseline demographic data were analyzed using a one-way Analysis of Variance (ANOVA). Overall MANOVA effects were examined as well as MANOVA univariate group effects for certain variables when significant interactions were seen. Greenhouse-Geisser univariate tests of within-subjects time and group x time effects and between-subjects univariate group effects were reported for each variable analyzed within the MANOVA model. Data were considered statistically significant when the

probability of type I error was 0.05 or less and statistical trends were considered when the probability of error ranged between $p>0.05$ to $p<0.10$. When a significant group, treatment and/or interaction alpha level was observed, Tukey's least significant differences (LSD) post-hoc analysis was performed to determine where significance was obtained. Prior to initiation of the study, we ran a *priori* power analysis which indicated a design with an n-size of 12 per group would provide sufficient power to identify previously reported changes in muscle creatine content and training adaptations in responses to creatine supplementation (>0.70).

Results

Subject Demographics

Forty-one participants were initially recruited for the study, completed consent forms and participated in the required familiarization session. However, of the original 41 participants, 36 completed the 28-day research study. Three participants dropped out due to time constraints, one due to an unrelated illness, and one due to apprehension of the muscle biopsy procedure. None of the participants dropped out of the study due to side effects related to the study protocol or supplementation. The baseline demographics for the participants are listed in Table 5. Of the participants that completed the study, they were on average 20.2±2 years old, 181±7 cm tall, weighed 82.1±12 kg, and had a body fat percentage of 14.7±5 %, with 3.8±3 years of resistance training experience. No significant differences in baseline demographics were observed among groups.

Table 5. Participant Demographics

Group	N	Age (years)	Height (cm)	Body Weight (kg)	Body Fat (%)	Training (years)
KA-L	12	19.8±1.8	180.1±8.4	83.4±13.6	17.0±4.9	3.0±2.5
KA-H	12	19.5±1.2	181.0±6.3	81.2±8.1	12.8±4.1	4.0±2.9
CrM	12	21.3±2.8	181.3±6.4	81.8±13.8	14.2±4.7	4.3±3.4
p-level		0.07	0.91	0.90	0.08	0.55

Values are means ± standard deviations.
Data were analyzed by one-way ANOVA.

Compliance, Side Effects, Training, and Diet

To the best of our knowledge, all participants followed the supplementation protocol properly and exhibited 100% compliance based off of the completed supplement checklists and empty supplement containers that were returned at each testing session. Furthermore, none of the participants reported any side-effects throughout the supplementation period following the assessment of the side-effect questionnaires. The workout logs allowed us to examine total training volumes for upper and lower body lifts throughout the supplementation period. A one-way ANOVA analysis was run to examine differences among the three groups and Table 6 shows the total training volumes for upper and lower body lifts for each group throughout the 28-day supplementation period. Results indicated there were no significant differences among groups in total upper body training volume ($p=0.89$) or lower body training volume ($p=0.55$).

Table 6. Training Volume

Group	Upper Body (kg)	Lower Body (kg)
KA-L	65,006±35,543	40,631±20,641
KA-H	74,445±42,340	32,930±20,258
CrM	69,227±62,251	32,665±19,471
p-level	0.89	0.55

Training logs were obtained on all participants (n=36 or 12 per group).
Values are means ± standard deviations.
Data were analyzed by one-way ANOVA.

A MANOVA analysis was run to determine differences among groups in dietary intake throughout the study. Table7 presents mean energy intake and macronutrient content for each group. MANOVA univariate analysis revealed a significant time effect for daily calorie intake ($p = 0.08$), suggesting that energy intake decreased throughout the study however no significant group x time interactions were observed ($p=0.81$). A significant time effect was also observed for daily protein intake ($p = 0.05$) again suggesting that protein intake tended to decrease during the study but no significant interactions were observed among groups ($p = 0.97$). No significant time effects were observed for daily carbohydrate ($p = 0.40$) and fat ($p = 0.19$) intake and with no group x time interactions observed for carbohydrate ($p = 0.38$) and fat ($p = 0.47$) intake as well.

Similar results were observed when assessing energy and macronutrient intake when expressed relative to body mass. There was a trend for total energy intake relative to body mass ($p = 0.06$) that suggested a decrease in energy intake over time however no group x time interaction was observed ($p = 0.06$). There was a significant time effect

observed for total protein intake relative to body weight ($p = 0.04$) suggesting a decrease in protein intake over time however no group x time interaction was observed ($p = 0.99$).

Table 7. Dietary Caloric and Macronutrient Intake

Variable	Group	Day 0	Day 7	Day 28		p-level
Calories (kcal/day)	KA-L	2,167±900	2,202±653	1,998±444	Group	0.29
	KA-H	2,506±645	2,604±670	2,321±677	Time	0.08
	CrM	2,511±582	2,372±735	2,312±394	G x T	0.81
Protein (g/d)	KA-L	126.3±76	126.2±58	112.4±46	Group	0.65
	KA-H	139.4±46	143.2±54	132.5±60	Time	0.05
	CrM	127.8±28	131.2±40	114.1±35	G x T	0.97
Carbohydrate (g/d)	KA-L	219.1±73	203.9±79	181.7±53	Group	0.53
	KA-H	221.9±74	216.0±91	206.1±86	Time	0.40
	CrM	231.0±72	226.1±93	242.6±66	G x T	0.38
Fat (g/d)	KA-L	78.6±38	84.7±27	71.6±16	Group	0.20
	KA-H	99.2±40	105.7±47	94.5±35	Time	0.19
	CrM	91.3±32	81.3±30	83.0±20	G x T	0.47
Calories (kcal/kg/d)	KA-L	26.2±10.0	26.6±7.9	24.4±7.2	Group	0.29
	KA-H	31.4±9.5	32.1±10.5	28.3±9.4	Time	0.06
	CrM	31.2±7.5	29.0±8.8	28.4±5.8	G x T	0.73
Protein (g/kg/d)	KA-L	1.50±0.8	1.52±0.7	1.36±0.6	Group	0.58
	KA-H	1.75±0.7	1.76±0.8	1.61±0.8	Time	0.04
	CrM	1.59±0.4	1.61±46	1.41±0.4	G x T	0.99
Carbohydrate (g/kg/d)	KA-L	2.69±1.0	2.48±0.9	2.21±0.7	Group	0.50
	KA-H	2.75±0.9	2.65±1.2	2.46±1.0	Time	0.24
	CrM	2.87±0.9	2.76±1.1	2.99±0.9	G x T	0.34
Fat (g/kg/d)	KA-L	0.96±0.4	1.02±0.3	0.87±0.2	Group	0.23
	KA-H	1.24±0.6	1.31±0.7	1.16±0.5	Time	0.14
	CrM	1.14±0.4	1.0±0.4	1.01±0.3	G x T	0.44

Nutritional records were analyzed on all participants (n=36 or 12 per group). Values are means ± standard deviations. Absolute and relative nutritional data were analyzed by MANOVA. Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels.

No time or group x time effect was observed for carbohydrate intake relative to body weight ($p = 0.24$) and ($p=0.34$), respectively. Similar results were observed for fat intake, with no time or group x time effects observed ($p = 0.44$), respectively.

Muscle Creatine Analysis

Table 8 presents muscle free creatine content data. Muscle samples were obtained from 36 participants however there was only sufficient sample to analyze samples for 25 out of the 36 participants. Subjects with missing baseline or day-28 data were not included in the analysis. For two subjects we were missing their Day 7 muscle biopsy so their last observed value was used as a replacement. A MANOVA was run on all muscle creatine data and the results are presented in table 8. The values listed in table 8 are expressed in mmol/kg DW and are presented as means ± standard deviations, mean changes from baseline and percent changes from baseline. All values for creatine content are expressed in mmol/kg DW. MANOVA univariate analysis revealed a significant time effect in muscle free creatine content expressed in absolute terms ($p=0.03$), suggesting an overall increase in muscle free creatine content for all groups over time 58.2±16.0, 59.6±18.6, and 70.1±16.2 mmol/kg DW for days 0, 7 and 28, respectively. A significant time effect was observed for delta changes from baseline for all groups (1.4± 20.7, 11.9± 24.0 mmol/kg DW; $p=0.03$), however no significant group x time interactions were observed (KA-L -8.0±22.3, 4.7±27.0; KA-H 1.0±12.8, 9.1±23.2; CrM 11.3±23.9, 22.3±21.0 mmol/kg DW). There was a significant time effect observed for percent changes from baseline (7.7±39.4, 30.4±46.2 %; $p=0.003$) however no

significant group x time interactions were observed (KA-L -6.4±37.8, 13.7±42.2; KA-H 6.2±29.2, 27.3±49.1; CrM 23.5±49.0, 50.4±44.8 %; p = 0.51).

Table 8. Muscle Creatine Levels

Variable	N	Group	Day 0	Day 7	Day 28		p-level
Cr	8	KA-L	65.8±15.4	57.9±16.1	70.5±20.9	Group	0.74
(mmol/kg DW)	9	KA-H	57.3±17.7	58.3±15.6	66.3±12.6	Time	0.03
	8	CrM	51.5±12.7	62.8±25.0	73.8±15.6	G x T	0.46
Cr	8	KA-L	0.0±0.0	-8.0±22.3	4.71±27.0	Group	0.14
(Δ mmol/kg DW)	9	KA-H	0.0±0.0	1.03±12.8	9.07±23.2	Time	0.03
	8	CrM	0.0±0.0	11.3±23.9	22.3±21.0	G x T	0.46
Cr	8	KA-L	0.0±0.0	-6.4±37.8	13.7±42.2	Group	0.20
(Δ %)	9	KA-H	0.0±0.0	6.2±29.2	27.3±49.1	Time	0.003
	8	CrM	0.0±0.0	23.5±49.0	50.4±44.8	G x T	0.51

Values are means ± standard deviations. Δ represents change from baseline values. Sufficient muscle samples were obtained to measure baseline and subsequent Cr on 25 participants. Missing day-7 data in participants with baseline and day-28 values were replaced using the last observed value method (n=2). Data were analyzed by MANOVA with repeated measures. Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels.

Although there were no overall group differences observed (p=0.14), pairwise comparison between the KA-L and CrM groups revealed that changes in muscle creatine tended to be greater in the CrM group (KA-L -1.1±4.3, CrM 11.2±4.3 mmol/kg DW, p=0.053 [mean±SEM]; KA-L 2.4±8.5, CrM 24.6±8.5 %, p=0.078 [mean±SEM]). Figure 2 represents percent changes in muscle creatine over time for each group. The results from the creatine analysis provides supporting evidence to reject hypothesis 1 which stated that there will be a significant difference between groups in phosphagen levels as measured by muscle biopsies after 7 and 28-days of supplementation.

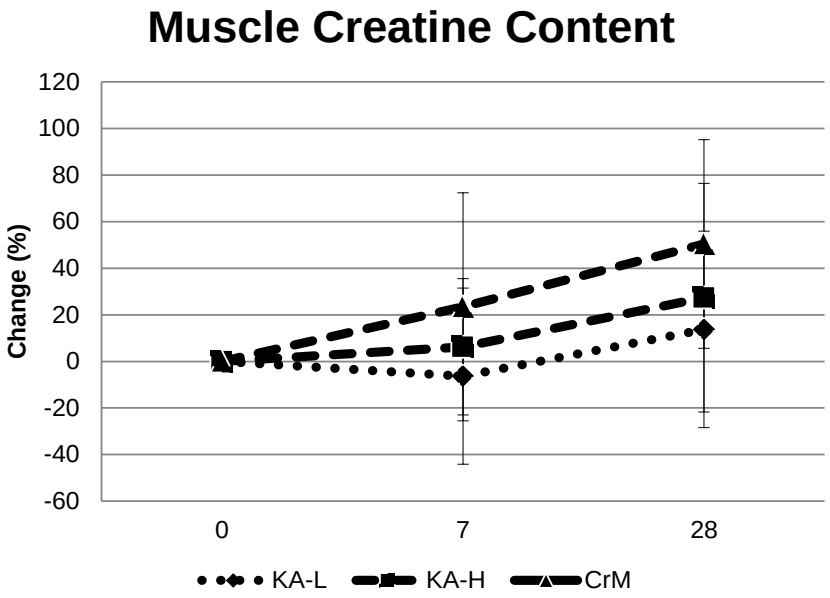

Figure 2. Percent Changes in Muscle Creatine

Body Composition

A MANOVA was run to assess changes in body composition variables which included body weight (kg), fat free mass (kg), fat mass (kg) and percent body fat. Table 9 displays the results from all body composition analysis. Bodyweight increased in all groups over time (1.0±1.9, 1.42±2.5 kg, $p<0.001$) with no significant group x time interaction effects observed among groups (KA-L 0.7±0.83, 0.9±1.6; KA-H 1.7±2.9, 2.3±3.7; CrM 0.6±1.1, 1.1±1.4 kg, $p=0.35$) after 7 and 28-days of supplementation, respectively. No significant differences were observed among groups over time for fat mass (-0.1±0.7, -0.1±1.3 kg, $p = 0.82$) and no group x time interactions were observed (KA-L 0.1±0.5, 0.2±1.1; KA-H -0.1±0.5, -0.1±1.3; CrM -0.3±0.7, -0.4±1.5 kg, $p = 0.73$).

Table 9. Body Composition

Marker	Group	Day				
		0	7	28		p-level
Body Weight	KA-L	83.4±13.6	84.1±14.0	84.3±13.6	Group	0.94
(kg)	KA-H	81.2±8.1	83.0±9.7	83.5±10.3	Time	0.001
	CrM	81.8±13.8	82.3±13.6	82.9±13.0	G x T	0.35
Fat Mass	KA-L	13.5±5.4	13.7±5.9	13.8±5.8	Group	0.11
(kg)	KA-H	9.7±3.2	9.6±3.1	9.6±3.1	Time	0.82
	CrM	11.0±5.3	10.7±5.4	10.6±4.4	G x T	0.73
Fat-Free Mass	KA-L	61.3±8.7	61.7±8.6	61.7±8.8	Group	0.77
(kg)	KA-H	63.5±8.0	64.4±8.0	64.7±8.4	Time	0.001
	CrM	62.3±9.8	63.0±9.6	63.4±9.9	G x T	0.43
Body Fat Percent	KA-L	17.0±4.9	17.0±5.5	17.2±5.4	Group	0.06
(%)	KA-H	12.8±4.1	12.5±3.8	12.5±3.6	Time	0.41
	CrM	14.2±4.7	13.7±5.0	13.7±4.2	G x T	0.77
Total Body Water	KA-L	37.8±5.0	37.2±4.4	35.9±3.3	Group	0.26
(%)	KA-H	37.4±2.9	35.1±2.6	34.1±1.7	Time	0.00
	CrM	36.7±2.7	35.8±3.0	33.9±1.5	G x T	0.71

Values are means ± standard deviations. DEXA body composition and BIA determined body water were determined on 36 participants (12 per group). Body composition variables were analyzed by MANOVA with repeated measures. Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels.

Fat-free mass significantly increased over time for all groups (0.67±1.0, 0.89±1.2 kg, $p<0.001$) with no significant group x time interaction effects observed among groups (KA-L 0.42±1.2, 0.37±1.3; KA-H 0.96±0.9, 1.2±1.4; CrM 0.6±0.8, 1.1±0.9 kg, $p=0.43$). Figure 3 represents changes in fat free mass over time for each group.

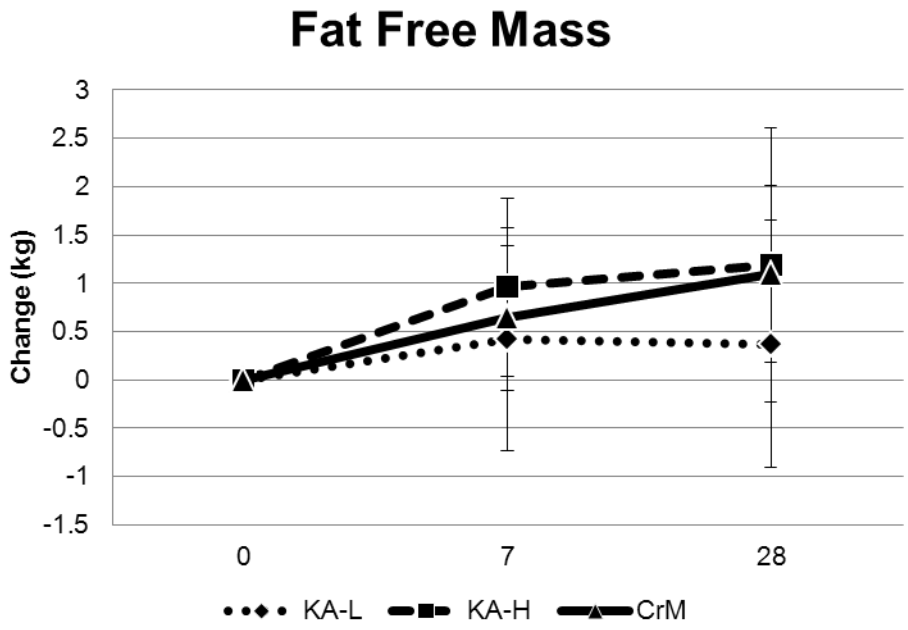

Figure 3. Changes in Fat Free Mass

No significant differences were observed over time in body fat percent for all groups (-0.28±1.0, -0.22±1.4 %, p=0.41) and no significant group x time interactions were observed among groups (KA-L -0.04±1.3, 0.15±1.2; KA-H -0.28±0.7, -0.31±1.6; CrM -0.53±0.9, -0.50±1.4 %, p=0.77) after 7 and 28-days of supplementation, respectively. Total body water expressed as a percentage of bodyweight significantly decreased over time for all groups (-1.25±3.7, -2.68±3.4 %, $p<0.001$) with no significant group x time interactions observed among groups (KA-L -0.58±4.1, -1.95±4.4; KA-H -2.25±2.0, -3.28±3.1; CrM -0.92±4.6, -2.82±2.6 %, p=0.71). Results from the body composition analysis provide evidence for a failure to reject the null hypothesis for Hypothesis 3, which stated there will be no significant differences between groups in body composition as measured by DEXA after 7 and 28-days of supplementation.

Training Adaptations

There was a significant increase in 1RM for bench press in all groups over time (97.6±22.3 to 101.3±22.6 kg, $p<0.001$) with no significant group x time interactions observed among groups in changes in bench press 1RM from baseline (KA-L 3.22±1.5, KA-H 3.3±6.8, CrM 4.5±3.7 kg, $p=0.73$). Figure 4 shows the changes in 1RM bench press over time.

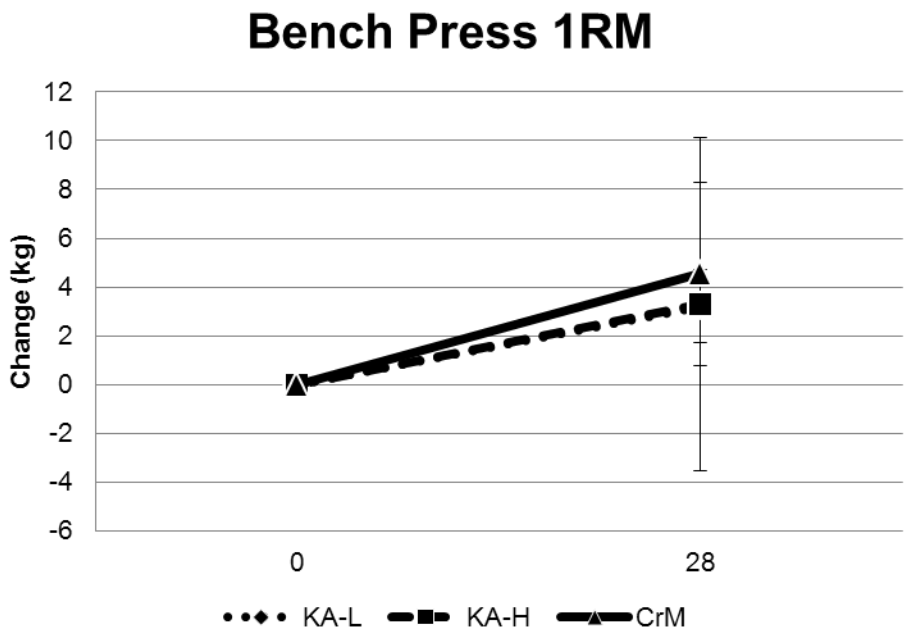

Figure 4. Changes in Bench Press 1RM

Table 10 shows upper and lower body 1RM strength data observed for each group. There was no significant difference observed in hip sled/leg press 1RM over time (449.5±162, 471.1±167, $p=0.33$) or a significant interaction observed among groups in changes in hip sled/leg press 1RM (KA-L 8.7±111, KA-H 68.8±96, CrM -13.3±185

kg, p=0.33). The results of the maximal strength analysis provide evidence which fails to reject the null hypothesis of hypothesis 5 which stated that there will be no significant difference between groups in strength as measure by 1RM on the leg press and bench press exercises after 28-days of supplementation.

Table 10. One Repetition Maximum Strength

Variable	N	Group	Day 0	Day 28		p-level
Upper	12	KA-L	95.3±25.4	98.6±24.7	Group	0.89
Body	11	KA-H	98.4±18.2	101.7±17.3	Time	0.001
(kg)	12	CrM	99.12±24.0	103.7±26.1	G x T	0.73
Lower	12	KA-L	445.3±182	454.1±155	Group	0.52
Body	12	KA-H	465.4±117	539.0±163	Time	0.35
(kg)	12	CrM	439.1±189	425.8±175	G x T	0.31

Values are means ± standard deviations. Data were analyzed by MANOVA with repeated measures. Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels.

Table 11 shows results for the anaerobic capacity observed for each group. A MANOVA analysis was run in order to assess changes in anaerobic capacity variables. Table 11 represents these variables expressed as means ± standard deviations. There was a significant time effect observed in all groups for average power (0.005), peak power (p=0.003), and total work (p=0.005), with no significant group x time interactions observed among groups. Mean power during the anaerobic capacity sprint test increased in all groups over time (-0.8±34.9, 19.1±44.5 W, p=0.005) after 7 and 28-days of supplementation with no significant group x time effects observed among groups (KA-L

-6.9±25.2, 2.1±40.6; KA-H 13.7±34.7, 28.0±45.8; CrM -8.1±41.6, 27.9±45.8 W, p=0.21). Peak power during the anaerobic capacity sprint test increased in all groups over time (157.5±326.0, 228.7±394.3 W, p=0.003) after 7 and 28-days of supplementation, however no significant group x time interaction was observed among groups (KA-L 119.2±291.8, 311.2±418.8; KA-H 209.2±262.8 287.1±382.6; CrM 148.6±419.9 92.8±375.7 W, p=0.48).

Table 11. Wingate Anaerobic Sprint Capacity

Variable		Group	Day				
	N		0	7	28		p-level
Mean	12	KA-L	658±136	651±134	660±138	Group	0.61
Power	11	KA-H	689±99	703±113	717±114	Time	0.005
(W)	12	CrM	660±119	652±108	688±105	G x T	0.21
Peak	12	KA-L	1,274±259	1,393±286	1,585±526	Group	0.50
Power	11	KA-H	1,329±285	1,538±389	1,616±378	Time	0.003
(W)	12	CrM	1,478±376	1,626±281	1,571±409	G x T	0.48
Total	12	KA-L	19,728±4,076	19,450±3,910	19,792±4,153	Group	0.59
Work	11	KA-H	20,681±2,968	21,093±3,387	21,523±3,432	Time	0.005
(J)	12	CrM	19,799±3,564	19,497±3,210	20,573±3,128	G x T	0.22

Values are means ± standard deviations. Data were analyzed by MANOVA with repeated measures. Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels.

Total work performed on the anaerobic capacity sprint test increased in all groups over time (-69±1,030, 552±1,361 J, p=0.02) after 7 and 28-days of supplementation with no significant group x time effects observed among groups (KA-L -278±676, 64±1,216; KA-H 412±1,041, 842±1,369; CrM -301±1,224, 775±1,463 J, p=0.32). Figure 5 presents the percent changes in total work done during the anaerobic sprint capacity tests over time. These results provide evidence to reject hypothesis 4

which stated that there will be a significant difference between groups in anaerobic capacity as measured by the 30 second Wingate test on a Lode cycle ergometer after 7 and 28 days of supplementation.

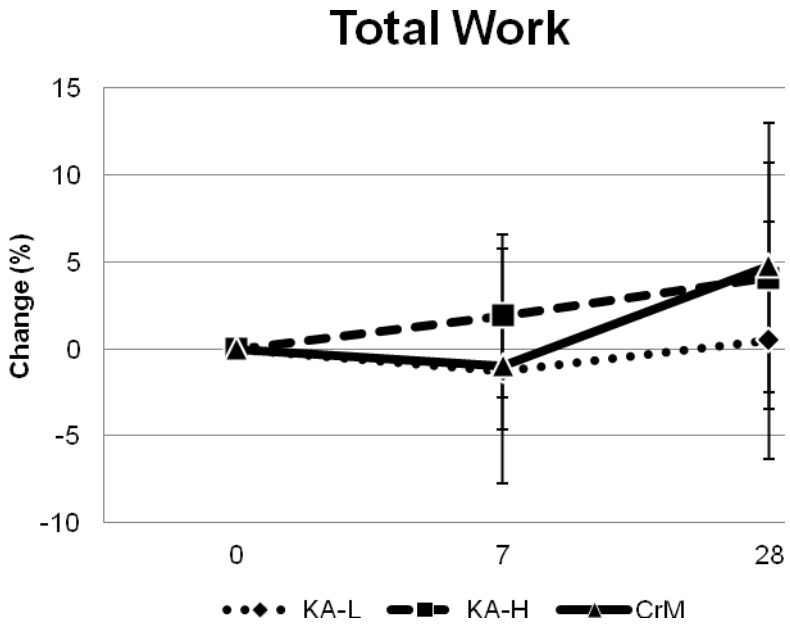

Figure 5. Percent Change in Total Work

Clinical Chemistry Panels

A MANOVA analysis was run in order to assess changes in blood lipid data. Table 12 presents blood lipid data observed throughout the study There was a significant time effect observed for high-density lipoprotein (HDL, p=0.03) , however no group x time interactions were observed among groups (p=0.64). There was also a significant time effect observed for the ratio of TCHL to HDL (p=0.04), however no significant group x time interactions were observed (p=0.09). No significant time effects

were observed for total cholesterol (TCHL, p=0.15), low-density lipoprotein (LDL, p=0.42), and triglycerides (TRIG, p=0.07). Furthermore, no group x time interactions in total cholesterol (TCHL, p=0.10) or triglycerides (TRIG, p=0.45) were observed.

Table 12. Serum Lipids and Glucose

Marker	N	Group	Day 0	Day 7	Day 28		p-level
TCHL	11	KA-L	149.1±25	153.0±23	149.9±28	Group	0.91
(mg/dl)	12	KA-H	153.3±26	152.3±28	157.5±22	Time	0.15
	12	CrM	156.3±20	147.3±19	158.9±21	G x T	0.10
HDL	11	KA-L	48.8±11.3	51.0±9.3	52.9±11.4	Group	0.42
(mg/dl)	12	KA-H	53.0±16.0	53.9±18.4	53.6±14.4	Time	0.03
	12	CrM	45.6±6.5	47.6±7.3	48.5±8.4	G x T	0.64
TCHL:	11	KA-L	3.16±0.7	3.09±0.6	2.92±0.7	Group	0.34
HDL	12	KA-H	3.03±0.6	2.95±0.5	3.04±0.5	Time	0.04
Ratio	12	CrM	3.48±0.6	3.15±0.6	3.36±0.7	G x T	0.09
LDL	11	KA-L	83.4±16*	86.5±16	81.4±18*	Group	0.66
(mg/dl)	12	KA-H	79.4±18*	82.7±19	83.7±16*	Time	0.42
	12	CrM	89.8±20	81.4±15†	92.5±17	G x T	0.005
TRIG	11	KA-L	84.5±33	77.3±30	78.5±37	Group	0.20
(mg/dl)	12	KA-H	105.1±37	78.4±26	101.1±27	Time	0.07
	12	CrM	104.1±28	92.1±30	89.6±30	G x T	0.45
Glucose	11	KA-L	93.0±5.1	90.5±8.2	93.6±4.7	Group	0.44
(mg/dl)	12	KA-H	91.1±6.6	92.7±8.1	90.4±6.9	Time	0.57
	12	CrM	90.5±9.6	89.6±5.5	88.3±6.3	G x T	0.67

Values are means ± standard deviations. Lipid data were analyzed by MANOVA with repeated measures. Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels. Glucose data were analyzed by repeated measures univariate ANOVA. † represents $p<0.05$ difference from baseline. * represents $p<0.05$ difference from CrM.

Some group x time effects were observed among groups in low-density lipoprotein (LDL) levels (p=0.005) with LDL levels significantly decreasing after the

loading phase in the CrM group. However, values remained low, near baseline and still within the normal range. No significant differences were observed among groups in blood glucose ($p=0.67$) over time.

A MANOVA analysis was run in order to assess changes in markers of catabolism. Table 13 shows markers of catabolism and bone status for each group over time. Significant time effects were observed for aspartate aminotransferase (AST, $p=0.02$), however no significant group x time interactions were observed ($p=0.68$). A significant time effect was observed for alanine aminotransferase (ALT, $p=0.05$), however no group x time interactions were observed ($p=0.48$). Univariate MANOVA found no significant group x time interactions in blood urea nitrogen (BUN, $p=0.75$), BUN to creatinine ratio ($p=0.24$), total protein ($p=0.84$), and total bilirubin (TBIL, $p=0.26$). Serum creatinine levels increased in all groups ($p<0.001$) over time with a significant group x time interaction demonstrating higher doses of creatine in the CrM and KA-H groups promoting significantly greater increases in serum creatinine ($p=0.03$) than the KA-L group. However, creatinine levels in the CrM and KA-H groups were only 0.1 – 0.2 mg/dL greater than the KA-L group, which were again well within normal values for active individuals, and of no clinical significance. MANOVA analysis of bone related markers found no significant time effects for bone mineral content ($p=0.49$), albumin (ALB, $p=0.73$), globulin (GLOB, $p=0.85$), the ratio of ALB to GLOB ($p=0.70$), calcium ($p=0.51$), or alkaline phosphatase (ALK, $p=0.29$). Likewise, no significant interactions among groups were observed in bone mineral content ($p=0.66$),

Table 13. Markers of Catabolism and Bone Status.

Marker	N	Group	Day 0	Day 7	Day 28		p-level
BUN	11	KA-L	16.0±5.3	15.3±4.9	15.6±5.1	Group	0.89
(mg/dl)	12	KA-H	16.1±3.3	16.6±3.9	16.6±3.6	Time	0.70
	12	CrM	16.4±3.2	15.7±2.7	16.1±4.7	G x T	0.75
Creatinine	11	KA-L	1.04±0.08	1.08±0.11	1.13±0.10†	Group	0.07
(mg/dl)	12	KA-H	1.07±0.14	1.23±0.18†*	1.26±0.13†*	Time	0.001
	12	CrM	1.11±0.19	1.28±0.20†*	1.23±0.15†*	G x T	0.03
BUN:CRN Ratio	11	KA-L	15.5±5.1	14.5±5.6	14.1±5.6	Group	0.83
	12	KA-H	15.1±3.4	13.7±3.4	13.3±3.4	Time	0.001
	12	CrM	15.2±3.7	12.4±2.6	13.2±3.8	G x T	0.24
AST	11	KA-L	25.4±9.6	26.5±8.4	29.5±12.9	Group	0.62
(U/L)	12	KA-H	27.3±10.5	25.6±8.3	32.0±12.0	Time	0.02
	12	CrM	24.9±7.9	23.8±7.5	26.3±7.8	G x T	0.70
ALT	11	KA-L	21.5±11.2	23.5±14.2	28.7±19.4	Group	0.50
(U/L)	12	KA-H	24.1±15.6	22.3±12.2	27.3±9.1	Time	0.05
	12	CrM	21.3±7.34	18.0±4.2	21.3±5.5	G x T	0.48
Total Protein	11	KA-L	7.4±0.6	7.4±0.4	7.4±0.4	Group	0.87
(g/dl)	12	KA-H	7.3±0.3	7.3±0.3	7.3±0.2	Time	0.88
	12	CrM	7.3±0.2	7.3±0.2	7.4±0.3	G x T	0.84
TBIL	11	KA-L	0.84±0.7	0.75±0.3	0.76±0.3	Group	0.60
(mg/dl)	12	KA-H	0.88±0.5	0.89±0.5	0.77±0.4	Time	0.90
	12	CrM	0.63±0.2	0.71±0.2	0.77±0.2	G x T	0.26
Bone Mineral	11	KA-L	2,517±404	2,503±409	2,505±398	Group	0.59
Content (g)	12	KA-H	2,632±457	2,604±466	2,615±456	Time	0.49
	12	CrM	2,446±344	2,456±0.2	2,441±351	G x T	0.66
Albumin	11	KA-L	4.80± 0.3	4.81±0.4	4.81±0.2	Group	0.95
(g/dl)	12	KA-H	4.83±0.2	4.74±0.2	4.78±0.1	Time	0.73
	12	CrM	4.82±0.2	4.80±364	4.79±0.2	G x T	0.89
Globulin	11	KA-L	2.60±0.4	2.63±0.3	2.55±0.3	Group	0.90
(g/dl)	12	KA-H	2.56±0.3	2.58±0.2	2.52±0.3	Time	0.85
	12	CrM	2.55±0.3	2.54±0.2	2.62±0.3	G x T	0.42
Alb:Glob Ratio	11	KA-L	1.88±0.3	1.85±0.2	1.90±0.2	Group	0.98
	12	KA-H	1.90±0.1	1.86±0.2	1.91±0.1	Time	0.70
	12	CrM	1.88±0.2	1.90±0.2	1.84±0.2	G x T	0.45
Calcium	11	KA-L	9.87±0.5	9.85±0.5	9.76±0.4	Group	0.42
(mg/dl)	12	KA-H	9.83±0.2	9.81±0.4	9.84±0.2	Time	0.51
	12	CrM	9.77±0.3	9.63±0.4	9.67±0.3	G x T	0.76
ALK	11	KA-L	82.0±16.4	84.1±20.5	83.9±17.0	Group	0.88
(U/L)	12	KA-H	81.1±29.7	83.8±30.3	87.1±27.6	Time	0.29
	12	CrM	78.9±20.7	80.6±26.4	78.8±23.1	G x T	0.65

Values are means ± standard deviations. Data were analyzed by MANOVA with repeated measures. Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels. † represents $p<0.05$ difference from baseline. * represents $p<0.05$ difference from KA-L.

albumin (ALB, p=0.89), globulin (GLOB, p=0.42), the ratio of ALB to GLOB (p=0.45), calcium (p=0.76), or alkaline phosphatase (ALK, p=0.65).

Table 14 presents serum electrolyte data. Univariate MANOVA analysis revealed some small time effects in chloride levels (p=0.008) and a trend toward an interaction in potassium levels (p=0.08) but the small changes observed would have no clinical significance. No significant interactions were observed in sodium levels (p=0.57).

Table 14. Serum Electrolyte Status.

Marker	N	Group	Day 0	Day 7	Day 28		p-level
Sodium	11	KA-L	140.1±2.3	139.9±1.1	140.0±1.3	Group	0.98
(mmol/L)	12	KA-H	139.9±2.3	139.7±2.4	140.3±2.1	Time	0.28
	12	CrM	140.8±2.1	139.3±1.4	139.7±1.6	G x T	0.57
Potassium	11	KA-L	4.54±0.3	4.86±0.4	4.82±0.3	Group	0.65
(mmol/L)	12	KA-H	4.89±0.5	4.71±0.6	5.00±0.3	Time	0.11
	12	CrM	4.74±0.4	4.93±0.4	4.81±0.4	G x T	0.08
Chloride	11	KA-L	103.3±2.2	103.0±2.4	103.8±1.9	Group	0.21
(mmol/L)	12	KA-H	102.4±2.2	101.5±2.2	102.6±2.4	Time	0.008
	12	CrM	104.3±2.2	102.3±1.7	103.1±1.8	G x T	0.21

Values are means ± standard deviations. Data were analyzed by MANOVA with repeated measures. Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels.

Finally, Table 15 shows whole blood markers assessed throughout the study. Univariate MANOVA analysis revealed no significant interactions observed among groups in white blood cell count (WBC, p=0.45), red blood cell count (RBC, p=0.64), hematocrit (p=0.65), hemoglobin (p=0.59), mean corpuscular volume (MCV, p=0.56), mean corpuscular hemoglobin (MCH, p=0.44), mean corpuscular hemoglobin

concentration (MCHC, p=0.68), red blood cell distribution width (RBCDW, p=0.92), or platelet count (p=0.48). These results provide evidence to for the failure to reject the null of hypothesis 2 which stated that there will be no significant difference between groups in markers of clinical health and safety after 7 and 28 days of supplementation.

Table 15. Whole Blood Markers.

Marker		Group	Day				
	N		0	7	28		p-level
WBC	9	KA-L	5.73±0.6	6.13±0.5	6.17±1.5	Group	0.95
($x10^3$/ul)	12	KA-H	5.83±1.1	5.76±0.9	6.36±1.1	Time	0.16
	12	CrM	5.97±1.2	5.73±1.0	5.98±1.2	G x T	0.45
RBC	9	KA-L	5.44±0.4	5.38±0.5	5.44±0.3	Group	0.28
($x10^6$/ul)	12	KA-H	5.10±0.4	5.18±0.3	5.23±0.3	Time	0.91
	12	CrM	5.42±0.5	5.41±0.5	5.35±0.7	G x T	0.64
Hematocrit	9	KA-L	48.4±3.4	47.9±4.3	48.1±2.9	Group	0.17
(%)	12	KA-H	46.5±3.2	47.0±2.8	47.4±1.8	Time	0.96
	12	CrM	45.9±2.3	46.1±2.5	45.2±5.4	G x T	0.65
Hemoglobin	9	KA-L	16.0±1.6	16.0±1.6	16.0±1.2	Group	0.21
(g/dl)	12	KA-H	15.2±1.2	15.7±1.0	15.6±0.7	Time	0.60
	12	CrM	15.1±0.9	15.2±1.1	14.9±2.0	G x T	0.62
MCV	9	KA-L	89.0±2.8	88.9±2.9	88.3±2.8	Group	0.10
(fL)	12	KA-H	91.1±3.5	90.8±3.1	90.7±3.6	Time	0.03
	12	CrM	85.4±9.2	85.7±9.5	85.0±9.1	G x T	0.56
MCH	9	KA-L	29.4±1.5	29.6±1.2	29.3±1.2	Group	0.34
(pg/cell)	12	KA-H	29.8±1.6	30.2±1.5	28.4±4.9	Time	0.20
	12	CrM	28.1±3.5	28.3±3.7	27.9±3.3	G x T	0.44
MCHC	9	KA-L	33.0±1.3	33.3±0.9	33.2±0.9	Group	0.73
(g/dl)	12	KA-H	32.8±0.9	33.3±0.8	32.9±0.6	Time	0.22
	12	CrM	32.9±1.1	32.9±1.3	32.9±0.8	G x T	0.68
RBCDW	9	KA-L	13.0±0.5	13.0±0.9	12.9±0.7	Group	0.34
(%)	12	KA-H	13.8±1.1	13.7±1.0	13.5±1.5	Time	0.41
	12	CrM	13.7±1.4	13.7±1.7	13.6±1.6	G x T	0.92
Platelet Count	9	KA-L	266±45	266±52	280±45	Group	0.12
($x10^3$/ul)	12	KA-H	253±54	248±62	269±65	Time	0.32
	12	CrM	222±69	222±74	216±65	G x T	0.48

Values are means ± standard deviations. White and red cell whole blood markers were analyzed by MANOVA with repeated measures. Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels.

CHAPTER V

SUMMARY AND CONCLUSIONS

The purpose of this study was to examine if ingesting 1.5 g/day of Kre-Alkalyn® (KA) for 28-days, or 28-days of creatine equivalent doses of KA was more efficacious than supplementation with CrM (20 g/d for 7-days and 5 g/d for 21-days) on muscle creatine retention, body composition, strength, anaerobic capacity and markers of clinical health. Furthermore, this study sought out to determine if KA had fewer side-effects than CrM. The results of the present study clearly show that a buffered form of creatine is no more efficacious than creatine monohydrate in terms of improving creatine retention, training adaptations and markers of clinical health.

Muscle Creatine

Claims by the manufacturers of Kre-Alkalyn, state that their product promotes greater bioavailability of creatine and therefore a greater amount of creatine can be absorbed by the muscles. The manufacturer's claim that KA has less conversion of creatine to creatinine during the digestive process compared to CrM because it is "*bufferd*" or "*pH-correct."[39]* They claim this despite the fact that previous research has shown that nearly 99% or orally ingested creatine is either taken up by the tissues or excreted in the urine[35, 36]. The manufacturer's claim that KA is "*up to ten times more powerful than ordinary Creatine*." It is because of this claim that the recommended dose of 1.5 g/d of KA (KA-L) as well as a creatine monohydrate equivalent dose (20 g/d for 7-days and 5 g/d for 21 days) of KA (KA-H) was used in order to compare KA to CrM.

The present study failed to show any significant differences in creatine retention between groups suggesting that KA is no more efficacious than CrM when it comes to improving the bioavailability of creatine. In the present study, muscle creatine content increased 34.6±50 % after 7-d of 20g of CrM, compared to -5.9±35 and 6.2±29 % following 7-d of supplementation with 1.5 g/d (KA-L) and 20 g/d (KA-H) of KA. Similar results have been found with previous research showing that acute ingestion of 20g of CrM can increase muscle creatine content 10-40% after 5-7 d of supplementation [13, 14, 17, 20]. When KA-L was compared to CrM there was a trend of CrM increasing creatine content to a greater extent compared to KA-L (KA-L -5.9±35; CrM 34.6±50 %, p=0.069) but results were not statistically significant. Similar results were seen after a maintenance dose of 5g for 21-d as the CrM group experienced an increase of 50.4±45% in creatine compared to 11.9±40 and 27.3±49 % in the KA-L and KA-H groups, respectively. When KA-L was compared to CrM alone, these changes were statistically significant (p=0.038). These results are directly in opposition to claims made by the manufacturers stating that a recommended dose of KA supplementation (1.5 g/d) promotes a greater creatine uptake compared to CrM supplementation. In fact, pairwise comparison of the mean group change from baseline in the CrM groups was 11 times greater than the change observed following recommended doses of KA (KA-L -1.1±4.3, CrM 11.2±4.3 mmol/kg DW [mean±SEM],p=0.053) after 7-days of supplementation, however the overall group effect was not statistically significant (p=0.14). Similar results were seen after 28-days of supplementation, again showing substantially greater increases in creatine content in the CrM group 22.3±21.0 mmol/kg

DW compared to 4.71±27.0 mmol/kg DW in the KA-L group. This is further evidence that KA is no more efficacious than CrM and the claims made by the manufacturers are not supported by the results of the present study.

Body Composition

Results of the present study indicated that there was a significant increase in bodyweight seen in all groups as is common after acute and long-term creatine supplementation. Typical weight gain from creatine supplementation ranges between 1-2 kg in total body weight [7, 14, 64]. Kreider et al. [19] suggested that short-term supplementation (5-7 days) with 20g/d can often lead to increases in body mass of up to 1.6 kg which is similar to the gains in body mass (KA-L 0.7±0.83, KA-H 1.7±2.9, CrM 0.56±1.1 kg) seen in the present study after 7-d of supplementation. There were no significant differences in bodyweight observed among groups. All groups also experienced a significant increase in fat-free mass with an average gain 0.67±0.9 and 0.89±1.2 kg, after 7 and 28-d, respectively. These gains in FFM are similar to those seen in previous creatine supplementation studies [19, 68, 112, 113]. No significant differences were observed in total body water, as a percentage of total bodyweight, among groups; which suggests the changes in body mass were not attributable to body water changes and likely due to increases in fat-free mass. Furthermore, no significant changes in body fat percent were observed, suggesting that the observed changes in bodyweight were of an equal ratio of fat-free mass to fat mass. Because significant differences were observed among total volume of training or dietary intake, the

differences observed in body weight and fat-free mass are likely attributable to the supplementation strategies utilized.

Training Adaptations

Several studies have shown improvements in strength and power following creatine supplementation [47, 60, 61, 77, 114]. In the present study, there was a significant increase in upper-body strength observed in all groups with no differences among groups. The average increase in bench press 1RM for all groups was approximately 4%. Similar improvements in bench press 1RM have been observed following creatine supplementation strategies. Earnest et al. found a 6% increase in bench press 1RM following 28 days of creatine supplementation in resistance trained males[80]. Similarly, Kelly et al. found an 8% increase in bench press 3RM following 26-days of creatine supplementation in resistance trained males. No significant changes in lower body strength were observed for any of the groups. This may be due to the fact that, on average, the participants had a greater training volume for upper body (~69,560 kg) compared to lower body (35,410 kg) throughout the duration of the study. This was an unexpected outcome from analyzing the workouts but may be typical for recreational weight lifters and perhaps a limitation of allowing participants to follow their own routines.

The manufacturers of Kre-Alkalyn® also claim that their product has a greater capacity to increase "raw" power and training capacity when compared to CrM. Previous research has shown improvements in anaerobic cycle ergometer performance in the range of 10-25% [88, 115-117]. For example, Ziegenfuss et al. found increases in

total work and peak power during consecutive high-intensity sprints on a cycle ergometer [118]. The results of the present study are in accordance with these findings. There was a significant increase in total work done during the Wingate test in all groups (-69±1,030, 552±1,361 J, p=0.02) after 7 and 28-days of supplementation which equated to approximately a 3% improvement after 28-days for all groups. However no significant differences were observed among groups. Similar improvements were seen in peak power (+20%) and mean power (+3%) after 28-days of supplementation for all groups with no significant differences observed among groups. Therefore, it can again be assumed that KA supplementation is no more effective than CrM supplementation in terms of improving anaerobic performance.

Markers of Clinical Health

Creatine supplementation has repeatedly been shown to have minimal negative side-effects with little to no effect on markers of clinical health and safety [21]. In the present study, neither manufacturers recommended doses or equivalent loading doses of KA resulted in any negative side-effects or health outcomes. These findings suggest that a buffered form of creatine is just as safe to consume as CrM with minimal side-effects. There was a trend that higher doses of supplemental creatine promoted greater increases in serum creatinine (KA-L: 1.04±0.08, 1.08±0.11, 1.1±0.1; KA-H: 1.1±0.14, 1.2±0.18, 1.3±0.13; CrM: 1.1±0.19, 1.3±0.2, 1.2±0.15 mg/dl, p=0.071) after 0, 7 and 28-days of supplementation, respectively. However, these values remained well within normal values for active individuals (i.e., <1.28±0.20 mg/dl). Furthermore, there were no significant differences in creatinine levels observed among groups throughout the study.

This is in direct opposition of the claim made by the manufacturers of Kre-Alkalyn® stating that because Kre-Alkalyn is a "buffered" creatine it won't convert to creatinine as does other forms of creatine. Furthermore, these results also refute the manufacturer's claims which state that KA is a safer form of creatine with less side-effects.

Cost Analysis

The results of this study indicate that CrM supplementation results in a greater creatine uptake when compared to the recommended dose of KA (1.5 g/d). When comparing the costs of these two products this difference becomes important. Kre-Alkalyn typically has 240 capsules per bottle with a recommended serving size of 1.5 grams per day (2 capsules). The average retail cost for a bottle is about $20.00 which comes about to be $0.16 cents per g or $161.11 per kg. Creatine monohydrate on the other hand typically sells for $20.00-$30.00 per kg ($0.02 to $0.03 cents per gram). Therefore, for a much cheaper price you can get an equivalent product with creatine monohydrate.

Future Direction and Recommendations for Additional Research

There are constantly new products appearing in today's nutritional supplement market. It is important that these products be tested by independent researchers before exaggerated claims are made my manufacturers and marketing strategies overshadow the true effects of the actual product. Furthermore, it is important that the safety of these products is also investigated especially with long-term supplementation before they are released into the market. Studies, such as the current one, should be done in order to monitor the safety and efficacy of new products. Acute and long-term supplementation

studies should be utilized in order to assess acute and chronic effects of the supplement on training adaptations and markers of clinical health. Future studies similar to the present one could be replicated but with a longer supplementation period or even a standardized exercise protocol throughout the study in order to assess the long-term adaptations to Kre-Alkalyn.

Conclusion

Supplementation of the diet with recommended doses of Kre-Alkalyn (1.5 grams/day) and equivalent (20 g/day for 7-days and 5 g/d for 21-days) doses does not promote greater increases in muscle creatine content or training adaptations in comparison to creatine monohydrate. Additionally, there was no evidence to support claims that Kre-Alkalyn is associated with fewer side effects or is a safer form of creatine to consume compared to creatine monohydrate. These findings refute claims that Kre-Alkalyn is a more efficacious and safer form of creatine than creatine monohydrate. However, it should me mentioned that there were some limitations to the current study design. There are also large variations seen when conducting human research and conduction human tissue assays. Similar variations are often seen following performance tests in which large standard deviations are common and sometimes make it difficult to detect statistical significance. Another limitation of the present study is a lack of a control group. The addition of a control group and/or more subjects may have increased the statistical power. In summary, results of the present study indicate that KA is no more effective than CrM and just as safe to consume.

REFERENCES

1. Greenhaff P: The nutritional biochemistry of creatine. The Journal of Nutritional Biochemistry 1997, 8:610-618.
2. Jager R, Purpura M, Shao A, Inoue, T, Kreider, R.: Analysis of the efficacy, safety, and regulatory status of novel forms of creatine. Amino Acids 2011, 40:1369-1383.
3. Kreider RB, Wilborn CD, Taylor, L, Campbell, B, Almada, A, et al.: ISSN Exercise & sport nutrition review: Research & recommendations. Journal of the International Society of Sports Nutrition 2010, 7(7).
4. Kreider RB and Jung YP: Creatine supplementation in exercise, sport, and medicine. Journal of Exercise Nutrition and Biochemistry 2011, 15:53-69.
5. Greenwood M, Farris J, Kreider R, Greenwood, L, Byars, A.: Creatine supplementation patterns and perceived effects in select Division I collegiate athletes. Clinical Journal of Sport Medicine. 2000, 10:191-4.
6. Sheppard HL, Raichada SM, Kouri KM, Stenson-Bar-Maor, L, Branch, JD.: Use of creatine and other supplements by members of civilian and military health clubs: A cross-sectional survey. International Journal of Sport Nutrition and Exercise Metabolism 2000, 10:245-59.
7. Greenhaff PL: Creatine and its application as an ergogenic aid. International Journal of Sport Nutrition 1995, 5 Suppl:S100-110.

8. Greenwood M, Kreider, R., Earnest, C., Rasmussen, C., & Almada, A.: Differences in creatine retention among three nutritional formulations of oral creatine supplements. Nutrition 2003, 6(2).

9. Spillane M, Schoch R, Cooke M, Harvey, T, Greenwood, M, Kreider, R, Willoughby, D.: The effects of creatine ethyl ester supplementation combined with heavy resistance training on body composition, muscle performance, and serum and muscle creatine levels. Journal of the International Society of Sports Nutrition 2009, 6:6.

10. Jager R, Harris RC, Purpura M, Francaux, M.: Comparison of new forms of creatine in raising plasma creatine levels. Journal of the International Society of Sports Nutrition 2007, 4(1):1-5.

11. Kreider RB, Willoughby, D., Greenwood, M., Parise, G., Payne, E., & Tarnopolsky, M.: Effects of serum creatine supplementation on muscle creatine and phosphagen levels. J Exerc Physiol Online 2003, 6: 24-33.

12. Volek JS and Rawson ES: Scientific basis and practical aspects of creatine supplementation for athletes. Nutrition 2004, 20:609-14.

13. Harris RC, Soderlund K, and Hultman E: Elevation of creatine in resting and exercised muscle of normal subjects by creatine supplementation. Clinical Science 1992, 83:367-74.

14. Hultman E, Soderlund K, Timmons JA, Cederblad, G, Greenhaff, PL.: Muscle creatine loading in men. Journal of Applied Physiology 1996, 81:232-7.

15. Greenhaff PL:Muscle creatine loading in humans: Procedures and functional and metabolic effects. 6th International Conference on Guanidino Compounds in Biology and Medicine. Cincinnati, OH 2001.

16. Greenhaff PL, Bodin K, Soderlund K, Hultman, E.: Effect of oral creatine supplementation on skeletal muscle phosphocreatine resynthesis. The American Journal of Physiology 1994, 266:E725-30.

17. Greenhaff PL, Casey A, Short AH, Harris, R.: Influence of oral creatine supplementation of muscle torque during repeated bouts of maximal voluntary exercise in man. Clinical Science 1993, 84:565-571.

18. Harris RC, Soderlund K, and Hultman E: Elevation of creatine in resting and exercised muscle of normal subjects by creatine supplementation. Clin Sci (Colch) 1992, 83:367-374.

19. Kreider RB, Ferreira M, Wilson M, Grindstaff, P, Plisk, S, Reinardy, J and Almada, A.: Effects of creatine supplementation on body composition, strength, and sprint performance. Medicine and Science in Sports and Exercise 1998, 30:73-82.

20. Volek JS, Duncan ND, Mazzetti SA, Staron, RS, Putukian, MA, Gomez, AL, Pearson, DR, Fink, WJ, and Kraemer, WJ.: Performance and muscle fiber adaptations to creatine supplementation and heavy resistance training. Medicine and Science in Sports and Exercise 1999, 31:1147-1156.

21. Buford TW, Kreider RB, Stout JR, Greenwood, M, Campbell, B, Spano, M, Ziegenfuss, T, Lopez, H, Landis, J and Antonio, J.: International Society of

Sports Nutrition Position Stand: Creatine supplementation and exercise. Journal of the International Society of Sports Nutrition 2007, 4(6):1-8.

22. Dalbo VJ, Roberts MD, Stout JR, Kerksick, CM.: Putting to rest the myth of creatine supplementation leading to muscle cramps and dehydration. Br J Sports Med 2008, 42:567-573.

23. Greenwood M, Greenwood L, Kreider R, Byars, A, Stahura, K.: Creatine supplementation does not increase perceptions of fatigue or adversely affect health status during three a day training. J Athletic Train 2002., 37:S82.

24. Greenwood M, Kreider RB, Greenwood L, Byars, A.: Cramping and injury incidence in collegiate football players are reduced by creatine supplementation. J Athl Train 2003, 38(3):216-219.

25. Greenwood M, Kreider RB, Melton C, Rasmussen, C, Lancaster, S, Cantler, E, Miller, P, Almada, A.: Creatine supplementation during college football training does not increase the incidence of cramping or injury. Mol Cell Biochem 2003, 244:83-88.

26. Kreider RB, Melton C, Rasmussen C, Greenwood, M, Lancaster, S, Cantler, E, Milnor, P, Almada, A.: Long-term creatine supplementation does not significantly affect clinical markers of health in athletes. Mol Cell Biochem 2003. 244(1), 95-104.

27. Kim HJ, Kim CK, Carpentier A, Poortmans, JR.: Studies on the safety of creatine supplementation. Amino Acids 2011, 40(5):1409-1418.

28. Braissant O, Henry H, Beard E, Uldry, J.: Creatine deficiency syndromes and the importance of creatine synthesis in the brain. Amino Acids 2011, 40(5):1315-1324.

29. Candow DG: Sarcopenia: Current theories and the potential beneficial effect of creatine application strategies. Biogerontology 2011, 12(4): 273-281.

30. Gualano B, Roschel H, Lancha-Jr AH, Brightbill, CE, Rawson, ES.: In sickness and in health: The widespread application of creatine supplementation. Amino Acids 2011, 1-11.

31. Klopstock T, Elstner M, and Bender A: Creatine in mouse models of neurodegeneration and aging. Amino Acids 2011, 40:1297-303.

32. Rawson ES and Venezia AC: Use of creatine in the elderly and evidence for effects on cognitive function in young and old. Amino Acids 2011, 40:1349-1362.

33. Tarnopolsky MA: Creatine as a therapeutic strategy for myopathies. Amino Acids 2011, 40:1397-1407.

34. Wallimann T, Tokarska-Schlattner M, and Schlattner U: The creatine kinase system and pleiotropic effects of creatine. Amino Acids 2011, 40:1271-1296.

35. Deldicque L, Decombaz J, Zbinden Foncea H, Vuichoud, J, Poortmans, J, Francaux, M.: Kinetics of creatine ingested as a food ingredient. European Journal of Applied Physiology 2008, 102(2):133-143.

36. Ganguly S, Jayappa S, and Dash AK: Evaluation of the stability of creatine in solution prepared from effervescent creatine formulations. AAPS PharmSciTech 2003, 4:E25.

37. Persky AM, Brazeau GA, and Hochhaus G: Pharmacokinetics of the dietary supplement creatine. Clinical Pharmacokinetics 2003, 42:557-74.

38. All American EFX Pharmaceutical: AA, Kre-alkalyn research booklet. All American EFX. Kre-Alkalyn®, All American Pharmaceutical, Billings, MT, USA.

39. EFX: AA, Kre-alkalyn - the world's most potent creatine. http://krealkalyn.com/ Kre-Alkalyn®, All American Pharmaceutical, Billings, MT, USA

40. Tallon MJ and Child R: Kre-alkalyn® supplementation has no beneficial effect on creatine-to-creatinine conversion rates. In Conference; Las Vegas, NV. Available at: http://www.aminoz.com.au/krealkalyna-waste-money-n-126.html. June 12, 2007.

41. McGuine TA, Sullivan JC, and Bernhardt DT: Creatine supplementation in high school football players. Clinical Journal of Sport Medicine. 2001, 11:247-53.

42. Ray TR, Eck JC, Covington LA, Murphy, RB, Williams, R, Knudtson, J.: Use of oral creatine as an ergogenic aid for increased sports performance: Perceptions of adolescent athletes. Southern Medical Journal 2001, 94(6):608-612.

43. LaBotz M and Smith BW: Creatine supplement use in an NCAA Division I athletic program. Clinical Journal of Sport Medicine. 1999, 9:167-169.

44. Houston ME: Biochemistry primer for exercise science. International Journal of Sports Science and Coaching 2007, 2:95-96.

45. Meyer RA, Sweeney HL, and Kushmerick MJ: A simple analysis of the "phosphocreatine shuttle". The American Journal of Physiology 1984, 246:C365-377.

46. Bogdanis G, Nevill, ME., Lakomy, HK., Boobis, LH.: Power output and muscle metabolism during and following recovery from 10 and 20 s of maximal sprint exercise in humans. Acta Physiol Scand 1998, 163:261-272.

47. Casey A, Constantin-Teodosiu D, Howell S, Hultman, E, Greenhaff, P..: Creatine ingestion favorably affects performance and muscle metabolism during maximal exercise in humans. The American Journal of Physiology 1996, 271(1):E31-E37.

48. Kreider RB, Leutholtz, B. C., Katch, F. I., & Katch, V. L. Exercise & Sport Nutrition. Fitness Technologies Press, Santa Barbara, CA. 2009.

49. Clark JF: Creatine and phosphocreatine: A review of their use in exercise and sport. Journal of Athletic Training 1997, 32:45-51.

50. Preen D, Dawson B, Goodman C, Lawrence, S, Beilby, J, Ching, S.: Effect of creatine loading on long-term sprint exercise performance and metabolism. Medicine and Science in Sports and Exercise 2001, 33:814-821.

51. Williams MH, Kreider R, and Branch JD: *Creatine: The Power Supplement*: Human Kinetics Publishers. Champaign, Illinois. 1999.

52. Abachi S, Abbott B, Abolins M, Acharya, BS, Adam, I et al.: Isolated photon cross section in the central and forward rapidity regions in pp-bar collisions at sqrt s=1.8 tev. Physical Review Letters 1996, 77:5011-5015.

53. Willoughby DS and Rosene JM: Effects of oral creatine and resistance training on myogenic regulatory factor expression. Medicine and Science in Sports and Exercise 2003, 35:923-929.

54. Willoughby DS and Rosene J: Effects of oral creatine and resistance training on myosin heavy chain expression. Medicine and Science in Sports and Exercise 2001, 33:1674-1681.

55. Thompson CH, Kemp GJ, Sanderson AL, Dixon, R, Syles, P, Taylor, D, Radda, G.: Effect of creatine on aerobic and anaerobic metabolism in skeletal muscle in swimmers. British Journal of Sports Medicine 1996, 30(3):222-225.

56. Odland LM, MacDougall JD, Tarnopolsky MA, Elorriaga, A, Borgmann, A.: Effect of oral creatine supplementation on muscle [pcr] and short-term maximum power output. Medicine and Science in Sports and Exercise 1997, 29(2):216-219.

57. Kreider RB: Effects of creatine supplementation on performance and training adaptations. Molecular and Cellular Biochemistry 2003, 244:89-94.

58. Branch JD: Effect of creatine supplementation on body composition and performance: A meta-analysis. International Journal of Sport Nutrition and Exercise Metabolism 2003, 13:198-226.

59. Dempsey RL, Mazzone MF, and Meurer LN: Does oral creatine supplementation improve strength? A meta-analysis. The Journal of Family Practice 2002, 51:945-951.

60. Volek JS, Kraemer WJ, Bush JA, Boetes, M, Incledon, T, Clark, KL, Lynch, JM.: Creatine supplementation enhances muscular performance during high-intensity resistance exercise. Journal of the American Dietetic Association 1997, 97(7):765-770.

61. Wright GA, Grandjean PW, and Pascoe DD: The effects of creatine loading on thermoregulation and intermittent sprint exercise performance in a hot humid environment. Journal of Strength and Conditioning Research. 2007, 21:655-660.

62. Tarnopolsky MA and MacLennan DP: Creatine monohydrate supplementation enhances high-intensity exercise performance in males and females. International Journal of Sport Nutrition and Exercise Metabolism 2000, 10:452-463.

63. Birch R, Noble D, and Greenhaff PL: The influence of dietary creatine supplementation on performance during repeated bouts of maximal isokinetic cycling in man. European Journal of Applied Physiology and Occupational Physiology 1994, 69:268-276.

64. Balsom PD, Soderlund K, Sjodin B, Ekblom, B.: Skeletal muscle metabolism during short duration high-intensity exercise: Influence of creatine supplementation. Acta Physio Scand. 1995, 154(3):303-310.

65. Dawson B, Cutler M, Moody A, Lawrence, S, Goodman, C, Randall, N.: Effects of oral creatine loading on single and repeated maximal short sprints. Australian Journal of Science and Medicine in Sport 1995, 27(3):56-61.

66. Kirksey B, Stone, M.H., Warren, B.J., Johnson, R.L., Stone, M., Haff, G.G.,... & Proulx, C.: The effects of 6 weeks of creatine monohydrate supplementation on performance measures and body composition in collegiate track and field athletes. The Journal of Strength & Conditioning Research 1999, 13:148-156.

67. Kelly VG, and Jenkins, D.G.: Effect of oral creatin supplementation on near maximal strength and repeated sets of high-intensity bench press exercise. Journal of Strength & Conditioning Research 1998, 12(2): 109-115.

68. Grindstaff PD, Kreider R, Bishop R, Wilson, M, Wood, L, Alexander, C, Almada, A.: Effects of creatine supplementation on repetitive sprint performance and body composition in competitive swimmers. International Journal of Sport Nutrition 1997, 7(4):330-346.

69. Deutekom M, Beltman JG, de Ruiter CJ, De Koning, JJ, De Haan, A.: No acute effects of short-term creatine supplementation on muscle properties and sprint performance. European Journal of Applied Physiology 2000, 82(3):223-229.

70. Febbraio MA, Flanagan TR, Snow RJ,Zhao, S, Carey, MF.: Effect of creatine supplementation on intramuscular tcr, metabolism and performance during intermittent, supramaximal exercise in humans. Acta Physio Scand. 1995, 155(4):387-395.

71. Green JM, McLester JR, Smith JE, Mansfield, E.: The effects of creatine supplementation on repeated upper- and lower-body wingate performance. Journal of Strength and Conditioning Research. 2001, 15(1):36-41.

72. Barnett C, Hinds M, and Jenkins DG: Effects of oral creatine supplementation on multiple sprint cycle performance. Australian Journal of Science and Medicine in Sport. 1996, 28:35-39.

73. Gilliam JD, Hohzorn C, Martin D, Trimble, M.: Effect of oral creatine supplementation on isokinetic torque production. Medicine and Science in Sports and Exercise 2000, 32(5):993-996.

74. Burke LM, Pyne DB, and Telford RD: Effect of oral creatine supplementation on single-effort sprint performance in elite swimmers. International Journal of Sport Nutrition 1996, 6:222-233.

75. Mujika I, Chatard JC, Lacoste L, Barale, F, Geyssant, A.: Creatine supplementation does not improve sprint performance in competitive swimmers. Medicine and Science in Sports and Exercise 1996, 28(11):1435-1441.

76. Jakobi JM, Rice CL, Curtin SV, Marsh, GD.: Contractile properties, fatigue and recovery are not influenced by short-term creatine supplementation in human muscle. Experimental Physiology 2000, 85(4):451-460.

77. Rawson ES and Volek JS: Effects of creatine supplementation and resistance training on muscle strength and weightlifting performance. Journal of Strength and Conditioning Research. 2003, 17:822-831.

78. Noonan D, Berg, K., Latin, R.W., Wagner, J.C., & Reimers, K.: Effects of varying dosages of oral creatine relative to fat free body mass on strength and body composition. The Journal of Strength & Conditioning Research. 1998, 12:104-108.

79. Vandenberghe K, Goris M, Van Hecke P, Vangerven, L, Hespel, P.: Long-term creatine intake is beneficial to muscle performance during resistance training. Journal of Applied Physiology 1997, 83(6):2055-2063.

80. Earnest CP, Snell PG, Rodriguez R, Almada, A, Mitchell, T.: The effect of creatine monohydrate ingestion on anaerobic power indices, muscular strength and body composition. Acta Physio Scand. 1995, 153(2):207-209.

81. Balsom PD, Harridge SD, Soderlund K, Sjodin, B, Ekblom, B.: Creatine supplementation per se does not enhance endurance exercise performance. Acta Physiologica Scandinavica 1993, 149:521-523.

82. Harris RC, Marlin DJ, Snow DH, Harkness, RA.: Muscle atp loss and lactate accumulation at different work intensities in the exercising thoroughbred horse. European Journal of Applied Physiology and Occupational Physiology 1991, 62(4):235-244.

83. Deldicque L, Atherton P, Patel R, Theisen, D, Nielens, H, Rennie, MJ, Francaux, M.: Effects of resistance exercise with and without creatine supplementation on gene expression and cell signaling in human skeletal muscle. Journal of Applied Physiology 2008, 104(2):371-378.

84. Burke DG, Candow DG, Chilibeck PD, MacNeil, L, Roy, BD, Tarnopalsky, M, Ziegenfuss, T.: Effect of creatine supplementation and resistance-exercise training on muscle insulin-like growth factor in young adults. International Journal of Sport Nutrition and Exercise Metabolism 2008, 18(4):389-398.

85. Cribb PJ, Williams AD, and Hayes A: A creatine-protein-carbohydrate supplement enhances responses to resistance training. Medicine and Science in Sports and Exercise 2007, 39:1960-1968.

86. Burke DG, Chilibeck PD, Davidson KS, Candow, DG, Farthing, J, Smith-Palmer, T.: The effect of whey protein supplementation with and without creatine monohydrate combined with resistance training on lean tissue mass and muscle strength. International Journal of Sport Nutrition and Exercise Metabolism 2001, 11:349-364.

87. Chwalbinska-Moneta J: Effect of creatine supplementation on aerobic performance and anaerobic capacity in elite rowers in the course of endurance training. International Journal of Sport Nutrition and Exercise Metabolism 2003, 13(2):173-183.

88. Nelson AG, Day R, Glickman-Weiss EL, Hegsted, M, Kokkonen, J, Sampson, B.: Creatine supplementation alters the response to a graded cycle ergometer test. European Journal of Applied Physiology 2000, 83(1):89-94.

89. Hickner RC, Dyck DJ, Sklar J, Hatley, H, Byrd, P.: Effect of 28 days of creatine ingestion on muscle metabolism and performance of a simulated cycling road race. Journal of the International Society of Sports Nutrition 2010, 7:26.

90. Poortmans JR, Kumps, A., Deuz, P., Fofonka, A., Carpentier, A., & Francaux, M.: Effect of oral creatine supplementation on urinary methylamine, formaldehyde, and formate. Medicine and Science in Sports and Exercise, 2005, 37:1717.

91. Kreider RB, Melton C, Rasmussen CJ, Greenwood, M, Lancaster, S, Cantler, E, Milnor, P, Almada, A.: Long-term creatine supplementation does not significantly affect clinical markers of health in athletes. Molecular and Cellular Biochemistry 2003, 244:95-104.

92. Vannas-Sulonen K, Sipila I, Vannas A, Simell, O, Rapola, J.: Gyrate atrophy of the choroid and retina. A five-year follow-up of creatine supplementation. Ophthalmology 1985: 92(12):1719-1727.

93. Stockler S, Marescau B, De Deyn PP, Trijbels, J, Hanefeld, F.: Guanidino compounds in guanidinoacetate methyltransferase deficiency, a new inborn error of creatine synthesis. Metabolism: Clinical and Experimental 1997, 46(10):1189-1193.

94. Allen PJ: Creatine metabolism and psychiatric disorders: Does creatine supplementation have therapeutic value? Neuroscience and Biobehavioral Reviews 2012, 36:1442-1162.

95. Kreider RB, Leutholtz, B. C., and Greenwood, M., Wolinsky, I., Driskell, JA. Creatine, in *Nutritional Ergogenic Aids*. 2004, CRC Press LLC: Boca Raton, FL: 81-104.

96. Hausmann ON, Fouad K, Wallimann T, Schwab, M.: Protective effects of oral creatine supplementation on spinal cord injury in rats. Spinal Cord 2002, 40(9):449-456.

97. Jacobs PL, Mahoney ET, Cohn KA, Sheradsky, LF, Green, BA.: Oral creatine supplementation enhances upper extremity work capacity in persons with cervical-level spinal cord injury. Archives of Physical Medicine and Rehabilitation 2002, 83(1):19-23.

98. Zhu S, Li M, Figueroa BE, Liu, A, Stavrovskaya, IG, Pasinelli, P, Flint Beal, M.: Prophylactic creatine administration mediates neuroprotection in cerebral ischemia in mice. The Journal of Neuroscience. 2004, 24(26):5909-5912.

99. Jowko E, Ostaszewski P, Jank M, Sacharuk, J, Zieniewicz, A, Wilczak, J, Nessen, S.: Creatine and beta-hydroxy-beta-methylbutyrate (hmb) additively increase lean body mass and muscle strength during a weight-training program. Nutrition 2001, 17:558-566.

100. Cribb PJ, Williams AD, Stathis CG, Carey, MF, Hayes, A.: Effects of whey isolate, creatine, and resistance training on muscle hypertrophy. Medicine and Science in Sports and Exercise 2007, 39(2):298-307.

101. Green AL, Hultman E, Macdonald IA, Sewell, DA, Greenhaff, PL.: Carbohydrate ingestion augments skeletal muscle creatine accumulation during creatine supplementation in humans. The American Journal of Physiology 1996, 271(5):E821-E826.

102. Peeters BM, Lantz, C.D., & Mayhew, J.L.: Effects of oral creatine monohydrate and creatine phosphate supplementation on maximal strength indices, body composition, and blood pressure. The Journal of Strength & Conditioning Research. 1999, 13:3-9.

103. Steenge GR, Simpson EJ, and Greenhaff PL: Protein- and carbohydrate-induced augmentation of whole body creatine retention in humans. J Appl Physiol 2000, 89:1165-1171.

104. Greenwood M, Kreider, R. B., Rasmussen, C., Almada, A. L. & Earnest, C. P.: D-pinitol augments whole body creatine retention in man. J Exerc Physiol Online 2001, 4:41-47.

105. Golini JM: *Oral creatine supplement and method for making same.* US Patent 6,399,661, B1, USA; Issue Date, June 4, 2002.

106. Kerksick CM, Wilborn CD, Campbell WI, Harvey, TM, Marcello, B, Roberts, MD, Parker, AG, et al.: The effects of creatine monohydrate supplementation with and without d-pinitol on resistance training adaptations. Journal of Strength and Conditioning Research. 2009, 23:2673-2682.

107. Lohman M, Tallroth K, Kettunen JA, Marttinen, MT.: Reproducibility of dual-energy x-ray absorptiometry total and regional body composition measurements using different scanning positions and definitions of regions. Metabolism 2009, 58:1663-1668.

108. Almada A, Kreider R, Ransom J, Rasmussen, C, Tutko, R, Milnor, P.: Comparison of the reliability of repeated whole body dexa scans to repeated spine and hip scans. J Bone Miner Res 1999, 14:SA243.

109. Evans WJ, Phinney SD, and Young VR: Suction applied to a muscle biopsy maximizes sample size. Med Sci Sports Exerc 1982, 14:101-102.

110. Harris RC, Hultman E, and Nordesjo LO: Glycogen, glycolytic intermediates and high-energy phosphates determined in biopsy samples of musculus quadriceps femoris of man at rest. Methods and variance of values. Scand J Clin Lab Invest 1974, 33:109-210.

111. Kerksick CM, Wilborn CD, Campbell BI, Roberts, MD, Rasmussen, CJ, Greenwood, M, Kreider, RB.: Early-phase adaptations to a split-body, linear periodization resistance training program in college-aged and middle-aged men. Journal of Strength and Conditioning Research. 2009, 23(2):962-971.

112. Stone MH, Sanborn K, Smith LL, O'bryant, HS, Hoke, T, Utter, AC, Garner, B.: Effects of in-season (5 weeks) creatine and pyruvate supplementation on anaerobic performance and body composition in American football players. International Journal of Sport Nutrition 1999, 9:146-165.

113. Kreider RB, Klesges R, Harmon K, Grindstaff, P, Ramsey, L, Bullen, D, Wood, L, Li, Y, Almada, A.: Effects of ingesting supplements designed to promote lean tissue accretion on body composition during resistance training. International Journal of Sport Nutrition 1996, 6:234-246.

114. Volek JS, Ratamess NA, Rubin MR, Gomez, A, French, D, McGuigan, M, Scheett, TP, Sharman, MJ, Hakkinen, K, Kraemer, WJ.: The effects of creatine supplementation on muscular performance and body composition responses to short-term resistance training overreaching. European Journal of Applied Physiology. 2004, 91(5):628-637.

115. Smith JC, Stephens DP, Hall EL, Jackson, AW, Earnest, CP.: Effect of oral creatine ingestion on parameters of the work rate-time relationship and time to exhaustion in high-intensity cycling. European Journal of Applied Physiology and Occupational Physiology 1998, 77(4):360-365.

116. Prevost MC, Nelson AG, and Morris GS: Creatine supplementation enhances intermittent work performance. Research Quarterly for Exercise and Sport 1997, 68:233-240.

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

118. Ziegenfuss TN, Rogers M, Lowery L, Mullins, N, Mendel, R, Antonio, J, Lemon, P.: Effect of creatine loading on anaerobic performance and skeletal muscle volume in NCAA Division I athletes. Nutrition 2002, 18(5):397-402.

APPENDIX A

CONSENT FORM

Comparison of Different Forms of Creatine on Creatine Availability, Retention, and Training Adaptations

Introduction

The purpose of this form is to provide you information that may affect your decision as to whether or not to participate in this research study. If you decide to participate in this study, this form will also be used to record your consent.

You have been asked to participate in a research project studying different forms of creatine. The purpose of this study is to determine the effects of ingesting loading doses of creatine monohydrate (CM) and Kre-alkalyn (KA) on muscle creatine retention and muscle phosphagen levels; and, the effects of 28-days of CM and KA supplementation on body composition, strength, and anaerobic capacity. You were selected to be a possible participant because you met all entrance criteria. This study is being sponsored/funded by AlzChem Trostberg GmbH.

What will I be asked to do?

If you agree to participate in this study, you will first be asked to sign an Informed Consent statement in compliance with the Human Subject's Protection Program (HSPP) at Texas A&M University and the American College of Sports Medicine. You will then be familiarized to the study requirements, food log recording and tests to be conducted during the study. This session will take approximately one hour to complete. You will then be provided eight 3 L urine collection containers in order to collect 24 hr. urine samples on days 0,1,2,3,4,5,6 and 7, and will be asked to record the number of times you urinate each day as well as your specific fluid intake. The 24 hr. baseline urine sample time parameter will be initiated at 8:00 a.m. the day before supplementation protocols begin. You will be asked to report daily to the Exercise & Sport Nutrition Lab (ESNL) between 7:00 and 8:00 a.m. in order to drop off your urine samples. Prior to reporting to the lab for baseline testing, you will record all food that you eat on dietary record forms for four days (including one weekend day). You will not exercise for 48 hours nor eat for 12 hours prior to reporting to the lab for baseline testing. You will then undergo a battery of tests as described in Table 1 below. You will fill out a Demographic Form, a Health History Form, an Exercise History Form and a Radiation Safety Form. You will also be required to report any adverse side effects that you may experience on a weekly basis. .

You will then continue with the tests as described in Table 1. You will first be weighed and have your height measured. You will then be asked to provide a muscle sample from your leg muscle for fiber type analysis. Muscle biopsies will be obtained using the Bergstroem technique (3 total biopsies during the study), which involves a ¼" incision on the skin and the use of a 5 mm biopsy needle. Local anesthetic will be used prior to the incision and biopsy. Percutaneous muscle biopsies (50-70 mg) will be obtained from the middle portion of the vastus lateralis muscle (thigh

muscle covering the outermost portion of the front of the leg) of one leg at the midpoint between the knee and hip joint at a depth between 1 and 2 cm. For the remaining biopsies, attempts will be made to extract tissue from approximately the same depth and area as the initial biopsy by using the pre-biopsy scar, depth markings on the needle, and a successive incision that will be made approximately 0.5 cm to the former from medial to lateral. All these procedures will be conducted again at 1 and 4 weeks into the study. You will then donate about 20 milliliters (4 teaspoons) of venous blood from a vein in your arm. Blood samples will be obtained by standard/sterile procedures using a needle inserted into a vein in your arm. Personnel who will be taking your blood are experienced in phlebotomy (procedures to take blood samples) and are qualified to do so under guidelines established by the Texas Department of Health and Human Services. This will take about 5 minutes. You will then have your total body water determined using a bioelectrical impedance analyzer (BIA). The BIA analysis will involve lying down on your back on a table and having two small electrodes placed on your right hand and your right foot. The analyzer wires will be attached and a small and safe current (500 micro-amps at a frequency of 5- kHz) will pass through your body so that the amount of water can be measured. This analyzer is commercially available and has been used in the health care/fitness industry as a means to assess body composition and body water for over 20 years. The use of this device has been approved by the Food and Drug Administration (FDA) to assess total body water and the current to be used has been deemed safe. Your body composition and bone density will then be determined by using a Discovery W dual energy x-ray absorptiometer (DEXA). This will involve lying down on your back on the DEXA exam table in a pair of shorts or a gown for about 6 minutes. A low dose of radiation will scan your entire body to determine the amount of fat weight, muscle weight, and bone weight. You will be exposed to an x-ray dose that is similar to the amount of natural background radiation a person would receive in one month while living in College Station. After this test, you will have resting blood pressure determined using a blood pressure cuff and stethoscope and heart rate determined by taking your pulse. You will then perform a one repetition maximum (1RM) test on the bench press using standard procedures. This will involve warming up and performing successive one repetition lifts on the bench press until you determine your 1 RM. You will then rest for 10-minutes and follow the same procedure in determining your 1 RM on the hip/leg sled. These tests will take about 20 minutes to complete. You will then be asked to perform a 30-second Wingate anaerobic capacity test on a bike at your maximal intensity against a resistance of 7.5 J/kg/rev. You will receive a five minute warm up before you will be asked to pedal as fast as possible for the entire 30 seconds. The same battery of tests will be performed at the post-study assessment 4 weeks into the study protocol. All the assessments minus the exercise tests will also be performed at 1 week into the study protocol. Each testing session will take between 1.5 and 3 hours to complete. In the event of an emergency during an exercise test proper emergency response protocols (calling 9-911 for serious injury or a medical emergency, calling Biosafety/EHS for cleanup assistance or spill team response, calling UPD for incidents in public areas, retrieving AED located in the lab, performing CPR or other First Aid techniques, etc.) will be followed by the Exercise & Sport Nutrition Laboratory (ESNL) Staff depending on the severity of the emergency.

After baseline testing, you will be matched into one of three groups according to age, body weight, and training status/experience and asked to ingest supplements containing CM (*Creapure*

AlzChem, Trostberg, Germany) or KA (*Kre-alkalyn, Billings, MT*). The initial 7 day loading dose will be 20 grams of CM, the purported equivalent dose of KA, and the manufacturers recommended dose. After the 7 day loading period you will be asked to ingest one 5 grams dose per day of CM, the purported equivalent dose of KA, or the manufacturers recommended dose of KA for 21 days. You will be asked to ingest the supplements at 8:00 a.m., 12:00 p.m., 4:00 p.m. and 8:00 p.m. during the loading phase and at 8:00 a.m. during the maintenance phase. The supplements will be provided with similar texture, taste, and appearance and placed in generic single serving packets for double-blind administration. Your compliance will be monitored by asking that you return empty containers of the supplement at the end of each testing session. In addition your compliance will be verified by administering and collecting daily questionnaires. After completing the compliance procedures you will be given the required supplement dosage for the next day. Everyone, regardless of group assignment, will be asked to keep a food record and food frequency log to monitor dietary compliance.

Please do your best to: 1) follow the instructions outline by the investigators; 2) show up to all scheduled testing and training sessions; and 3) follow the diet prescribed and do not take any other nutritional supplements or performance enhancing aids during this study (i.e., vitamins/minerals, creatine, HMB, androstenedione, DHEA, etc). In addition, please do not take any non-medically prescribed medications and report any medication that is prescribed for you to take during this study. If you take any other nutritional supplements or medications during the course of the study that may affect vitamin/mineral status, body composition, or strength you may be removed from the study.

Table 1. Overview of Research Design

Familiarization and Entry	Baseline Day 0 (T1)	Loading Phase Day 7 (T2)	Training Phase Day 28 (T3)
Familiarization session Informed Consent Form Demographic Form Health History Form Exercise History Form 4-day Dietary History Distribute Eight 3 L Urine Containers General Exam to Determine Qualifications to Participate in Study Determination of Height and Body Weight Randomization Into 3 Groups (CM, KA recommended, KA equivalent) Instructions for Supplementation	Baseline Muscle Biopsy Baseline Fasting Blood Sample Turn in Final Urine Container Body Weight Body Water (BIA) DEXA Body Composition 1 RM Bench Press 1 RM Leg Press Wingate Anaerobic Capacity Test Loading Phase of Supplementation Begins Maintain Training Log	4-Day Diet History Submit Training Log Muscle Biopsy Fasting Blood Sample Body Weight Body Water (BIA) DEXA Body Composition Wingate Anaerobic Capacity Test Low-Dose Maintenance Phase of Supplementation Begins	4-Day Diet History Submit Training Log Muscle Biopsy Fasting Blood Sample Body Weight Body Water (BIA) DEXA Body Composition 1 RM Bench Press 1 RM Leg Press Wingate Anaerobic Capacity Test

What are the risks involved in this study?

The risks associated with this study are: You will be exposed to a low level of radiation during the DEXA body composition tests, which is similar to the amount of natural background radiation you would receive in one month while living in College Station. In addition, a very low level of electrical current will be passed through your body using a bioelectrical impedance analyzer (BIA). This analyzer is commercially available and has been used in the health care/fitness industry as a means to assess body composition and body water for over 20 years. The BIA and DEXA analyzers have been shown to be safe methods of assessing body composition and total body water and are approved by the FDA. You will donate about 4 teaspoons (20 milliliters) of venous blood three (3) times during the study using standard phlebotomy procedures. This procedure may cause a small amount of pain when the needle is inserted into the vein as well as some

bleeding and bruising. You may also experience some dizziness, nausea, and/or faint if you are unaccustomed to having blood drawn. The biopsy procedure carries the risk of soreness (100%), infection (<1%), and permanent numbness (<<1%). The exercise tests that will be performed may cause symptoms of fatigue, shortness of breath, and/or muscular fatigue/discomfort. The exercise tests may also cause short-term muscle soreness and moderate fatigue for several days following the tests. You may also experience muscle strains/pulls during the exercise testing and/or training program. However, exercise sessions will be conducted by trained personnel and monitored to ensure you follow appropriate exercise guidelines. The likelihood of any of these occurring is slim.

What are the possible benefits of this study?
The possible benefit you may receive from participation in this study is increased physical fitness and improvements in body composition. You may also gain insight about your health and fitness status from the assessments that will be performed.

Do I have to participate?
No. Your participation is voluntary. You may decide not to participate or to withdraw at any time without your current or future relations with Texas A&M University being affected.

Will I be compensated?
You will receive $150 ($50 for each testing session) upon completion of the study. Disbursement will occur upon completion of all sessions and after all study related materials (food logs, training logs, etc.) are turned in. Those forced to terminate the study prior to completion will be compensated on a pro-rated basis depending on the total number of sessions completed.

Who will know about my participation in this research study?
This study is confidential. The records of this study will be kept private. No identifiers linking you to this study will be included in any sort of report that might be published. Research records will be stored securely and only Mr. Christopher Rasmussen and Dr. Richard Kreider will have access to the records.

Whom do I contact with questions about the research?
If you have questions regarding this study, you may contact Dr. Richard Kreider, 945-1333, rkreider@hlkn.tamu.edu or Mr. Christopher Rasmussen, 458-1741, crasmussen@hlkn.tamu.edu.

Whom do I contact about my rights as a research participant?
This research study has been reviewed by the Human Subjects' Protection Program and/or the Institutional Review Board at Texas A&M University. For research-related problems or questions regarding your rights as a research participant, you can contact these offices at (979)458-4067 or irb@tamu.edu.

Signature

Please be sure you have read the above information, asked questions and received answers to your satisfaction. You will be given a copy of the consent form for your records. By signing this document, you consent to participate in this study.

Signature of Participant: _____ **Date:** _____

Printed Name: _____

Signature of Person Obtaining Consent: _____ **Date:** _____

Printed Name: _____

APPENDIX B

PERSONAL INFORMATION WORKSHEET

Texas A&M University
EXERCISE & SPORT NUTRITION LABORATORY

Personal Information

Name:
_____

Address:
_____

City: _____ State: _____ Zip Code _____ SS# _____

Home Phone: (___) _____ Work Phone: (___) _____

Beeper: (___) _____ Cell Phone: (___) _____

Fax: (___) _____ E-mail address:_____

Birth date: _____ / _____ / _____ Age: _____ Height: _____ Weight: _____

Exercise History/Activity Questionnaire

1. Describe your typical occupational activities.

2. Describe your typical recreational activities

3. Describe any exercise training that you routinely participate.

4. How many days per week do you exercise/participate in these activities?

5. How many hours per week do you train?

6. How long (years/months) have you been consistently training?

APPENDIX C

MEDICAL HISTORY QUESTIONNAIRE

Texas A&M UNIVERSITY

EXERCISE & SPORT NUTRITION LABORATORY

Medical History Inventory

Directions. The purpose of this questionnaire is to enable the staff of the Exercise and Sport Sciences Laboratory to evaluate your health and fitness status. Please answer the following questions to the best of your knowledge. All information given is **CONFIDENTIAL** as described in the **Informed Consent Statement.**

Name:_____ Age _____Date of Birth_____

Name and Address of Your
Physician:_____

MEDICAL HISTORY

Do you have or have you ever had any of the following conditions? (Please write the date when you had the condition in the blank).

____Heart murmur, clicks, or other cardiac findings?
____Asthma/breathingdifficulty?
____ Frequent extra, skipped, or rapid heartbeats?
____Chest Pain of Angina (with or without exertion)?
____Bronchitis/Chest Cold?
____Cancer, Melanoma, or Suspected Skin Lesions?
____High cholesterol?
____Diagnosed high blood pressure?
____Heart attack or any cardiac surgery?
____Leg cramps (during exercise)?
____Chronic swollen ankles?
____Varicose veins?
____Frequent dizziness/fainting?
____Muscle or joint problems?
____High blood sugar/diabetes?
____Thyroid Disease?
____Low testosterone/hypogonadism?
____Glaucoma?
____Bleeding Disorders
____Stroke or Blood Clots?
____Emphysema/lung disease?
____Epilepsy/seizures?
____Rheumatic fever?
____Scarlet fever?
____Ulcers?
____Pneumonia?
____Anemias?
____Liver or kidney disease?
____Autoimmune disease?
____Nerve disease?
____Psychological Disorders?

Do you have or have you been diagnosed with any other medical condition not listed?
_____
_____

Please provide any additional comments/explanations of your current or past medical history.

_____
_____

Please list any recent surgery (i.e., type, dates etc.).

_____
_____

List all prescribed/non-prescription medications and nutritional supplements you have taken in the last 3 months.

_____
_____

What was the date of your last complete medical exam?

_____
_____

Do you know of any medical problem that might make it dangerous or unwise for you to participate in this study? (including strength and maximal exercise tests) ____ If yes, please explain: _____

_____
_____

Recommendation for Participation (for ESNL use only):

____ No exclusion criteria presented. Subject is ***cleared*** to participate in the study.
____ Exclusion criteria is/are present. Subject is ***not cleared*** to participate in the study.

Signed: _____ Date: _____