ACUTE AND CHRONIC ANALYSIS OF THE SAFETY AND EFFICACY OF DOSE

DEPENDENT CREATINE NITRATE SUPPLEMENTATION AND EXERCISE

PERFORMANCE

A Dissertation

by

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Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

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December 2015

Major Subject: Kinesiology

ABSTRACT

Creatine monohydrate (CrM) and nitrate are popular supplements for improving exercise performance; yet they have not been investigated in combination. We performed two studies to determine the safety and exercise performance-characteristics of creatine nitrate (CrN) supplementation.

In Study 1, 13 participants ingested 1.5 g CrN (CrN-L), 3 g CrN (CrN-H), 5 g CrM or a placebo (PL) in a crossover study to determine supplement safety. Hepatorenal and muscle enzymes, heart rate, blood pressure and side effects were measured before supplementation, 30 minutes after ingestion, and then hourly for 5 hours post-supplementation. In Study 2, 48 participants received the same CrN treatments vs. 3 g CrM in a double-blind, 28-day trial inclusive of a 7-day interim testing period and loading sequence (4 servings/d). Day-0 and day-28 measured bench press performance, Wingate testing and a 6x6-s bicycle ergometer sprints. Data were analyzed using a general linear model and results are reported as mean ± standard deviation or mean change ± 95% confidence interval (CI).

Both studies yielded several significant, yet stochastic changes in blood markers that were not indicative of potential harm or consistent for any treatment group. Equally, all treatment groups reported a similar number of minimal side effects. In Study 2, there was a significant increase in plasma nitrates for both CrN groups by day-7, subsequently abating by day-28. Muscle creatine increased significantly by day-7 in the CrM and CrN-H groups, but decreased by day-28 for CrN-H. By day-28, there were significant

increases in bench press lifting volume (kg) for all groups (PL, 126.6, 95% CI 26.3, 226.8; CrM, 194.1, 95% CI 89.0, 299.2; CrN-L, 118.3, 95% CI 26.1, 210.5; CrN-H, 267.2, 95% CI 175.0, 359.4, kg). Only the CrN-H group was significantly greater than PL ($p<0.05$). Similar findings were observed for bench press peak power (PL, 59.0, 95% CI 4.5, 113.4; CrM, 68.6, 95% CI 11.4, 125.8; CrN-L, 40.9, 95% CI -9.2, 91.0; CrN-H, 60.9, 95% CI 10.8, 111.1, Watts) and average power.

Creatine nitrate was well-tolerated, demonstrated similar performance benefits to 3 g CrM, and was void of significant hemodynamics or blood enzymes changes associated with supplement safety.

DEDICATION

I would like to dedicate this work to my parents for their encouragement throughout my childhood to pursue higher education.

ACKNOWLEDGEMENTS

I would never have been able to finish my dissertation without the guidance of my committee members, help from friends, and support from my family and wife.

I would like to thank my committee chair, Dr. Richard Kreider, and my committee members, Dr. Jim Fluckey, Dr. Steve Riechman, and Dr. Stephen Smith, for their guidance and support throughout the course of this research and time at Texas A&M University. Additionally, I would like to thank Dr. Conrad Earnest for his guidance and timely email responses to my many, many questions throughout this project. I would also like to thank Drs. Dillon Walker and Sunday Simbo for their assistance during the early morning biopsy procedures and encouragement throughout my research. Thanks also go to my friends, especially Kyle Levers and Nick Barringer, and colleagues and the department faculty and staff for making my time at Texas A&M University a great experience.

I would like to thank Drs. Paul La Bounty and Peter Horvath. Both played a pivotal role in my research career. Dr. La Bounty introduced me to the Exercise and Sport Nutrition Lab at Baylor University where I first became interested in sports nutrition research. Dr. Horvath was a great mentor during my time at SUNY Buffalo. I had a truly great and unforgettable experience in Dr. Horvath's lab. My interest in a career in research was eventually solidified because of him.

Finally, thanks to my wife for her patience, love, and, most importantly, her company in the lab during the hours and hours of data analysis. When the situation

v

arises I can now unpretentiously use the renowned words of Dr. Peter Venkman who once said, "Back off, man. I'm a scientist."

NOMENCLATURE

μM	Micromolar
1RM	1 Repetition Maximum
ADP	Adenosine Diphosphate
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
AP	Average Power
ATP	Adenosine Triphosphate
AV	Average Velocity
BP	Blood Pressure
BRJ	Beetroot Juice
BUN	Blood Urea Nitrogen
CEE	Creatine Ethyl Ester
CK	Creatine Kinase
Cr	Creatine
CrC	Tri-Creatine Citrate
CrM	Creatine Monohydrate
CrN-L	Creatine Nitrate (1.5 grams)
Crn	Creatinine
CrN-H	Creatine Nitrate (3 grams)
CrP	Creatine Phosphate

CrPyr	Creatine Pyruvate
DBP	Diastolic Blood Pressure
DXA	Dual Energy X-ray Absorptiometry
FCr	Free Creatine
FFM	Fat-Free Mass
FM	Fat Mass
g	Grams
g/kg	Grams per Kilogram Body Weight
HR	Heart Rate
J	Joules
J/kg	Joules per kilogram
LBM	Lean Body Mass
LDH	Lactate Dehydrogenase
mL	Milliliter
MP	Mean Power
PCr	Phosphocreatine
PL	Placebo
PP	Peak Power
SBP	Systolic Blood Pressure
W	Watts
W/kg	Watts per Kilogram Body Weight

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

INTRODUCTION

Background

Creatine (Cr) was discovered as an organic component of meats over 150 years ago. Cr (2-[Carbamimidoyl(methyl)amino]acetic acid) is a nitrogenous amine that humans can endogenously synthesize from three amino acids – glycine, arginine, and methionine (126). Animal products such as meats and fish are primary sources of dietary creatine. Nevertheless, a typical western diet does not lead to a significant increase in muscle creatine concentrations (8, 42). Therefore, providing supplemental creatine should lead to increases in total creatine concentrations, which, in turn, can improve exercise performance. Cr is a high-energy buffer that plays an important role during high intensity exercise that requires rapid adenosine triphosphate (ATP) synthesis. This rapid production of ATP is mostly met by the phosphagen energy system. In this energy system creatine kinase catalyzes phosphocreatine and adenosine diphosphate (ADP) to creatine and ATP.

The form of Cr most extensively investigated is creatine monohydrate (CrM) (19). Several supplementation protocols have been employed to increase muscle Cr stores. Some protocols recommend a loading phase and a maintenance phase. The loading phase consists of providing a higher dose of Cr, usually 20 – 25 g for 5 – 7 days, while the maintenance phase consists of a lower dose, usually 3 – 5 g/d for the remainder of the supplementation period. Researchers have reported a 20% increase in muscle Cr

1

stores after the brief loading phase period (58). Alternatively, other supplementation protocols have omitted the loading phase altogether. This type of supplementation protocol has been shown to increase muscle Cr stores as well, but at a much slower rate. Hultman et al. (58) reported a 20% increase in muscle Cr stores after supplementing with 3 – 5 g/d for one month. Similar Cr supplementation protocols have been shown to increase total creatine (TCr) concentration by 10 – 40% in healthy adults (44). Increased TCr concentration, when accompanied by a resistance training program, leads to improved strength, power, and lean body mass (52, 117, 120, 121). Furthermore, there is substantial evidence suggesting CrM is safe with no detrimental effects on health.

Despite supporting evidence, novel forms of Cr appear in the marketplace with claims of being more efficacious than CrM. Research does not support the majority of the claims purported by the novel forms of Cr. For example, Kre-Alkalyn[®], a buffered form of Cr, claims to enhance the delivery of usable Cr (40). A recent study by Jagim et al. (63), compared the effects of CrM and Kre-Alkalyn[®] on muscle Cr content, body composition, and adaptations to training. Results indicated that Kre-Alkalyn[®] did not promote greater changes in any variable measured when compared to CrM.

Nitrate supplements have received much attention due to their effects on vasodilation, blood pressure, improved work efficiency, and reduce phosphocreatine (PCr) degradation (79, 80, 118). Nitrate supplementation is most commonly consumed as beetroot juice (BRJ) or sodium nitrate (55). Nitrate doses have been prescribed in absolute and relative amounts ranging from 300 – 600 mg (55) and 0.1 mmol/kg/d, respectively (78, 81). In relative terms, a 70 kg person prescribed 0.1 mmol/kg/d would

ingest 7 mmol/d which is approximately 435 mg nitrate. Exercise performance has been measured after short term (2 − 3 h prior to exercise) and longer term (2 − 7 d) supplementation. Muggeridge et al. (88), observed lowered oxygen consumption during submaximal exercise as well as significantly faster time performance after consuming approximately 310 mg nitrate 3 h prior to testing. Larsen et al. (78), observed a reduction in oxygen cost during submaximal exercise performance on a cycle ergometer after supplementing with nitrate (0.1 mmol/kg/d) for 3 d. Nitrate supplementation research has mainly been shown beneficial in endurance exercise (55).

Nitrate supplementation research and its effect on anaerobic exercise are lacking. Additionally there is currently no research that has examined nitrate in combination with Cr supplementation. A novel form of Cr, creatine nitrate (CrN), has gained some recent attention (66), but to our knowledge there are no studies that have examined the effects of CrN supplementation and anaerobic exercise performance. The existing separate evidence of Cr and nitrate research suggests that the novel form of Cr, CrN, may potentially yield greater improvements in anaerobic exercise performance when compared to supplementation with CrM.

Research related to the safety and efficacy of CrN currently is lacking. Thus, the present study examined the acute safety and chronic efficacy of CrN supplementation on exercise performance. Study 1 examined the hemodynamic, hematologic, and dose effects of ingesting two doses of CrN compared to CrM and placebo (PL). Study 2 examined the effects of 28 days of two doses of CrN compared to CrM and PL on body composition and exercise performance in recreationally active males.

Statement of the Problem

CrN will increase exercise performance and related performance indices in a dose dependent manner and be equal in effectiveness to CrM. Furthermore, CrN ingestion will not adversely affect hepatorenal function or hemodynamic indices following acute and chronic ingestion.

Purpose of the Study

The aim was to examine the acute (5 h) hemodynamic (heart rate and blood pressure) and hematologic profiles (blood chemistries) for two doses of CrN compared to CrM and PL as well as compare their effects on muscle Cr content, body composition and anaerobic exercise performance after 28 d of supplementation.

General Study Overview

This study was carried out in two trials and both were randomized, double-blind, placebo-controlled. In Study 1, in a crossover fashion, we determined acute (5 h) hemodynamic (heart rate and blood pressure) and hematologic profiles after the administration of four supplements – 1) placebo (PL: 6.5 g dextrose), 2) creatine monohydrate (5 g CrM, 1.5 g dextrose), 3) creatine nitrate-lower dose (CrN-L; 1.5 g CrN [1 g Cr, 0.5 g nitrate], 5 g dextrose) and 4) creatine nitrate-higher dose (CrN-H; 3 g CrN [2 g Cr, 1 g nitrate], 3.5 g dextrose). In Study 2, we determined changes in muscle creatine content, body composition, and anaerobic exercise performance after 28 d of supplementation. Participants were randomly assigned in counter-balanced order to 1) PL (6.5 g dextrose), 2) creatine monohydrate (CrM: 3 g CrM, 0.5 g flavoring, 2 g dextrose), 3) CrN-lower dose (1.5 g CrN [1 g Cr, 0.5 g nitrate], 0.5 g flavoring, 3.5 g

4

dextrose), and 4) CrN-higher dose (3 g CrN [2 g Cr, 1 g nitrate], 0.5 g flavoring, 2 g dextrose). Study 2 consisted of a loading phase (7 d) and maintenance phase (21 d) of supplementation. During the loading phase participants ingest 4 doses/d while the maintenance phase consisted of ingesting one dose/d. In other words, participants ingested 1) PL (26 g dextrose/d for 7 d and 6.5 g dextrose/d for 21 d), 2) CrM (12 g CrM/d for 7 d and 3 g CrM/d for 21 d), 3) CrN-L (6 g CrN/d for 7d and 1.5 g CrN/d for 21 d) and 4) CrN-H (12 g CrN/d for 7 d and 3 g CrN/d for 21 d) in a randomized, double-blind manner. Muscle Cr, body composition, and blood chemistries were assessed at days 0, 7, and 28 to determine acute and chronic effects and safety. Anaerobic power variables were assessed and compared between groups using a bench press test outfitted with a Tendo FitroDyne Unit and an anaerobic sprint test on a cycle ergometer at day 0 and after 28 d of supplementation.

Hypotheses

Study 1

H1:   There will be significant differences among groups in plasma creatine concentrations.

H2:   There will be significant differences among groups in plasma nitrate concentrations.

H3:   There will be no significant differences among groups in markers of clinical health.

H4:   There will be no significant difference among groups in heart rate.

H5:   There will be significant differences among groups in blood pressure.

5

H6:   There will be no significant differences among groups in side effects symptoms.

Study 2

H7:   There will be a significant difference among groups in plasma creatine concentration after 7 and 28 d of supplementation

H8:   There will be a significant difference among groups in plasma nitrate concentration after 7 and 28 d of supplementation

H9:   There will be a significant difference among groups in muscle creatine concentrations after 7 and 28 d of supplementation.

H10:   There will be no significant difference among groups in body composition as measured by dual x-ray absorptiometry (DXA) after 7 and 28 d of supplementation.

H11:   There will be a significant difference among groups in upper body power as measured by the bench press test after 28 d.

H12:   There will be a significant difference among groups in anaerobic capacity as measured by the repeated sprints test on a cycle ergometer after 28 d.

H13:   There will be no significant difference among groups in markers of clinical health and safety after 7 and 28 d of supplementation.

H14:   There will be no significant difference among groups in side effects symptoms after 7, 14, 21, and 28 d of supplementation.

Delimitations

This study was conducted under the following guidelines:

1. Thirteen (n=13) recreationally active males ages 18 – 40 y were recruited for Study 1.

2. Forty-eight (n=48) recreationally active males ages 18 – 40 y were recruited for Study 2.

3. Subjects refrained from the consumption of dietary supplements, and/or ergogenic aids (excluding daily vitamins and protein supplements) for at least three months prior to initiating testing.

4. Eligible participants took part in a familiarization session during which time they were informed of the study protocol, filled out necessary forms including an informed consent form. Only those participating in Study 2 completed a 1 repetition max bench press test and a practice anaerobic sprint test on a cycle ergometer.

5. Participants were advised to maintain a consistent workout regimen throughout the duration of the study and recorded all workouts in a workout log.

6. Percutaneous muscle biopsies (only Study 2) were obtained from the *vastus lateralis* at d -1, 7 and 27 and analyzed for muscle creatine content.

7. Participants refrained from strenuous exercise, alcohol, and non-steroidal anti-inflammatory drugs at least 48 h prior to each testing session.

8. Participants were fasted for at least 8 hours for Study 1 and fasted for at least 12 h for Study 2.

9. Participants performed to their maximal ability on all strength and anaerobic sprints tests.

10. Participants were instructed to consume all supplements and report any side-effects in a weekly questionnaire.

Limitations

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

2. 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 efforts during performance during testing may not have been 100% at each testing session.

4. Participants may not have followed the supplementation instructions.

5. All participants were instructed to maintain a consistent training program and keep a workout record. However, exercise habits during the duration of the study may have changed and therefore changes in performance 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 or 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 THE LITERATURE

Introduction

Creatine monohydrate (CrM) is the most studied ergogenic aid in the last few decades. It continues to be one of the most popular dietary supplements among amateur and elite athletes. It is estimated that ~25% of high school athletes and ~45% of male division I collegiate athletes consume creatine (Cr) supplements (77, 85, 102). Cr supplementation can improve strength, power, and lean body mass when combined with resistance training in young (69, 92) and older adults (18, 48). The benefits of Cr supplementation have mainly been validated through research involving anaerobic exercise. Theoretically Cr supplementation may also benefit endurance exercise via increase in glycogen storage (90). To date, CrM is the most researched form of Cr; however, novel forms of Cr are created and investigated in hopes of surpassing the efficacy of CrM (62). The benefits of Cr supplementation are also being seen in clinical research as a therapeutic agent to alleviate symptoms associated with certain in-born errors of metabolism, Alzheimer's, and Parkinson's disease (19, 111).

Cr was discovered as an organic component of meats over 150 years ago by a French scientist, Michel Engene Chevreul in 1832 (104). A few years later in 1847, the German scientist, Justus von Liebig, identified Cr's chemical structure as methyl-guanidino-acetic acid. In the late 1920s, approximately a century after the discovery of Cr, phosphocreatine (PCr) was discovered. In 1981, Sipila et al. (107), treated patients

with gyrate atrophy with 1.5 g of Cr/d for one year. One characteristic of gyrate atrophy is progressive atrophy of skeletal muscle. Patients ingesting oral Cr supplementation reported increased strength and were observed to have increase muscle fiber thickness. Cr research was sparse until the 1990s. In 1992, Harris et al. (52) and Greenhaff et al. (45), showed the promising effects of Cr supplementation using double-blind, placebo-controlled investigation. They showed that Cr supplementation increased total creatine (TCr) concentration in the muscle, which, in turn, could be responsible for increasing muscular performance. Interestingly enough that same year Olympic Gold medalists Lindford Christie and Sally Gunnell, both sprint athletes, mentioned using Cr supplementation during their training for the Olympics (104).

Cr (2-[Carbamimidoyl(methyl)amino]acetic acid) is a nitrogenous amine and a natural constituent of meats, that humans can endogenously synthesize from three amino acids – glycine, arginine, and methionine (126). The biological synthesis of Cr follows a two-step process that involves arginine:glycine amidinotransferase (AGAT) and S-adenosyl-methionine:N-guanidinoacetate methyltransferase (GAMT). The first step in Cr synthesis is catalyzed by AGAT and involves the production of guanidoacetic acid and ornithine from glycine and arginine (127, 130). This action mainly takes place in the kidneys, but has also noted in the pancreas. In the second step guanidoacetic acid is then transferred to the liver where it is methylated by methionine to yield Cr via the action of GAMT (130). Cr is then transported via circulation and stored, primarily in the skeletal muscle.

The human body has many different storage sites for Cr, although the primary storage site is skeletal muscle. Approximately 95% of TCr is stored in skeletal muscle (8). Elevated Cr concentrations are also found in the heart and eyes (130). The brain, endothelial cells, and macrophages are known to contain intermediate concentrations of Cr, while low concentrations are found in the lungs, spleen, kidney, liver, blood cells, and plasma (130). On average, a 70-kg person stores approximately 120 g of TCr in their body (122). Some researchers have reported a maximum TCr content of approximately 160 g (44). Free creatine (FCr) and phosphocreatine (PCr) (the phosphorylated form of creatine) are the two components that make up TCr. Approximately $60 - 70\%$ of Cr is found as PCr while the other $30 - 40\%$ remains as FCr (127).

Although humans can synthesis Cr, dietary sources such as animal products can provide significant amounts of Cr. Meats and fish are the primary sources of dietary Cr, with raw meat containing the greatest concentration of Cr (3.93 g/kg raw meat) (51). However, there is evidence to suggest that the cooking process results in the degradation of Cr within the meat (51). Those following a vegetarian diet have lower Cr concentrations in the muscle when compared to those consuming an omnivorous diet (106). Humans typically endogenously synthesize approximately 1 g of Cr per day, while a common omnivorous diet can provide an additional 1 g of Cr (122, 130). The Cr pool turn-over rate is approximately 2 g/d and is lost as creatinine (122). Creatinine is the end product of Cr metabolism via a spontaneous, nonenzymatic process. (129). Creatinine, once formed, enters the circulatory system, and is eliminated from the body through the urine (96).

12

Cr is a high-energy buffer that is a constituent of the phosphagen energy-system, which is one of three energy systems that yields adenosine triphosphate (ATP) (39). The phosphagen system yields ATP during short, explosive movements lasting up to 30-seconds (126). This energy system utilizes one catalytic enzyme, creatine kinase. In this reversible reaction, creatine kinase can convert adenosine diphosphate (ADP) and PCr to Cr and ATP. ATP is vital for muscle movement and without it muscle contraction could not occur. The glycolytic and oxidative phosphorylation systems are energy systems that also yield ATP.

Creatine Supplementation Protocol

Various supplementation protocols have been undertaken over the years to optimize TCr stores. Harris et al. (52), were the first to demonstrate that 20 g CrM/d increased muscle Cr of the quadriceps femoris muscles. Generally, Cr supplementation occurs in two phases − the loading phase, followed by a maintenance phase. The initial phase of Cr supplementation is referred to as the loading phase, which consists of ingesting 20 − 30 g Cr for 5 − 7 d (19, 37, 38, 49, 57, 83, 108, 119, 124). More recent recommendations suggest ingesting Cr at 0.3 g/kg body weight/d for at least 3 d (19). The loading phase is typically divided into four equal doses distributed evenly throughout the day (100). The maintenance phase typically consists of ingesting a lesser amount of Cr. It is recommended to ingest 3 − 5 g of Cr supplementation to maintain elevated TCr stores (19). Alternative dosing strategies recommend ingesting 0.03 g of Cr/kg body weight/d during the maintenance phase. Some long-term supplementation

protocols that eliminated the loading phase of supplementation have shown increases in strength and power (94).

This supplementation protocol has been shown to increase muscle Cr stores in most, but not all studies. Increases in TCr stores by 20 – 40% have been observed in those who normally consume relatively less meat, while the increase in TCr is slightly less (10 – 20%) in those who regularly consume meats (44). Approximately 20 – 30% of subjects do not respond to Cr supplementation (42). Greenhaff et al. (44), suggests that some individuals may already have TCr concentrations near the physiological limit and thus do not respond to additional Cr supplementation.

Mechanisms of Action

Several theories explain the increased benefits in exercise performance when Cr supplementation is combined with resistance training (20, 28, 74, 82). Cr supplementation may create a more anabolic environment by influencing hormone concentrations and certain proteins vital in the protein synthesis pathway (20, 59). Cr may contain anti-catabolic properties, which can also impact net protein synthesis (93). Cr supplementation can increase the ability to perform high-intensity exercise, which can lead to greater training adaptations over time (74).

In 2005, Deldicque et al. (31), in a double-blind, cross-over study hypothesized that Cr (21 g • d^{-1} • 5 d^{-1}) supplementation would result in an anabolic environment after an acute bout of high-intensity resistance exercise. After 5 days of supplementation, participants performed 10 sets of 10 leg press repetitions at 70% of 1 repetition maximum (1RM). Percutaneous muscle biopsies of the *vastus lateralis* were collected

before exercise and 3 and 24 h post-exercise. Cr supplementation resulted in increased expression of mRNA for IGF-I and IGF-II in resting muscle. Furthermore, phosphorylated eukaryotic initiation factor-4e binding protein-1 (4E-BP1) was greater in the Cr group than in the PL group at 24 h post-exercise. There were no significant differences between treatments in IGF-I, IGF-II, and $p70^{s6k}$ post-exercise, but that may have been due to the small number of participants (n=6).

In 2008, the same group of researchers later examined the changes in gene expression after 5 d of Cr supplementation (21 g $\bullet$ d^{-1} $\bullet$ 5 d^{-1}) in combination with an acute bout of heavy resistance exercise (10 sets of 10 repetitions of one leg extension at 80% 1RM) (31). Percutaneous muscle biospies of the *vastus lateralis* were collected before, immediately after and 24 h and 72 h after exercise. Cr supplementation increased collagen 1 (α_1) mRNA by 250% and myosin heavy chain (MHC) mRNA I at rest by 80%, while MHCII mRNA increased by 70% immediately after exercise. The increased mRNA expression of collagen 1 (α_1), MHCI, and MHCII may provide a more anabolic environment for skeletal muscle mass accretion.

A different study compared changes in muscle IGF-I after combining Cr supplementation (0.25 g $\bullet$ kg lean body mass $\bullet$ d^{-1} $\bullet$ 7 d^{-1}[*loading phase*]; 0.06 g $\bullet$ kg lean body mass $\bullet$ d^{-1} $\bullet$ 49 d^{-1}[*maintenance phase*]) with an 8-week resistance training program (20). Results showed the Cr group had greater concentrations of IGF-1 (78%) when compared to PL group (55%), although the increase was not significantly different (p =0.06). IGF-I has been of interest as it has been shown to increase protein synthesis and

stimulate certain proteins important in the mechanistic target of rapamycin (mTOR) pathway, which plays a pivotal role in protein synthesis (2, 31, 53).

Cr supplementation has been reported to have anti-catabolic properties. Parise et al. (93), examined the effects of CrM supplementation on protein metabolism in young men and women. In a double-blind, placebo-controlled study, participants were randomly assigned to ingest CrM (20 g • d^{-1} • 5 d^{-1}; 5 g • d^{-1} • 3 – 4 d^{-1}) or PL (glucose polymer). Stable isotope leucine infusion was used to measure protein metabolism before and after supplementation. Acute CrM supplementation decreased the rate of leucine oxidation as well as the rate of leucine appearance in plasma only in men. It was not clear why Cr supplementation had anti-catabolic effects in men, but not in women.

The available research suggests that Cr supplementation facilitates higher intensity exercise performance, which can then lead to greater training adaptations over time (74). It is important to note that very little ATP is stored in skeletal muscle (100). Stored ATP typically supports a few seconds activity and therefore must be continuously replenished by the different energy systems. Under aerobic conditions or low-intensity exercise, glycolysis and oxidative phosphorylation, from the breakdown of glycogen, blood sugar, and triglycerides are upregulated to resynthesize ATP. During maximal anaerobic conditions or high intensity exercise lasting less than 10 sec, intramuscular PCr provides high energy phosphates for ATP resynthesis (8, 100, 106, 120). PCr serves as an energy reservoir ready to yield ATP when immediate energy is needed to support human movement. In a reversible reaction catalyzed by creatine kinase, PCr combines with adenosine diphosphate (ADP) to yield ATP and Cr. Some suggest that fatigue

from short-term, high intensity exercise results from the inability to sustain ATP resynthesis (23). Theoretically, Cr supplementation can be used to maximize TCr concentration in the skeletal muscle facilitating higher intensity work. In turn, total work and power output may be sustained longer as the increased TCr allows for greater ATP resynthesis needed to meet the demands of high intensity exercise. In other words, Cr supplementation can result in a greater pool of PCr, which can then yield greater amounts of ATP resynthesized.

Muscular Strength

It appears that the earliest Cr-related research involving muscular performance dates back to the early 1930s. In 1934, Boothby (24) reported that a glycine-rich diet could delay the onset of muscular fatigue. In 1939, Ray and colleagues (101) hypothesized that gelatin, a glycine-rich product, supplementation would results in greater TCr concentrations in the muscle, which would in turn improve exercise performance. In this study, men were observed to have reduced muscular fatigue after a glycine-rich diet. In 1940, Chaikelis (24) examined the effects of glycocoll (glycine) on muscular strength in a single-blind, placebo-controlled study. Results indicated that 10-weeks of glycocoll supplementation increased total strength measures. Later that century in 1981, Sipila et al. (107), provided Cr supplementation (1.5 g Cr • d^{-1}) for one year as a nutritional therapy for gyrate atrophy. Participants reported increases in strength and one participant beat his personal record in the 100-meter sprint by 2 sec.

Improvements in strengths have been observed after long-term Cr supplementation. Vandenburghe et al. (117), examined the effects of Cr supplementation

on muscle strength in young women. After being matched by strength and body mass, participants followed a supervised, resistance training program for 10 wk while ingesting Cr (20 g Cr • d^{-1} • 4 d^{-1}; 5 g Cr • d^{-1} • 10-wk^{-1}) supplementation or PL (maltodextrin). 10 wk of detraining followed the supplementation and resistance training period. Cr supplementation promoted 20 − 25% greater strength gains in 1RM of leg press, leg extension, and squats. It was also determined that strength gains remained elevated in the Cr group than the PL following the 10 wk detraining period. In an investigation of similar length, Pearson and colleagues (94) examined the effects of Cr supplementation on collegiate football players. In double-blind fashion, participants received Cr (5 g Cr • d^{-1} • 10 wk^{-1}) supplementation or PL during a 10-week resistance training program (94). Strength increased in bench press, squats, and power clean by approximately 3%, 12%, and 6%, respectively. The increase in strength gains were attributed to increases in TCr concentrations.

Longer investigations have also reported improved strength performance after Cr supplementation. Volek et al. (120), observed significantly increased bench press and squat 1RM after 12-weeks of Cr (25 g Cr • d^{-1} • 7 d^{-1}; 5 g Cr • d^{-1} • 11 wk^{-1}) and heavy resistance training in young, resistance-trained males. In a different study Volek et al. (120), observed significant increases in bench press (24%) and squat (32%) performance with Cr supplementation in conjunction with 12 wk of heavy resistance training. The resistance-trained participant were matched according to strength and anthropometric measures and in a double-blind manner, were randomly assigned to a Cr (25 g Cr • d^{-1} • 7 d^{-1}; 5 g Cr • d^{-1} • 11 wk^{-1}) group of PL (powdered cellulose). Becque et

al. (10), observed an ~12% increase in arm flexor 1RM after Cr supplementation (20 g Cr • d^{-1} • 5 d^{-1}; 2 g Cr • d^{-1} • 6 wk^{-1}) during a 6-week resistance training program. The greater strength gains associated with Cr supplementation were attributed to an increase in TCr, which in turn could lead to greater ATP resynthesis during exercise training.

Improvements in strength have also been observed with shorter periods of supplementation. Brenner et al. (17), reported that creatine supplementation (20 g Cr • d^{-1} • 7 d^{-1}; 2 g Cr • d^{-1} • 28 d^{-1}) significantly improved 1RM bench press strength (17%) gain in 16 female college lacrosse players during pre-season training. Earnest et al. (34), examined the effects Cr supplementation on strength indices in experienced weight-trained males. In a double-blind manner participants received Cr (20 g Cr • d^{-1} • 14 d^{-1}) or PL (glucose). The Cr group significantly increased bench press 1RM by 6% and bench press repetition (70% of 1RM) by 35%. These increases were not observed in the PL group.

Muscular Power and Work

Muscular power is important in many sports, especially in tasks such as striking, throwing, and jumping. Cr supplementation has also been observed to increase muscular power in conjunction with resistance training. It is unclear why some researchers have observed increases in muscular power after Cr supplementation, while others have not (26, 113).

Cr supplementation has been observed to improve upper body power output. In 2004, Volek et al. (121), studied the effects of Cr supplementation on muscular performance in young, resistance-trained males. In a double-blind, randomized study

using Cr (0.3 g Cr • kg body mass^{-1} • d^{-1} • 7 d^{-1}; 0.05 g Cr • kg body mass^{-1} • d^{-1} • 21 d^{-1}) supplementation or PL during 4 wk of resistance training. Power measures included ballistic bench press throws and jump squat exercises. Bench press throws were significantly greater in the Cr group, but not the PL group. Izquierdo et al. (60), also observed increase power output (~20%) during bench press performance after 5 d of Cr (20 g Cr • d^{-1} • 5 d^{-1}) supplementation. No changes in exercise performance were observed with PL supplementation. However, some researchers have failed to notice differences in upper body power outputs during arm-Wingate tests following short-term Cr (20 g Cr • d^{-1} • 5 d^{-1}) supplementation (41).

Chronic high-intensity exercise can result in greater exercise performance over time; some researchers have observed increases in power output after relatively short supplementation periods of Cr supplementation. Tarnopolsky et al. (113), reported increased relative (W/kg) and absolute (W) peak power on a cycle ergometer after four days of CrM (20 g Cr • d^{-1} • 4 d^{-1}) in young, recreationally active men and women. In this double-blind, cross-over study participants consumed CrM or PL (glucose) and performed an anaerobic cycling test consisting of two, 30 sec maximal cycling bouts separated by a 4 min rest period. After a similarly short supplementation period, Dawson et al. (30), noticed a significant increase in peak power on a cycle ergometer in the Cr group when compared to PL. Participants received Cr (20 g Cr • d^{-1} • 5 d^{-1}) and performed repeated sprints (6 x 6 sec sprints, with 30 sec recovery) one and three days after Cr supplementation. Cr group performed significantly greater total work (kJ) during the repeated sprints. Similarly, Wiroth and coworkers (128) observed a

significant increase maximal power and total work in sedentary young and elderly men when performing five all-out 10 sec sprints on a cycle ergometer after supplementation following Cr (15 g Cr • d^{-1} • 5 d^{-1}) supplementation.

Improvements in muscular power have also been observed in elite level athletes. After 42 d of Cr (0.3g Cr • kg^{-1} • d^{-1} • 42 d^{-1}) supplementation, Kirksey and colleagues (71) reported greater gains in vertical jump and power output during sprints on a cycle ergometer in Division IAA male and female track and field athletes. Similarly, Jones et al. (65), reported that Cr (20 g Cr • d^{-1} • 5 d^{-1}; 5 g Cr • kg^{-1} • d^{-1} • 10 wk^{-1}) supplementation improved sprinting performance during a cycle ergometer test (5, 15 sec sprints with 15 sec recovery) and a sport-specific ice skating task (6 x 80-m sprints) in 16 elite ice-hockey players. Bemben et al. (12), investigated the effects of Cr supplementation in conjunction with resistance training on body composition in NCAA division I football athletes. Participants received Cr (20 g Cr • d^{-1} • 5 d^{-1}; 5 g Cr • d^{-1} • 58 d^{-1}), PL (sodium phosphate monohydrate), or no supplementation (control group) during preseason training. Peak power and total work on the Wingate test performance increased by approximately 20% with Cr supplementation, whereas no significant differences were observed in the PL and control group.

Numerous studies have reported on the performance-enhancing ability of Cr using explosive, high intensity exercise. Balsom et al. (6), assigned participants to ingest Cr (25 g Cr • d^{-1} • 6 d^{-1} + 6 g glucose) or PL (glucose only) before a high intensity cycle ergometer protocol consisting of ten, 6 sec bouts (30 sec rest between bouts). With Cr supplementation, the difference in sprint performance were noticeable after the forth

21

sprint bout, but were only significantly different after the seventh sprint bout. In a later study, the same researchers recruited recreationally active males to perform repeated bouts of high intensity exercise on a cycle ergometer before and after Cr (25 g Cr • d^{-1} • 6 d^{-1}) supplementation (7). The sprint protocol consisted of five, 6 sec sprints (30-sec rest between bouts), followed by a 40 sec rest period, then one, 10 sec sprint occurred. Following supplementation there were no differences in cycling performance during the five, 6 sec sprint; however, the Cr group showed a significantly greater power output during the 10 sec sprint bout. The authors conclude that the ability to maintain a greater total power output during the entire cycle ergometer protocol was a direct result of significantly greater PCr stores.

Increases in anaerobic performance in aerobically-trained athletes have also been reported. Engelhardt et al. (35), examined triathletes aerobic and anaerobic performance on an incremental cycle ergometer test interspersed with high intensity sprints following Cr (6 g Cr • d^{-1} • 5 d^{-1}) supplementation. Aerobic performances during the two, 30 min cycling bouts were not affected by Cr supplementation; however, an increase in power output was observed during the interval sprints. In a similar study, Vandebuerie et al.(116), also examined the effects of Cr (25 g Cr • d^{-1} • 5 d^{-1}) on sprinting performance and endurance capacity in elite cyclist. Participants performed a cycling session for 150 minutes at their lactate threshold, followed by 5, 10 sec sprints (2 min recovery between sprints). Sprint performance in Cr group significantly increased peak and mean power output by ~9% for all sprints.

Even while following similar supplementation protocols, several studies have reported no increases in anaerobic performance. Odland et al. (91), studied the effect of Cr supplementation on power output during one 30 sec Wingate test. In a crossover design, participants were randomly assigned to Cr (20 g Cr • d^{-1} • 3 d^{-1}), control (no beverage), and PL (beverage only). There were no power output differences during the Wingate test between any of the three groups.

In a different study, Casey et al. (22), recruited nine males subjects to perform two bouts of 30 sec sprints on a cycle ergometer before and after Cr (20 g Cr • d^{-1} • 5 d^{-1}) supplementation. There were no significant differences in peak work (J/kg) before and after Cr supplementation. Earnest et al. (34), also observed no significant differences in peak power during three Wingate tests following Cr (20 g Cr • d^{-1} • 5 d^{-1}) supplementation. Using a greater amount of Cr (30 g Cr • d^{-1} • 5 d^{-1}) supplementation, Snow and colleagues (108) evaluated repeated sprint performance on a cycle ergometer. They also reported no differences in sprinting performance. Interestingly, Earnest et al. (34), and Casey et al. (22), both reported significant increases in total work following Cr supplementation.

Other researchers have used different testing modalities to observe changes in lower body power following Cr supplementation. Claudino et al. (26), examined the effect of Cr supplementation on muscular power in professional Brazilian elite soccer player. In a double-blind, placebo-controlled study, participants received CrM (20 g Cr • d^{-1} • 7 d^{-1}; 5 g Cr • d^{-1} • 6 wk^{-1}) or PL (dextrose) during pre-season training. Lower limb power output was determined by observing ground reaction forces during counter-

movement jumps. Jumping performance was slightly lower in PL group, but the difference between groups was not significantly different.

Body Composition

Increases in total body mass and lean body mass have been reported following Cr supplementation. In 1940, Chaikelis (24) reported increases in body mass after participants supplemented with the Cr precursor, glycine. Many athlete and recreationally active individuals desire to improve the lean mass-to-fat mass ratio. Increasing lean mass has the potential to improve sports performance. It is well known that the muscular strength and power has the potential to predict success in combat sports such as Judo, wrestling, and boxing.

Changes in body composition following Cr supplementation have also been reported in National Collegiate Athlete Association (NCAA) football athletes. Kreider et al. (75), examined the effects of Cr supplementation on body composition in NCAA division IA football players during off-season training. In a double-blind study, participants were matched according to total body weight and assigned to ingest Cr (15.75 g CrM $\bullet$ d^{-1} $\bullet$ 28 d^{-1}) or PL (glucose + taurine + disodium phosphate + potassium phosphate) for 28 d. It is important to note that the Cr formula contained the same ingredients found in PL supplement. Total body mass significantly increased in both groups, but total body mass and fat-free mass was significantly greater in Cr group following 28 d of supplementation.

Using a similar cohort, Bemben et al. (12), investigated the effects of Cr supplementation in conjunction with resistance training on body composition in NCAA

24

division I football athletes. In a double-blind, placebo-controlled study, participants received Cr (20 g Cr • d^{-1} • 5 d^{-1}; 5 g Cr • d^{-1} • 58 d^{-1}), PL (sodium phosphate monohydrate), or no supplementation (control group) during preseason training (12). Total body mass and lean body mass (hydrostatic weighing) were significantly increased by approximately 4% in the Cr group, but not in the PL or control group. Furthermore, total body water was significantly increased (5%) in the Cr group.

Different investigations by Volek and colleagues (120) have also reported body composition changes following Cr supplementation. Resistance-trained men were matched according to physical characteristics and baseline strength parameters before commencing Cr (25 g Cr • d^{-1} • 7 d^{-1}; 5 g Cr • d^{-1} • 11 wk^{-1}) supplementation and a 12-week periodized, heavy resistance exercise program. Total body mass was significantly greater in Cr group when compared to PL after one-week (1.7 kg) and 12-weeks (5.2 kg) of supplementation. Lean body mass was also significantly greater at wk 1 (1.5 kg) and wk 12 (4.3 kg) in Cr group when compared to PL. A few years later, Volek et al. (121), examined body composition (DXA) following a shorter supplementation period. Young, resistance-trained men received Cr (0.3 g Cr • kg body $mass^{-1}$ • d^{-1} • 7 d^{-1}; 0.05 g Cr • kg body $mass^{-1}$ • d^{-1} • 21 d^{-1}) supplementation or PL during a 4 wk resistance training period. The Cr group, when compared to PL, experienced a significant increase in body mass (2.5 kg) and lean body mass (LBM) of the legs (1.6 kg). Becque and coworkers (11) reported less dramatic changes in body composition examined after 6 wk of Cr (20 g Cr • d^{-1} • 5 d^{-1}; 5 g Cr • d^{-1} • 37 d^{-1}) supplementation. They observed a significant

increase in total body mass (2 kg) and lean body mass (1.6 kg) following supplementation.

Aerobic Performance

The majority of the literature suggests Cr supplementation is mainly beneficial during anaerobic exercise. The research on Cr supplementation and aerobic performance is less voluminous. Creatine supplementation does not seem to be as beneficial to endurance activity as it is to anaerobic exercise.

Some researchers postulated that increased PCr concentrations at the end of an endurance event could theoretically benefit an athlete performing a final sprint to the finish line (54). In a study by Hickner et al. (54), endurance-trained cyclist performed a two hour session on a cycle ergometer before and after receiving Cr (3 g Cr $\cdot$ d^{-1} $\cdot$ 28 d^{-1}) supplementation or PL. The two hour cycling session consisted of lower intensity cycling (60 – 65% VO$_{2peak}$) interspersed with high intensity sprints (110% VO$_{2peak}$). Sprinting time performance and power output was not significantly influenced by Cr supplementation, although Cr group was found to have significantly greater TCr and PCr concentration prior to the cycling bout. Following similar expectations Engelhardt et al. (35), examined triathletes aerobic performance on an incremental cycle ergometer test interspersed with high intensity sprints following Cr (6 g Cr $\cdot$ d^{-1} $\cdot$ 5 d^{-1}) supplementation. The results indicated that aerobic performance during the two, 30 min cycling bouts were not affected by Cr supplementation.

In a similar study using greater amounts of Cr, Vandebuerie et al. (116), examined the effects of Cr (25 g Cr $\cdot$ d^{-1} $\cdot$ 5 d^{-1}) on endurance capacity in elite cyclist.

26

Participants performed a cycling session for 150 min at their lactate threshold, followed by five, 10 sec sprints (2 min recovery between sprints). Endurance performance was not influenced by Cr supplementation; however, Cr supplementation increased sprinting performance (peak and mean power) in all five sprints.

Theoretically, Cr supplementation may benefit endurance performance via its action on muscle glycogen stores. Nelson et al. (90), observed an significant increase in muscle glycogen content when Cr loading preceded carbohydrate loading. Young men carbohydrate loaded (6.6 g of carbohydrate/kg) for 3 d, consumed their normal mixed diet with Cr supplementation (20 g CrM/d) for 7 d, followed by a second carbohydrate loading protocol for three days. The researchers concluded that the Cr-induced increase in muscle cell volume allowed for greater glycogen storage. Furthermore, Cr supplementation may improve recovery after muscle damage. Cooke et al. (27), reported significantly greater isokinetic and isometric knee extension strength after exercise-induced muscle damage. Participants ingested 0.3 g/kg/d of CrM for 5 d (loading dose) and 0.1 g/kg/d for 14 d (maintenance dose). After loading period, participants performed an exercise regimen meant to induce muscle damage which consisted of 4 x 10 eccentric-only repetitions at 120% of their 1RM on leg press, leg extension, and leg flexion exercise machine. They observed significantly lower (~84%) plasma creatine kinase concentration 48, 72, 96 h, and 7 d after exercise.

Different Forms of Creatine

CrM is the most researched and effective form of Cr supplementation (19). CrM first hit consumers in the early 1990s. Since then, novel forms of Cr have appear in the

marketplace with claims of being more efficacious than CrM. Novel forms of Cr have appeared in salt-form such as creatine pyruvate, creatine citrate, creatine malate, creatine phosphate, magnesium creatine, creatine orotate, and Kre-Alkalyn® (1, 28, 86, 97, 98). Ester forms of creatine (creatine ethyl ester, creatine gluconate) have also are available. The claims of the aforementioned forms of Cr vary greatly. Some claim greater solubility in solution and bioavailability, although the bioavailability of CrM is close to 100% (61). Other manufacturers claim greater shelf life stability although CrM shows little quality degradation over several years (62). Furthermore, others claim improved efficacy on exercise performance (28, 61).

Kre-Alkalyn®, a buffered form of Cr claimed to "enhance the delivery of usable creatine to the person taking the supplement, and overcomes the problem caused when creatine is converted to creatinine. The higher the pH, the more creatine a human will ingest" (40). In a recent study, Jagim et al. (63), compared the effects of CrM and Kre-Alkalyn® on muscle Cr content, body composition, and adaptations to training. In a double-blind manner, participants were randomly assigned to ingest CrM (20 g Cr • d^{-1} • 7 d^{-1}; 5 g Cr • d^{-1} • 21 d^{-1}), a recommended dose of Kre-Alkalyn® (KA-L; 1.5 g Cr • d^{-1} • 28 d^{-1}), or Kre-Alkalyn® with dose equivalent to CrM group (20 g Cr • d^{-1} • 7 d^{-1}; 5 g Cr • d^{-1} • 21 d^{-1}). Results indicated that FCr content in muscle significantly increase in all group; however, there were greater changes in muscle Cr content with CrM supplementation. Furthermore, it was determined that Kre-Alkalyn® did not promote greater changes in body composition, strength, or anaerobic performance than CrM supplementation.

Effervescent creatine citrate claims to have more optimal properties of digestion and absorption, thereby allowing greater creatine retention in the body. Greenwood et al. (46), examined whole body Cr retention after comparing several forms of Cr supplementation, including effervescent creatine citrate. No differences were observed in whole body Cr retention between CrM and effervescent creatine citrate; however, the group receiving CrM + dextrose was shown to have significantly greater whole body retention of Cr.

Manufacturers have also attempted to suspend Cr in solution with claims of greater transport to the muscle. To test this claim Kreider et al.(73), compared the influence of liquid ATP Advantage™ Creatine Serum (CS) and CrM on muscle ATP, FCr, PCr, and TCr concentration after 5 d of supplementation. In a randomized and double-blind manner, participants were assigned to ingest 5 mL CS (2.5 g of CrM equivalent), 5 mL PL, 40 mL CS (20 g of CrM equivalent), 40 mL PL, or 20 g of CrM (control). Results indicated that the recommended dose of CS as well as the higher dose of CS (8 times greater than recommended dose) is not effective at increasing FCr or PCr concentrations. Furthermore, FCr concentration significantly increased only with CrM supplementation. According to this study, findings did not support the claims purported by the liquid creatine product.

Some researchers have noticed that salt-forms of Cr dissolve more easily in solution than CrM, and therefore there may be a basis for greater uptake when Cr is in its salt-form. Jager et al. (61), examined various forms of Cr to determine uptake kinetics. Participants, in a balanced cross-over study, ingested isomolar amounts of Cr as creatine

monohydrate (CrM; 5 g), tri-creatine citrate (CrC; 6.7 g), or creatine pyruvate (CrPyr; 7.3 g). Plasma Cr concentrations were significantly greater for CrPyr 1 h after ingestion. Mean peak concentrations were found to be significantly greater in CrPyr when compared to CrC, but CrPyr was not greater than CrM. The findings may suggest that different forms of Cr have slightly different absorption kinetic. Also, it is unclear if the kinetics of Cr absorption would influence muscle Cr content and exercise performance as neither were performed in this study.

Creatine ethyl ester (CEE) has also been purported to increase the bioavailability of Cr. In 2009, Spillane et al. (109), examined the effects of CEE supplementation during a standardized, resistance training program. Participants ingested CrM (0.3 g Cr • kg^{-1} • d^{-1} • 5 d^{-1}; 0.075 g Cr • d^{-1} • d^{-1}• 42 d^{-1}), CEE (0.3 g Cr • kg^{-1} • d^{-1} • 5 d^{-1}; 0.075 g Cr • d^{-1} • d^{-1}• 42 d^{-1}), or PL for 7 wk. Plasma Cr concentrations were not significantly greater with CEE when compared to CrM or PL. Although muscle Cr content increased with CrM and CEE, there was no significant difference between the two groups. Researchers conclude that CEE was not as effective at increase plasma and muscle Cr content when compared to CrM. Furthermore, CEE did not seem to improve body composition or muscular strength and power when compared to CrM supplementation.

Creatine phosphate (CrP) supplementation appears to have some beneficial effects. Peeters et al. (95), examined different forms of Cr on strength, body composition, and blood pressure. Resistance-trained participants were matched according to strength measured and were then were assigned to ingest CrM (20 g Cr • d^{-1} • 3 d^{-1}; 10 g Cr • d^{-1} • 39 d^{-1}), CrP (20 g Cr • d^{-1} • 3 d^{-1}; 10 g Cr • d^{-1} • 39 d^{-1}), or PL. The

findings indicated similar increases in 1RM bench press strength, total body mass, and lean body in CrM and CrP groups. The increases in 1RM bench press strength, total body mass, and lean body mass were greater in CrM when compared to CrP, but the differences were not significant. Although CrP supplementation resulted in comparable gains in this study others have reported that CrP is more expensive to produce, thus making CrM supplementation a more economical choice (19).

Creatine nitrate (CrN) is a novel form of Cr that has gained some recent attention. A recent study examined the safety profile of CrN (66). Participants were randomly assigned to consume either 1 g or 2 g of CrN/d for 28 d. A complete blood and chemistry panel was determined on blood samples collected before and after supplementation. Joy et al. (66), reported that all hematological safety markers were within normal physiological range following supplementation and therefore, concluded CrN supplementation to be safe to consume. Figure 1 shows the chemical structure of CrM, while Figure 2 shows the chemical structure of CrN.
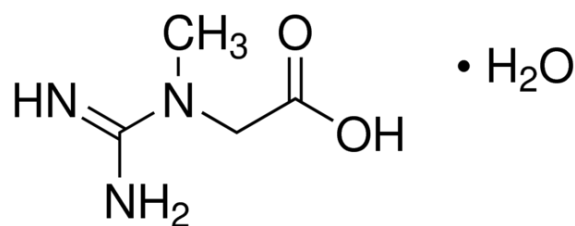
Figure 1. Chemical Structure of Creatine Monohydrate
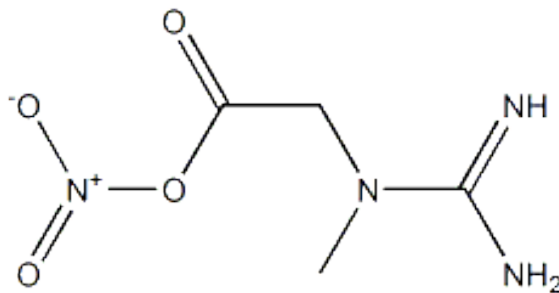
Figure 2. Chemical Structure of Creatine Nitrate

Nitrates and Nitrites

Inorganic nitrate (NO_3^-) is an ion exhibiting limited synthesis in the body, therefore usually obtained from the diet via green leafy vegetables, while nitrites (NO_2^-) are also found in food including as processing additives, but to a much lesser degree. Dietary sources of nitrates can get metabolized to nitrites. In turn, nitrates and nitrites can serve as precursors to the bioactive compound nitric oxide (NO). Dietary nitrate and nitrites are the main precursor to NO via the nitric oxide-independent pathway, which was discovered in 1990 (13, 84). NO is well known for its vasodilatory effect and thus its influence on blood pressure (14). The ergogenic aid potential of nitrate supplementation lies in its ability to modulate muscle force production, blood flow, and mitochondrial respiration (110). Many investigators have relied on sodium nitrate and beet root juice (BRJ) to supply dietary sources of nitrates.

One of the first to investigate nitrate supplementation and its effect on blood pressure (BP) was Larsen and colleagues (79). In a randomized, double-blind, crossover

study, Larsen et al., examined the effects of sodium nitrate (0.1 mmol • kg^{-1} • d^{-1}) and PL (sodium chloride) on BP in young, healthy volunteers. Researchers found that nitrate supplementation did not affect heart rate (HR) or systolic BP (SBP). On the other hand, diastolic BP (DBP) and mean arterial pressure were significantly reduced by 3.7 mm Hg and 3.2 mm Hg, respectively. Webb et al. (123), also measured BP following nitrate supplementation. In an open label, crossover study, participants ingested 0.5 L of BRJ (1,395 mg nitrate) or water. Serial sampling of BP was taken over the course of 6 h and again at 24 h post supplementation. BRJ supplementation resulted in significant increase in plasma nitrate concentrations after 30 min and remained elevated for 6 h (peaked at 1.5 h). Plasma nitrite concentrations were found to be significantly elevated at 3 h and 4 h post-supplementation. Significant reductions in SBP were observed at 2.5 h (10.4 mm Hg), while DBP was significantly reduced at 3 h (8.1 mm Hg) post-supplementation. BRJ supplementation was found to have no impact on HR. However, not all investigators have reported the same results. Murphy and coworkers (89) administered whole beetroot (>500 mg nitrate) to men and women and observed no changes in HR or BP 60 min post-administration.

Changes in endurance performance have been reported in moderately trained individuals (78, 81). Larsen et al. (78), reported reduced oxygen consumption and improved mechanical efficiency during work rates intensities of $40 - 80\%$ of VO_{2peak} with sodium nitrate (0.1 mmol • kg^{-1} • d^{-1} • 3 d^{-1}). In a different study, Larsen and colleagues (81) reported significantly reduced oxygen consumption at VO_{2peak} after nitrate supplementation equivalent to the amount found in 100-300 g of nitrate-rich food.

Participants performed incremental exercise to volitional fatigue with arm and leg cranking ergometers on two different ergometers after supplementation. It was concluded that the increase NO production, via nitrate supplementation, is modulating mitochondrial respiration and therefore directly influencing endurance exercise. Improvements in mitochondrial respiration were determined as the ratio of oxygen consumed per ATP produced.

Sodium nitrate supplementation was also used in conjunction with endurance performance in highly trained individuals. Bescos et al. (15), investigated the impact of highly trained cyclist and triathletes ingesting sodium nitrate (10 mg $\cdot$ kg^{-1}) before a cycle ergometer test. The exercise test consisted of a submaximal and maximal exercise portion. The submaximal exercise bouts, each lasting six minutes, corresponded to 2, 2.5, 3, and 3.5 W$\cdot$ kg^{-1} body mass (3 min recovery between bouts). A continuous incremental exercise test to exhaustion (maximal exercise) followed the submaximal exercise bout. The findings show a significant reduction in VO_{2peak} without influencing time to exhaustion or maximal power outputs.

Other researchers have used beetroot juice as a way to provide supplemental nitrates. Bailey et al. (5), have also reported changes in oxygen consumption during submaximal exercise following nitrate supplementation. In this double-blind, placebo-controlled, crossover study participants consumed 500 mL of BRJ (~695 mg nitrate) or PL (blackcurrant cordial) for 6 days. Various submaximal and maximal exercise tests occurred on the last three days of supplementation. There was a significant reduction in systolic blood pressure (-6 mm Hg), but no changes in mean arterial pressure or diastolic

blood pressure were observed. Findings suggest a reduction in muscle fractional oxygen extraction during submaximal exercise only with BRJ supplementation. Furthermore, there appears to be a reduction in oxygen consumption and a concomitant increase in time-to-exhaustion with BRJ supplementation.

Some have investigated the effects of nitrate supplementation in longer duration endurance activity. Wilkerson et al. (125), examined the effects of nitrate supplementation on time trial performance in well-trained cyclist (influence of acute dietary nitrate supplementation on 50 mile time trial performance in well-trained cyclist). Participants received a single bolus of 0.5 L of BRJ (~385 mg nitrate) or PL 2.5 hrs before a 50-mile time trial. BRJ supplementation was found to have no effect on systolic, diastolic, or mean arterial blood pressure. Time trial performance was not found to be significantly different between groups, although BRJ supplementation results in slightly reduced time to completion (BRJ: 136.7 min versus PL: 137.9 min). Similarly, mean power output during the trial was no different between groups.

Most research determining the ergogenic value of nitrate supplementation has been completed with aerobic exercise. Additionally, others have examined the effects of nitrate supplementation on aerobic exercise under hypoxic conditions. Muggeridge et al. (88), investigated the effects of a single dose of BRJ on submaximal and time trial performance in simulated altitude (~2500 meters). In a double-blind, crossover study, young, amateur cyclist performed 15 min of submaximal cycling at moderate-intensity work rate followed by a 16.1 km time trial after ingesting BRJ (310 mg nitrate) or PL

supplementation. Finding indicated a significant decrease in oxygen consumption during submaximal cycling and improved time-trial performance with BRJ supplementation.

Vanhatalo et al. (118), hypothesized that nitrate supplementation would ameliorate the negative effects of hypoxia on muscle metabolism and oxidative function. To test this hypothesis, recreationally active participants performed knee-extension exercises under normoxia and hypoxia conditions after nitrate supplementation (~575 mg nitrate) or PL. Muscle metabolism assessed via phosphorous magnetic resonance spectroscopy (^{31}P-MRS) showed greater PCr recovery with BRJ supplementation during hypoxic conditions. Under hypoxic conditions, BRJ supplementation resulted in a significant increase in tolerance during high-intensity knee-extension exercise when compared to PL. In support of these findings, Bailey et al., also reported a significant reduction (36%) in PCr degradation and a contaminant decline (21%) in inorganic phosphate (known to depress contractile function) accumulation during low- and high-intensity exercise with BRJ (~315 mg nitrate) supplementation (4).

Dietary nitrates are found primarily in plant-based foods such as fruits and vegetables (56). The dietary approach to stop hypertension (DASH) diet typically emphasizes a diet rich in fruits, vegetables, and low-fat dairy products. The DASH diet is recommended in national guidelines due to the health benefits associated with a diet rich in fruits and vegetables (25). The DASH diet recommends 4 – 5 daily servings each of fruits and vegetables. Celery, lettuce, red beetroot, and spinach are known to contain very high concentrations of nitrate (>250 mg nitrate/100 g food), while artichokes, asparagus, green beans, tomato, and watermelon are known to contain very low

concentrations of nitrate (<20 mg nitrate/100 g food) (105). Researchers have reported that the daily nitrate concentration of the DASH diet can vary from ~175 to ~1220 mg nitrate by consuming low-nitrate or high-nitrate fruits and vegetables (56). Ingesting approximately 1220 mg nitrate can be accomplished by consuming the following: 1 cup raw spinach, 0.5 cup cooked collard greens, 0.5 cup vegetable juice, 1 medium banana, 0.25 cup raisins, 1 medium orange, and 0.5 cup pomegranate juice. The World Health Organization current acceptable daily intake for dietary nitrate is $0 - 3.7$ mg nitrate/kg body weight (25). For a 70 kg individual this equates to approximately 260 mg nitrate. Some researchers have criticized the rationale limiting nitrate consumption from plant foods (56).

CHAPTER III

METHODS

Experimental Design and Approach to Problem

A longitudinal research design was employed to assess two different studies. Study 1 examined the acute hemodynamic and dose effect of ingesting two doses of creatine nitrate (CrN) compared to creatine monohydrate (CrM) and placebo (PL). Study 2 examined the effects of 28 days of two doses of CrN compared to CrM and PL on muscle creatine (Cr) content, body composition, and exercise performance in recreationally active males.

Study 1 – Acute Supplementation

Independent and Dependent Variables

Independent variables − The independent variables consisted of 1) Placebo (PL: 6.5 g dextrose), 2) Creatine Monohydrate (CrM: 5 g CrM, 1.5 g dextrose), 3) Creatine Nitrate-lower dose (CrN-L: 1.5 g CrN [1 g Cr, 0.5 g nitrate], 5 g dextrose), and 4) Creatine Nitrate-higher dose (CrN-H: 3 g CrN [2 g Cr, 1 g nitrate], 3.5 g dextrose)

Dependent variables − The dependent variables include body composition (DXA), heart rate (HR), blood pressure, blood chemistries, and side effects questionnaire.

Study Site

All laboratory testing was conducted in the Exercise & Sport Nutrition Laboratory. This laboratory is located in the Department of Health and Kinesiology at Texas A&M University in College Station, Texas.

Familiarization

Participants were required to have been actively involved in a resistance training program for at least six months consisting of upper and lower body training. Participants were required to have not had a history of treatment for metabolic disease (e.g., diabetes, hypertension, thyroid disease, arrhythmias, and/or cardiovascular disease. Furthermore, participants were required to not have currently used prescription medication, a history of smoking, or to drink excessively (i.e., 12 alcoholic drinks per week or more). Lastly, participants were required to have not had used creatine supplements in the six weeks prior to starting the study. Participants meeting entrance criteria were scheduled for familiarization session.

Prior to testing, participants were required to participate in a familiarization session in which guidelines of were explained and outlined. Participants were familiarized with the study protocol including measurements of body composition measurements, heart rate, blood pressure, and blood collection procedures. Information related to dietary records and side effect questionnaires were also explained during this session. Participants meeting entry criteria were asked to grant consent to continuation in the study by signing an informed consent form as approved by the Institutional Review Board of Texas A&M University. Once consent was granted, participants were asked to

complete a medical history questionnaire which was to be reviewed by a registered nurse. After the familiarization session, the participants were scheduled to return to the lab for four different testing sessions (i.e., testing session $1 - 4$). At each testing session the participants received one of four different supplements – 1) PL (6.5 g dextrose), 2) CrM (5 g CrM, 1.5 g dextrose), 3) CrN-L (1.5 g CrN [1 g Cr, 0.5 g nitrate], 5 g dextrose) or 4) CrN-H (3 g CrN [2 g Cr, 1 g nitrate], 3.5 g dextrose).

Participants

Thirteen male participants volunteered to participate in Study 1 (22 ± 5 y, 177.8 ± 7.4 cm, 84.1 ± 18.9 kg). The study was conducted using a double-blind, randomized, and crossover design separated by 1 wk. All testing sessions were started at the same time of day for each testing session.

Experimental Design

Participants were asked to fast for 8 h and refrain from exercise, alcohol, non-steroidal anti-inflammatory drugs, and high nitrate-containing foods 48 h prior to all testing sessions. Participants turned in their food records upon arrival to the lab. Next, participants were weighed and body composition was determined via dual energy x-ray absorptiometry (DXA) scan. Initial measures (time 0) were assessed, followed by supplementation ingestion and serial sampling (0.5, 1, 2, 3, 4, 5 h) post-supplementation. The collected measures included HR, blood pressure, and blood samples.

In a randomized, double-blind, and crossover manner, participants ingested 1) PL (6.5 g dextrose), 2) CrM (5 g CrM, 1.5 g dextrose), 3) CrN-L (1.5 g CrN [1 g Cr, 0.5 g nitrate], 5 g dextrose) or 4) CrN-H (3 g CrN [2 g Cr, 1 g nitrate], 3.5 g dextrose).

HR was determined by palpation of the radial artery using standard procedures and blood pressure was also assessed using standard procedures (115). Participants provided a fasted blood sample (8 h) via venipuncture, using intravenous (IV) catheterization (BD Insyte Autoguard, Bection, Dickinson and Company, Franklin Lakes, NJ) from the antecubital vein in the forearm according to standard phlebotomy procedures. Blood samples were collected before supplementation (time 0) and 0.5, 1, 2, 3, 4, 5 h post-supplementation.

Self-reported side effects questionnaire were completed at each time point following supplementation. Dietary intake was not controlled, but participants recorded their dietary intake prior to each testing session. Figure 3 outlines the events of Study 1. Figure 4 outlines an individual testing session within Study 1.

Study 1 – Procedures

Supplementation Protocol

Participants were assigned in a double-blind, randomized, and crossover fashion to ingest 1) PL (6.5 g dextrose), 2) CrM (5 g CrM, 1.5 g dextrose), 3) CrN-L (1.5 g CrN [1 g Cr, 0.5 g nitrate], 5 g dextrose) or 4) CrN-H (3 g CrN [2 g Cr, 1 g nitrate], 3.5 g dextrose) with at least a 7 d washout period between testing sessions. Subjects consumed one serving of the supplement mixed with approximately 8 oz of water. All supplements were supplied to the investigator in a double-blind fashion. Initial measures

Familiarization
Session
(FAM)

Testing
Session
(T1)

Testing
Session
(T2)

Testing
Session
(T3)

Testing
Session
(T4)

7d washout
between testing

7d washout
between testing

7d washout
between testing
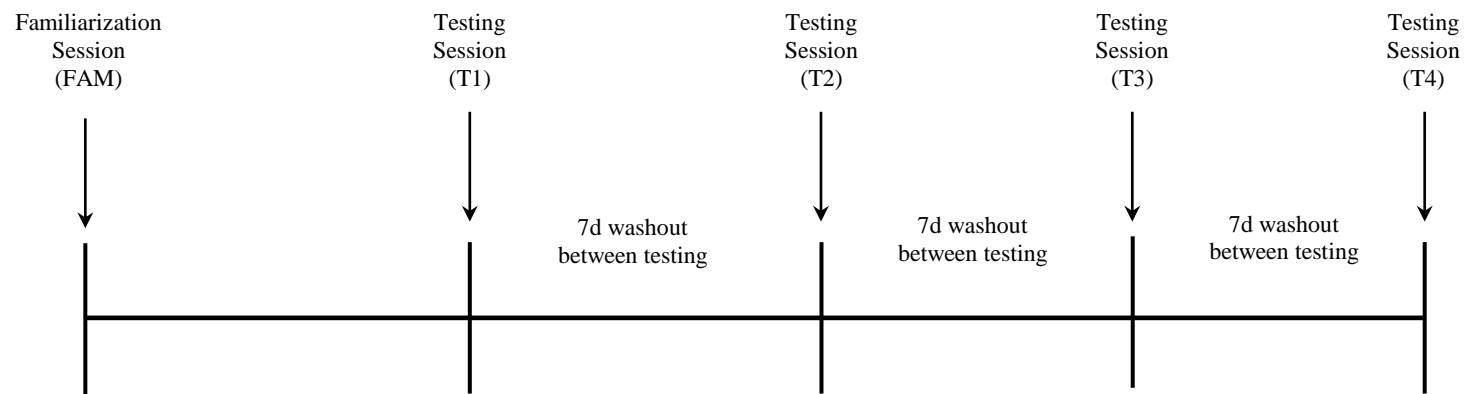
Figure 3. Study 1 Timeline

Arrive After
8 h Fast

Scheduled
Next
Testing
Session

0 h   Supplement   **0.5 h**   **1 h**   **2 h**   **3 h**   **4 h**   **5 h**

Weight &
Body
Composition
(DXA)

Blood Sample (BS),
Blood Pressure (BP),
& Heart Rate (HR)

BS, BP, HR,
&
Side Effect
Questionnaire

BS, BP, HR,
&
Side Effect
Questionnaire

BS, BP, HR,
&
Side Effect
Questionnaire

BS, BP, HR,
&
Side Effect
Questionnaire

BS, BP, HR,
&
Side Effect
Questionnaire

BS, BP, HR,
&
Side Effect
Questionnaire

Figure 4. Study 1 Testing Session Timeline

43

(time 0), followed by supplement ingestion and serial sampling (0.5, 1, 2, 3, 4, 5 h) occurred at each testing session. Certificate of analysis from Thermo-Life International can be found in appendix E.

Dietary Records and Analysis

After familiarization and prior to baseline testing, participants completed a dietary record to include 3 weekdays and 1 weekend day. Participants were asked to record all food and beverage consumption except water. Dietary recording then took place prior to each successive testing session corresponding to wk 1, 2, 3, and 4. All food logs were entered and analyzed by a registered dietitian using dietary analysis software (ESHA Food Processor Version 8.6, Salem, OR).

Body Composition Testing

Body composition testing took place at each testing session which corresponds to wk 1, 2, 3, and 4. Participants reported to the Exercise and Sport Nutrition Laboratory (ESNL) on the assigned testing session day. Prior to testing, participants were asked to fast for at least 8 h. Height and weight were recorded to the nearest 0.01 lb and 0.1 in., respectively, using a self-calibrating digital scale (Health-O-Meter, Bridgeview, IL, USA) in socks or bare feet. Body composition was then determined using dual energy e-ray absorptiometry (DXA) technology (Hologic Discovery W DXA APEX Systems software version 4.0.2, Waltham, MA). Previous studies indicate DXA to be an accurate and reliable means to assess changes in body composition (3). For determination of body composition, participants removed all metal objects that are known to interfere with measurement. Participants were then positioned in the supine position based on

44

manufacture's guidelines by a trained technician. DXA measurement was then performed; taking approximately $6 - 8$ min. Analysis was immediately performed by a trained technician to determine body composition. Test/retest reliability studies performed on male athletes with DXA yielded mean deviation for total bone mineral content and total fat-free/soft tissue mass of 0.31-0.45%, with a mean intra-class correlation of 0.985 (3).

Blood Collection

Participants abstained from exercise, alcohol, and non-steroidal anti-inflammatory drugs 48 h prior to each testing session. Participants provided a fasted blood sample (8 h) via venipuncture, using intravenous (IV) catheterization (BD Insyte Autoguard, Bection, Dickinson and Company, Franklin Lakes, NJ) from the antecubital vein in the forearm according to standard phlebotomy procedures. Approximately 20 mL of whole blood was collected at each time point (i.e., 0, 0.5, 1, 2, 3, 4, 5 h) in three, pre-chilled, 10-mL (18 mg K_2 ethylene-diaminetera-acetic acid) tubes (BD Hemogard, Franklin Lakes, New Jersey). The 10-mL EDTA tubes were pre-chilled on ice and immediately placed back on ice after each blood sampling period. Collection tubes were centrifuged at 3000 x g for 10 min at 4°C within 3 min of collection. Plasma was subsequently extracted and stored at -80°C for later analysis.

Plasma Creatine and Nitrate Assessment

Calorimetric assay kits were used to measure plasma Cr (Sigma-Aldrich, St. Louis, MO) and plasma nitrate (Cayman Chemical, Ann Arbor, MI) concentrations. Test-to-test variability of performing these assays yielded mean coefficient of variation

(C_v) values for plasma nitrate ($\pm3.21\%$) and plasma Cr ($\pm6.66\%$) with a test/retest correlation of r=0.98 and r=0.99 for plasma nitrate and Cr, respectively.

Hematologic Profile

Plasma was analyzed for alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT), creatinine, blood urea nitrogen (BUN), creatine kinase (CK), lactate dehydrogenase (LDH), glucose, and blood lipids (total cholesterol (TCHL), high density lipoprotein [HDL], low density lipoprotein [LDL], triglycerides [TG]) using a Cobas® c 111 (Roche Diagnostics, Basel, Switzerland). The Cobas® automated clinical chemistry analyzer was calibrated according to manufacturer guidelines. This analyzer has been known to be highly valid and reliable in previously published reports (64). The internal quality control for the Cobas c 111 was performed using two levels of control fluids purchased from manufacturer to calibrate acceptable SD and C_v values for all aforementioned assays. Samples were re-run if the observed values were outside control values and/or clinical norms according to standard procedures.

Heart Rate Measurement

Resting heart rate was determined at each time point (i.e., 0, 0.5, 1, 2, 3, 4, 5 h) by palpitation of the radial artery using standard procedure (115). HR measurement at time 0 was performed immediately following the DXA, in the supine position. The remaining HR measurements occurred in the seated position prior to blood sampling. Trained technicians counted the pulse for 15 sec to determine the number of beats per minute.

Blood Pressure Measurement

Blood pressure was determined at each time point (i.e., 0, 0.5, 1, 2, 3, 4, 5 h) by auscultatory method using standard procedure (115). This proceeded immediately after determining heart rate. Blood pressure measurement at time 0 was performed in the supine position. The remaining blood pressure measurements occurred in the seated position prior to blood sampling.

Side Effect Assessment

Table 1 presents the side effects questionnaire used. Participants were given questionnaires at each testing session to determine how well participants tolerated supplementation; how well participants followed the supplementation protocol; and if participants experienced any symptoms as a result of the supplement. Compliance to the supplementation protocol was monitored by supplement logs and verbal confirmation. The side effect questionnaire was completed immediately after the each of the following blood sampling time points (0.5, 1, 2, 3, 4, 5 h). Participants were asked to rank the frequency and severity of their symptoms – dizziness, headache, tachycardia, heart skipping or palpitations, shortness of breather, nervousness, blurred vision, and unusual or adverse effects. Participants were asked to rank their symptoms with 0 (none), 1 minimal: 1-2/wk), 2 (slight: 3-4/wk), 3 (occasional: 5-6/wk), 4 (frequent: 7-8/wk), or 5 (severe: 9 or more/wk). We reported frequency and severity ranked greater than or equal to 2 (slight: 3-4/wk).

Table 1: Study 1: Side Effects Questionnaire

Time Point	0.5-h	1-h	2-h	3-h	4-h	5-h
Rate the *frequency* of the following symptoms according to the scale where: 0 = none 1 = minimal (1-2 per/wk) 2 = slight (3-4 per/wk) 3 = occasional (5-6 per/wk) 4 = frequent (7-8 per/wk) 5 = severe (9 or more per/wk)						
Dizziness?						
Headache?						
Tachycardia?						
Heart skipping or palpitations?						
Shortness of breath?						
Nervousness?						
Blurred Vision?						
Any other unusual or adverse effects?						
Rate the *severity* of the following symptoms according to the scale where: 0 = none 1 = minimal 2 = slight 3 = moderate 4 = severe 5 = very severe						
Dizziness?						
Headache?						
Tachycardia?						
Heart skipping or palpitations?						
Shortness of breath?						
Nervousness?						
Blurred Vision?						
Any other unusual or adverse effects?						

Study 2 – Chronic Supplementation

Independent and Dependent Variables

Independent variables – The independent variables consisted of 1) placebo (PL: 6.5 g dextrose), 2) creatine monohydrate (CrM: 3 g CrM, 0.5 g flavoring, 2 g dextrose), 3) CrN-lower dose (1.5 g CrN [1 g Cr, 0.5 g nitrate], 0.5 g flavoring, 3.5 g dextrose), and 4) CrN-higher dose (3 g CrN [2 g Cr, 1 g nitrate], 0.5 g flavoring, 2 g dextrose).

Dependent variables – The dependent variables include muscle Cr content, body composition (DXA), blood chemistries, bench press performance, anaerobic sprint performance on a cycle ergometer, and side effects questionnaire.

Study Site

All laboratory testing was conducted in the Exercise & Sport Nutrition Laboratory. This laboratory is located in the Department of Health and Kinesiology at Texas A&M University in College Station, Texas.

Familiarization

Participants were required to have been actively involved in a resistance training program for at least six months consisting of upper and lower body training. Participants were required to have not had a history of treatment for metabolic disease (e.g., diabetes, hypertension, thyroid disease, arrhythmias, and/or cardiovascular disease. Furthermore, participants were required to not have currently used prescription medication, a history of smoking, or to drink excessively (i.e., 12 alcoholic drinks per wk or more). Lastly, participants were required to have not had used Cr supplements in the 6 wk prior to starting the study. Participants meeting entrance criteria were scheduled for familiarization session.

Prior to testing, participants were required to participate in a familiarization session in which guidelines of were explained and outlined. Participants were familiarized with the study protocol including measurements of body composition measurements, blood collection procedures, biopsies, and exercise tests measures. Participants also completed a 1 repetition maximum (1RM) bench press test and a

practice anaerobic sprint practice test. Strength tests were performed using a standard isotonic Olympic bench press according to standard procedures (68). Participants followed a standard warm-up consisting of 10 repetitions using 50% of their estimated 1RM, 5 repetitions using 70% of their estimated 1RM, and 1 repetition using 90% of their estimated 1RM. Previous research in our lab on resistance-trained participants have yielded a low day-to-day mean coefficients of variation and high reliability for the bench press (1.1%, intra-class, r=0.99). Information related to dietary records and side effect questionnaires were also explained during this session. Participants meeting entry criteria were asked to grant consent to continuation in the study by signing an informed consent form as approved by the Institutional Review Board of Texas A&M University. Once consent was granted, participants were asked to complete a medical history questionnaire which was to be reviewed by a registered nurse. After the familiarization session, the participants were scheduled to return to the lab and assigned to a treatment group. The four treatment groups were 1) PL (6.5 g dextrose), 2) CrM (3 g CrM, 0.5 g flavoring, 2 g dextrose), 3) CrN-L (1.5 g CrN [1 g Cr, 0.5 g nitrate], 0.5 g flavoring, 3.5 g dextrose), and 4) CrN-H (3 g CrN [2 g Cr, 1 g nitrate], 0.5 g flavoring, 2 g dextrose). After the familiarization session participants were scheduled for subsequent testing sessions and were asked to start the standardized resistance training program 2 wk prior to their next laboratory visit. Exercise testing occurred on d 0 and d 28.

Participants

Forty-eight, recreationally active, males volunteered to participate in Study 2 (21 ± 3 y, 176.8 ± 5.8 cm, 77.4 ± 20.9 kg). All testing sessions were started at the same time

of day for each testing session. Participants were asked to follow a standardized resistance training program two weeks before their baseline testing session (d 0) and continued throughout the remained of the supplementation period. Participants were asked not to change their dietary intake throughout the investigative period.

Experimental Design

Participants arrived to the laboratory on d -1, 0, 7, 27, and 28; a total of five laboratory visits. A percutaneous muscle biopsy of the *vastus lateralis* was obtained from the participant's right leg on testing sessions corresponding to d -1, 7, and 27 using a modified Bergstrom needle biopsy technique following standard procedures (36). Participants were asked to fast for 12 h and refrain from exercise, alcohol, and non-steroidal anti-inflammatory drugs 48 h prior to d 0, 7, and 28. Participants turned in their food records upon arrival to the lab on d 0, 7, and 28. Next, participants were weighed and body composition was determined via dual energy x-ray absorptiometry (DXA) scan. Total body water was determined next via bioelectrical impedance analysis (BIA). A fasted blood sample was collected after determining body composition. On d 7, participants completed the testing session with a muscle biopsy. On d 0 and 28, after the blood sample collection, participants continued with the exercise tests, which consisted of a bench press test and an anaerobic sprint test.

After baseline testing (d 0) participants were randomly assigned, in a double blind fashion and matched to body weight, to ingested 1) PL (6.5 g dextrose), 2) CrM (3 g CrM, 0.5 g flavoring, 2 g dextrose), 3) CrN-L (1.5 g CrN [1 g Cr, 0.5 g nitrate], 0.5 g flavoring, 3.5 g dextrose), and 4) CrN-H (3 g CrN [2 g Cr, 1 g nitrate], 0.5 g flavoring, 2

g dextrose). A side effect questionnaire was completed weekly after the first week of supplementation. Figure 5 outlines the Study 2 timeline.

Study 2 – Procedures

Supplementation Protocol

Participants were assigned in a double-blind and counter-balanced manner to ingest of 1) PL (6.5 g dextrose), 2) CrM (3 g CrM, 0.5 g flavoring, 2 g dextrose), 3) CrN-L (1.5 g CrN [1 g Cr, 0.5 g nitrate], 0.5 g flavoring, 3.5 g dextrose), and 4) CrN-H (3 g CrN [2 g Cr, 1 g nitrate], 0.5 g flavoring, 2 g dextrose). Participants were asked to ingest 4 doses per day (at approximately 0800, 1200, 1600, and 2000 h) on d 0 through 7 (loading phase). During the loading phase participants ingested a total of 1) PL (26 g dextrose/d), 2) CrM (12 g CrM+2 g flavoring+8 g dextrose/d), 3) CrN-L (6 g CrN+2 g flavoring+14 g dextrose/d), and 4 CrN-H (12 g CrN+2 g flavoring+8 g dextrose/d). Thereafter, participants ingested supplements one dose, one time per day (maintenance phase) for the remainder of the study (d 8 – 28). During the maintenance phase participants ingested a daily total of 1) PL (6.5 g dextrose), 2) CrM (3 g CrM, 0.5 g flavoring, 2 g dextrose), 3) CrN-L (1.5 g CrN [1 g Cr, 0.5 g nitrate], 0.5 g flavoring, 3.5 g dextrose), and 4) CrN-H (3 g CrN [2 g Cr, 1 g nitrate], 0.5 g flavoring, 2 g dextrose). All supplements were provided by Nutrabolt International (Bryan, TX).

Training Protocol and Analysis

All participants were required to follow the same resistance training routine. The resistance training routine consisted of exercise 4-d/wk split into two upper
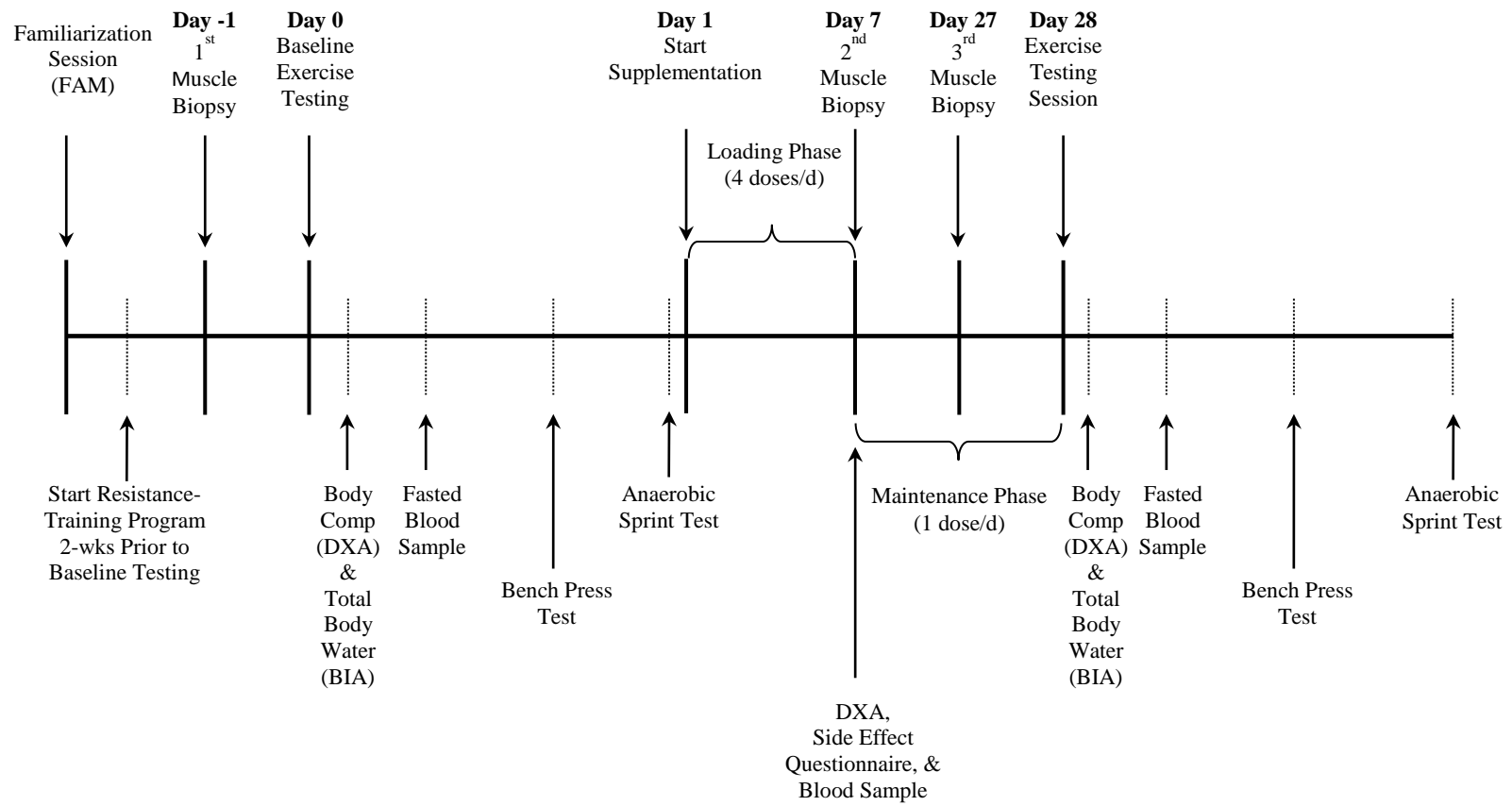
Figure 5. Study 2 Timeline

and two lower body workouts per week for a total of 6 wk. The 6 wk training protocol was periodized in 3 wk increments consisting of selected exercises for the following muscle groups: chest (two exercises for a total of six sets), back (two exercise for a total of six sets), shoulders (one exercise for a total of three sets), biceps (one exercise for a total of three sets), triceps (one exercise for a total of three sets), abdominals (one exercise for a total of three sets), quadriceps (two exercises for a total of six sets), hamstrings (two exercises for a total of six sets), and calves (one exercise for three sets). Each exercise consisted of three sets of 10 repetitions (wk 1 – 3) or 8 repetitions (wk 4 – 6) performed with as much weight as the participant could perform per set.

Training logs were completed and maintained by each participant. The participants recorded the amount of weight lifted during each set on a training log. Training sessions were monitored by a training partner or fitness instructor who signed off that the session was completed. Total lifting volume was calculated for each subject per exercise session and for the entire training program.

Dietary Records and Analysis

Participants recorded all caloric intakes from food and beverages for 4 d prior to three labs – d 0, 7, and 28. Participants were asked to record all food and beverage consumption except water. After familiarization and prior to the first performance testing session (d 0), participants completed a dietary record to include 3 weekdays and 1 weekend day. Dietary recording then took place prior to testing on d 7 and 28. All food records were entered and analyzed by a registered dietitian using dietary software

(ESHA Food Processor Version 8.6, Salem, OR). All participants were instructed to maintain their normal mixed diet throughout the training and supplementation period.

Blood Collection

Approximately 20 mL of whole blood was collected at each testing session on d 0, 7, and 28. Three, 10-mL (18 mg K_2 ethylene-diaminetera-acetic acid) tubes (BD Hemogard, Franklin Lakes, New Jersey) were pre-chilled. The 10-mL EDTA tubes were pre-chilled on ice and immediately placed back on ice after each blood sampling period. Two collection tubes were centrifuged at 3000 x g for 10 min at 4°C within 3 min of collection. One collection tube was stored at -4°C for approximately 5 h for complete blood count on whole blood. Plasma was subsequently extracted and stored at -80°C for later analysis.

Plasma Creatine and Nitrate Analysis

Calorimetric assay kits were used to measure plasma Cr (Sigma-Aldrich, St. Louis, MO) and plasma nitrate (Cayman Chemical, Ann Arbor, MI) concentrations. Test-to-test variability of performing these assays yielded mean C_v values for plasma nitrate (±4.46%) and plasma Cr (±4.95%) with a test/retest correlation of r=0.98 and r=0.99 for plasma nitrate and Cr, respectively.

Muscle Biopsies

A percutaneous muscle biopsy of the *vastus lateralis* was obtained from the participant's right leg on testing sessions corresponding to d -1, 7, and 27 using a modified Bergstrom needle biopsy technique following standard procedures (36). Muscle samples were assessed for Cr concentrations.

In the supine position, the region around the biopsy site was shaved and sterilized with 3 povidone-iodine swab sticks (Professional Disposables International, Inc, Orangeburg, NY). Lidocaine HCl (1%) was injected underneath the skin, followed by an injection through the fascia and to the epidermis using a 10 mL syringe to anesthetize the biopsy region. After approximately $5-10$ min a small incision of about 0.5 cm was made at the biopsy site using a sterile scalpel (Aspen Surgical, Caldedonia, MI). Pressure was usually applied with sterile gauze after the incision was made. A 5mm biopsy needle was inserted into the incision and into the 'belly' of the *vastus lateralis* muscle. Once the biopsy needle was pushed through the fascia and settled into the correct location suction, using 60 mL syringe, a small muscle sample was collected with the biopsy needle. The biopsy needle was in the muscle for approximately $5-20$ sec until the procedure was completed.

After the biopsy was obtained, the sample was removed from the needle by forcing air through the syringe connected to the biopsy needle. The muscle was quickly blotted on a sterile cover sponge to remove excess blood and then snap frozen into liquid nitrogen. The sample was then stored at -80°C for later analysis.

Immediately following the biopsy procedure, pressure was applied for at least 10 min to the incision site to prevent unwarranted bleeding. After bleeding was thwarted, steri-strips (3M Health Care, St. Paul, MN) were applied ensure closure of the incision. A tegaderm film (3M Health Care, St. Paul, MN) was placed over the steri-strips, followed by gauze and a self-adherent pressure bandage. Participants were provided with

a biopsy care kit including multiple steri-strips, tegaderm films, and the contact information of the study coordinator.

Biochemical Analysis for Muscle Creatine

Muscle samples were analyzed using mass spectrophotometer for muscle Cr concentrations. Samples were analyzed for Cr on methods developed by Harris and colleagues (50, 52, 57). The previously stored muscle samples were placed in a vacuum centrifuge (Jouan RC1010 SpeedVac Concentrator, Abbott Laboratories, Abbott Park, IL) and centrifuged for approximately 4 h. Following the dehydration process, the samples were powdered using a mortar and pestle and then placed into pre-weight microcentrifuge tubes. Perchloric acid (0.5 M) and 1mM ethylenediaminetetraacetic acid (EDTA) solution was used to extract the muscle metabolites. The acid solution was added to the microcentrifuge tubes containing the powered muscle. The microcentrifuge tube was placed on ice for 15 min while periodically vortexing. Samples were then centrifuged at 5,000 rpm for 5 min. The supernatant was transferred into a pre-weighed microcentrifuge tube. A 2.1 M $KHCO_3$ basic solution was used to neutralize the samples. The samples were then centrifuged a second time at 5,000 rpm for 5 min. The supernatant was removed and placed into a label microcentrifuge tube and stored at -80°C.

The samples were allowed to thaw at room temperature while periodically vortexing. Extracts were then assayed for Cr concentrations in presence of 50 mM imidazole buffer, pH 7.4; 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 individually added into 1.5 mL cuvettes. The assay was then carried out using 200 μL buffer, 100 μL potassium chloride, 25 μL NADH, 20 μL ATP, 10 μL phosphoenolpyruvate, 2 μL pyruvate kinase, 2 μL lactate dehydrogenase, 150 μL water, and 100 μL of muscle extract. Changes in absorbance were recorded with a Beckman Coulter DU 7400 Diode Array Spectrophotometer (Brea, California, USA) at a wavelength of 339 nm. 20 μL of creatine kinase (25 U/mg) was added after initial reading. The solution was read every 5 min for 20 min for post-reaction absorbance values. Test-to-test variability of performing these assays yielded mean C_v values for muscle Cr (±3.69%) with a test/retest correlation of r=0.946.

Body Composition Assessment

Upon arrival to the lab on d 0, 7, and 28, total body mass was measured on a calibrated digital scale with a precision of ± 0.02 kg (Health-O-Meter, Bridgeview, IL, USA). Next, body composition was determined (excluding cranium) with a Hologic 4500W DXA (Hologic, Bedford, MA, USA) by using procedures previously described (67, 70). A low dose of radiation is used to scan the entire body to determine body composition and bone density. The DXA scans regions of the body (limbs, trunk, and head) to determine bone mass, fat mass, and fat-free mass within each region. The values for each region are subtotaled to determine whole-body composition. Body fat percentage was determined by dividing fat mass by the total scanned mass (bone mass, fat mass, and fat-free mass). Test/retest reliability studies performed on male athletes with DXA yielded mean deviation for total bone mineral content and total fat-free/soft tissue mass of 0.31-0.45%, with a mean intra-class correlation of 0.985 (3).

58

Body Water Measurement

Total body water was determined immediately after DXA scan using an ImpediMed DF50 bioelectrical impedance analysis (ImpediMed, San Deigo, CA). While supine, the participant's hand, wrist, ankle, and foot were properly cleaned with an alcohol wipe. Next, four electrodes are then attached to the hand, wrist, ankle and foot. The participant's height, weight, and age information is then added to the BIA device. The device then determined total body water, intracellular water, and extracellular water.

Exercise Testing Session

Upon arrival to the lab on d 0 and 28, the following tests were conducted in the order listed: (1) body mass; (2) body composition; (3) total body water; and (4) donation of approximately 20 mL of fasting venous blood from antecubital vein. After these assessments, the participants then began the exercise performance tests. The bench press test preceded the anaerobic sprint test. The performance test consisted of two sets of ten repetitions at 70% of 1RM. One additional set of repetitions to failure using the same load was also performed. Participants had a 2 min rest period between sets. Participants were asked to focus on exploding upward during the concentric phase of the lift. Power output was measured using a Tendo Fitrodyne (Tendo Sport Machines, Slovak Republic; test C_v=2.1%, intra-class, r=0.99). Peak power, average power, and average velocity were measured during each repetition of the three sets.

After completion of the third set, participants rested for approximately 5 min before starting the anaerobic sprint test on a Lode Excalibur Sport 925900 cycle ergometer (Lode BV, Groningen, The Netherlands). The anaerobic sprint test consisted

of the following: three-minute warm up consisted of pedaling at $60 - 70$ rpm against a resistance of 50 watts (W) for the first minute, 75 W for the second minute, and 100 W for the third minute of the warm-up. The six, 6 sec sprints with 30 sec rests in between followed immediately after the warm-up period. The sprints were followed by a 3 min rest period. The anaerobic sprint test concluded with a 30 sec Wingate test (Figure 6).

A standardized work rate of 7.5 J/kg/rev was set on the cycle ergometer during the sprints and the Wingate test. The participants were asked to pedal as fast as possible prior to application of the workload and sprint at an all-out maximal capacity throughout the 6 sec sprints and the 30 sec Wingate test. Test-to-test variability in performing repeated Wingate anaerobic capacity tests in our lab have yielded correlation coefficients of $r=0.98\pm15\%$ for mean power. Participants practiced the anaerobic sprint test during the familiarization session to minimize learning effects during which seat height, pedal position, and handlebar height was assessed and recorded.

Blood Chemistries

Plasma was analyzed for ALP, AST, ALT, creatinine, BUN, CK, LDH, glucose, and blood lipids (TCHL, HDL, LDL, TG) using a Cobas® c 111 (Roche Diagnostics, Basel, Switzerland). The Cobas® automated clinical chemistry analyzer was calibrated according to manufacturer guidelines. This analyzer has been known to be highly valid
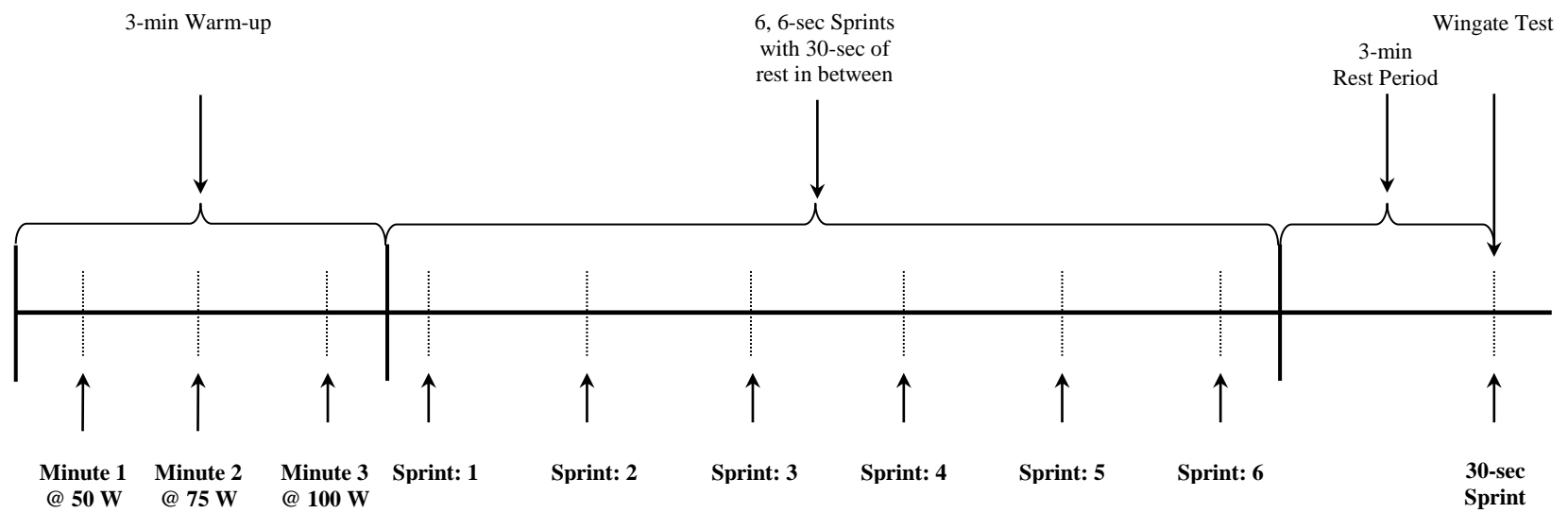
Figure 6. Anaerobic Sprint Test on Cycle Ergometer

and reliable in previously published reports (64). The internal quality control for the Cobas c 111 was performed using two levels of control fluids purchased from manufacturer to calibrate acceptable SD and C_v values for all aforementioned assays. Samples were re-run if the observed values were outside control values and/or clinical norms according to standard procedures.

Complete blood count with platelet differential were run on whole blood (hemoglobin, hematocrit, red blood cell (RBC) counts, MCV, MCH, MCHC, RDW, white blood cell (WBC) counts, lymphocytes, granulocytes, and mid-range absolute count (MID) using a Abbott Cell Dyn 1800 (Abbott Laboratories, Abbott Park, IL, USA) automated hematology analyzer. The internal quality control for Abbott Cell Dyn 1800 was performed using three levels of control fluids purchased from manufacturer to calibrate acceptable SD and C_v values for all whole blood cell parameters.

Side Effect Assessment

The same side effects questionnaires were used during Study 1 and Study 2. During Study 2, participants were completed the side effects questionnaire after 7, 14, 21, and 28 d of supplementation. The questionnaires were completed to determine how well participants tolerated supplementation; how well participants followed the supplementation protocol; and if participants experienced any symptoms as a result of the supplement. Participants were asked to rank the frequency and severity of their symptoms – dizziness, headache, tachycardia, heart skipping or palpitations, shortness of breather, nervousness, blurred vision, and unusual or adverse effects. Participants were asked to rank their symptoms with 0 (none), 1 minimal: 1-2/wk), 2 (slight: 3-4/wk), 3

(occasional: 5-6/wk), 4 (frequent: 7-8/wk), or 5 (severe: 9 or more/wk). Compliance to the supplementation protocol was monitored by supplement logs and verbal confirmation. After completing the first performance testing session (d 0) the participants were given the required supplements and written directions on how to properly ingest the supplements during the supplementation period.

Data Analysis

All data were analyzed using the statistical software SPSS 22.0. Study data were analyzed using a repeated measured 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 analysis of variance (ANOVA). Participant baseline demographic data were analyzed using one-way 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 $p < 0.10$. Post-hoc LSD pairwise comparisons using Cohen's d were used to determine effect magnitude. When a significant group, treatment and/or interaction alpha level was observed, Tukey's least significant difference (LSD) post-hoc analysis was performed to determine where significance was obtained. Data are presented as mean ± SD and mean change ± 95% confidence interval as appropriate.

Prior to initiation of the study, we ran a priori power analysis which indicated a design with an N-size of 12 during Study 1 and an N-size of 11 per group during Study 2 would provide sufficient power to identify previously reported changes in the independent variables.

CHAPTER IV

RESULTS

Study 1

Subject Demographics

Twenty-two participants were initially recruited for Study 1, completed consent forms, and participated in the required familiarization session. However, of the original 22 participants, 13 completed Study 1 (Figure 7). Seven participants dropped out after the familiarization session, six due to time constraints and one due to a pre-existing medical condition that excluded them from participating. One participant was dropped after being randomized into a treatment group due to missing scheduled testing sessions. 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 2. Of the participants that completed the study, they were on average 23 ± 2 y old, 177.8 ± 7.4 cm tall, 84.1 ± 18.9 kg, 26.6 ± 5.9 BMI, and a body fat percentage of 17.3 ± 8.4 %.

Table 2. Study 1 - Participant Demographics

N	Age (yrs)	Height (cm)	Body Weight (kg)	BMI	Body Fat (%)
13	23±2	177.8±7.4	84.1±18.9	26.6±5.9	17.3±8.4

Values are means ± standard deviations (SD). Data were analyzed by one-way ANOVA.
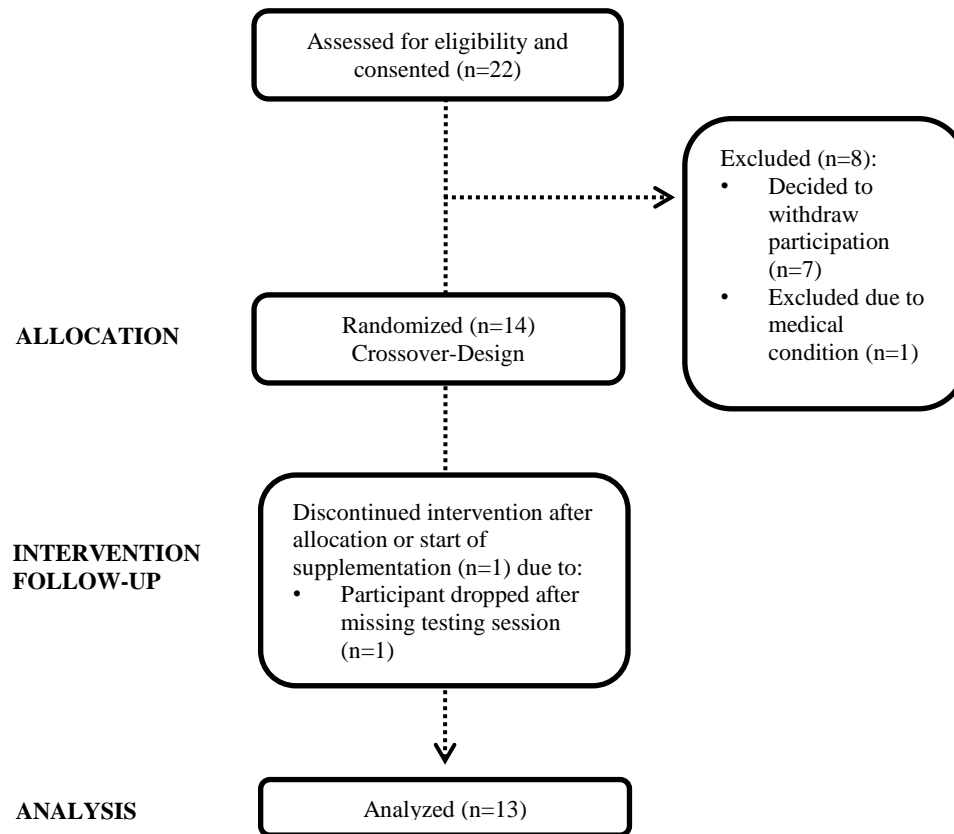
Figure 7. Consort Diagram for Study 1 Participation

Body Composition

Table 3 presents body composition (DXA) data. MANOVA analysis revealed an overall Wilks' Lambda group (p=0.62). Body composition was determined for demographic purposes. Univariate analysis revealed no significant differences among groups in total body weight, fat mass, lean mass, fat-free mass, and body fat percentage.

Table 3. Body Composition (DXA)

Variable	Group	Mean±SD	Mean (SEM)	Group p-level
Body Weight (kg)	PL	83.6±18.6	83.6±5.2	0.34
	CrM	83.7±17.9	83.7±5.0	
	CrN-L	84.1±18.9	84.1±5.3	
	CrN-H	84.1±18.3	84.1±5.1	
	Overall	83.9±17.9		
Fat Mass (kg)	PL	14.0±10.9	14.0±3.0	0.67
	CrM	14.2±11.1	14.2±3.1	
	CrN-L	14.3±11.5	14.3±3.2	
	CrN-H	14.1±11.6	14.1±3.1	
	Overall	14.2±10.9		
Fat-Free Mass (kg)	PL	63.0±8.1	63.0±2.3	0.55
	CrM	62.8±7.3	62.8±2.0	
	CrN-L	62.9±7.7	62.9±2.1	
	CrN-H	63.4±7.6	63.4±2.1	
	Overall	63.0±7.5		
Body Fat (%)	PL	16.8±7.7	16.8±2.1	0.85
	CrM	16.9±8.2	16.9±2.3	
	CrN-L	16.9±8.5	16.9±2.4	
	CrN-H	16.6±8.3	16.6±2.3	
	Overall	16.8±7.9		

Values are means ± standard deviations (SD). MANOVA analysis revealed overall Wilks' Lambda group effects (p=0.62). Greenhouse-Geisser univariate group p-levels are reported.

Diet

Tables 4 and 5 present absolute and relative, respectively, caloric and macronutrient intake data. MANOVA analysis revealed an overall Wilks' Lambda group effect (p=0.78). Univariate analysis showed no significant differences observed among groups in absolute or relative caloric or macronutrient intake.

Table 4. Absolute Dietary Intake and Macronutrient Composition

Variable	Group	Mean±SD	Mean (SEM)		p-level
Calories (kcal/d)	PL	2,532±716	2,532±198	Group	0.48
	CrM	2,615±769	2,615±213		
	CrN-L	2,408±691	2,408±191		
	CrN-H	2,318±522	2,318±144		
	Overall	2,468±671			
Protein (g/d)	PL	129.9±74.7	129.9±20.7	Group	0.89
	CrM	130.4±66.6	130.4±18.5		
	CrN-L	124.6±63.5	124.6±17.6		
	CrN-H	126.8±65.5	126.8±17.9		
	Overall	127.9±65.5			
Carbohydrate (g/d)	PL	280.6±89.6	280.6±24.8	Group	0.35
	CrM	299.3±137.5	299.3±38.1		
	CrN-L	262.8±68.6	262.8±19.0		
	CrN-H	238.9±62.5	238.9±17.3		
	Overall	270.4±94.2			
Fat (g/d)	PL	95.6±40.3	95.7±11.2	Group	0.33
	CrM	94.5±26.4	94.5±7.3		
	CrN-L	83.5±38.1	83.5±10.6		
	CrN-H	82.4±35.7	82.4±9.9		
	Overall	89.0±35.0			

Values are means ± standard deviations (SD). MANOVA analysis revealed overall Wilks' Lambda group effects (p=0.78). Greenhouse-Geisser univariate group p-levels are reported.

Table 5. Relative Dietary Intake and Macronutrient Composition

Variable	Group	Mean±SD	Mean (SEM)		p-level
Calories (kcal/kg/d)	PL	31.3±10.5	31.3±2.9	Group	0.32
	CrM	32.8±12.4	32.8±3.5		
	CrN-L	28.9±7.1	28.9±2.0		
	CrN-H	28.1±6.6	28.1±1.8		
	Overall	30.3±9.4			
Protein (g/kg/d)	PL	1.61±0.95	1.61±0.26	Group	0.43
	CrM	1.62±0.87	1.62±0.24		
	CrN-L	1.50±0.78	1.50±0.21		
	CrN-H	1.93±2.00	1.93±0.55		
	Overall	1.67±1.22			
Carbohydrate (g/kg/d)	PL	3.52±1.36	3.52±0.38	Group	0.26
	CrM	3.84±2.35	3.84±0.65		
	CrN-L	3.16±0.68	3.16±0.19		
	CrN-H	2.90±0.78	2.90±0.22		
	Overall	3.36±1.45			
Fat (g/kg/d)	PL	1.16±0.52	1.16±0.14	Group	0.24
	CrM	1.16±0.35	1.16±0.10		
	CrN-L	0.99±0.38	0.99±0.11		
	CrN-H	0.99±0.41	0.99±0.12		
	Overall	1.07±0.42			

Values are means ± standard deviations (SD). MANOVA analysis revealed overall Wilks' Lambda group effects (p=0.78). Greenhouse-Geisser univariate group p-levels are reported.

Plasma Creatine and Nitrate

Figure 8 shows mean plasma Cr concentration at baseline and 0.5, 1, 2, 3, 4, and 5 h post-supplementation. Values reported are means ± standard deviation. MANOVA analysis revealed overall Wilks' Lambda group ($p<0.001$), time ($p<0.001$), and group x

time (p<0.001). Univariate analysis also revealed a significant group, time, and group x

time interaction (all, p<0.001). Tests of within-subject contrast indicates

Plasma Creatine Concentration
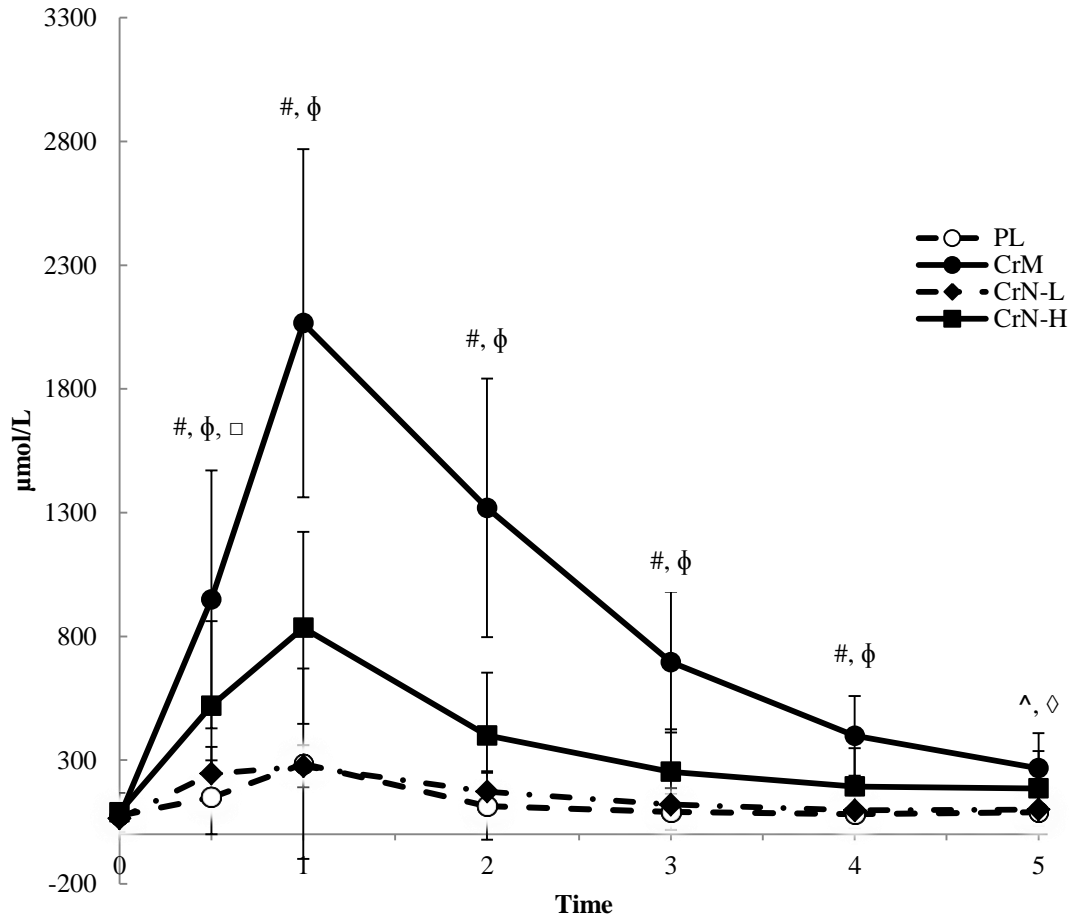
Figure 8. Comparison of Plasma Creatine Concentrations After Acute Supplementation
Values are means ± standard deviations. Values reported are means ± standard deviation. MANOVA
analysis revealed overall Wilks' Lambda group (p<0.001), time (p<0.001), and group x time (p<0.001).
Greenhouse-Geisser group p-levels are reported. p<0.05 considered significant.   (#) denotes a
significantly greater plasma Cr concentration with CrM compared to PL, CrN-L, and CrN-H. (φ) denotes
a significantly greater plasma Cr concentration with CrN-H compared to CrN-L and PL.(□) denotes a
significantly greater plasma Cr concentration with CrN-L compared to PL. (^) denotes significantly
greater plasma Cr concentration with CrM compared to CrN-L and PL. (◊) denotes significantly greater
plasma Cr concentration with CrN-H compared to CrN-L.

both a linear (both, $p<0.001$) and quadratic (both, $p<0.001$) relationship for overall time and group x time interaction. There were no significant differences ($p>0.05$) in plasma Cr concentration among groups at baseline (PL: 76.1±38.3 µM; CrM: 71.6±48.5 µM; CrN-L: 64.4±30.3 µM; CrN-H: 89.5±78.1 µM). Plasma Cr concentrations were significantly increased ($p<0.001$) from baseline with CrM and CrN-H at all time-points (i.e., 0.5, 1, 2, 3, 4, 5 h). Plasma Cr concentration were significantly greater ($p<0.01$) at 0.5, 1, 2, and 3 h post-supplementation with CrN-L compared to baseline, while plasma Cr concentration with PL did not significantly change ($p>0.05$) over time.

Plasma Cr concentration with CrM was significantly ($p<0.001$) greater than all other groups at 0.5 h (PL: 149.5±149.4 µM; CrM: 949.6±520.8 µM; CrN-L: 245.5±108.1 µM; CrN-H: 519.8±342.0 µM), 1 h (PL: 285.4±384.4 µM; CrM: 2,065.9±703.3 µM; CrN-L: 275.1±84.9 µM; CrN-H: 835.0±388.1 µM), 2 h (PL: 113.9±135.3 µM; CrM: 1319.4±522.2 µM; CrN-L: 173.0±82.8 µM; CrN-H: 399.8±253.8 µM), 3 h (PL: 90.8±72.8 µM; CrM: 696.2±284.8 µM; CrN-L: 120.4±66.0 µM; CrN-H: 252.8±172.2 µM), and 4 h (PL: 81.9±57.2 µM; CrM: 398.2±161.5 µM; CrN-L: 97.5±57.8 µM; CrN-H: 193.8±153.9 µM) post supplementation. At 5 h post-supplementation plasma Cr with CrM (267.1±141.4 µM) was only significantly greater than CrN-L (100.3±47.5 µM) and PL (89.7±52.9 µM). Plasma Cr with CrN-H was significantly greater ($p<0.05$) than CrN-L and PL at 0.5,1, 2, 3, and 4 h post supplementation. At 5 h post-supplementation, plasma Cr concentration was significantly ($p=0.03$) greater with CrN-H compared to CrN-L, but not significantly

different than CrM and PL. CrN-L was only significantly greater (p=0.04) than PL at 0.5 h post-supplementation.

Figure 9 shows the plasma Cr area under the curve (AUC) after acute supplementation. Values reported are means ± standard deviation. MANOVA analysis revealed overall Wilks' Lambda group (p=0.001). Plasma Cr AUC with CrM (5,634.4 ± 1,949.8 μmol/L) was significantly greater (p<0.05) than PL (1,012.4±1882.2 μmol/L, p=0.001), CrN-L (2,342.0±3,133.3 μmol/L, p=0.004), and CrN-H (1,761.7±3,408.8 μmol/L, p=0.007). Tests of within-subjects contrasts indicates a linear (p<0.001) relationship between groups. Plasma Cr AUC values for CrM, CrN-L, and CrN-H were 457%, 131%, and 74% greater than plasma Cr AUC with PL. There were no significant differences in plasma Cr AUC among PL, CrN-L, and CrN-H. Results from the plasma Cr analysis provides supporting evidence which fails to reject the null hypothesis of hypothesis 1 which stated that there will be significant differences among group in plasma Cr concentrations after acute supplementation.

Figure 10 shows plasma nitrate concentration at baseline and 0.5, 1, 2, 3, 4, and 5 h post supplementation. Values reported are means ± standard deviation. MANOVA analysis revealed overall Wilks' Lambda group (p<0.001), time (p<0.001), and group x time (p<0.001). Univariate analysis revealed significant group (p<0.001), time (p<0.001), and group x time (p<0.001) effects. Tests of within subject contrasts indicates linear (both, p<0.001) and quadratic (both, p<0.01) relationship for overall time and group x time. There were no significant differences (p>0.05) in plasma nitrate concentration among groups at baseline (PL: 5.4±3.0 μM; CrM: 4.8±2.5 μM; CrN-L:

Plasma Creatine AUC
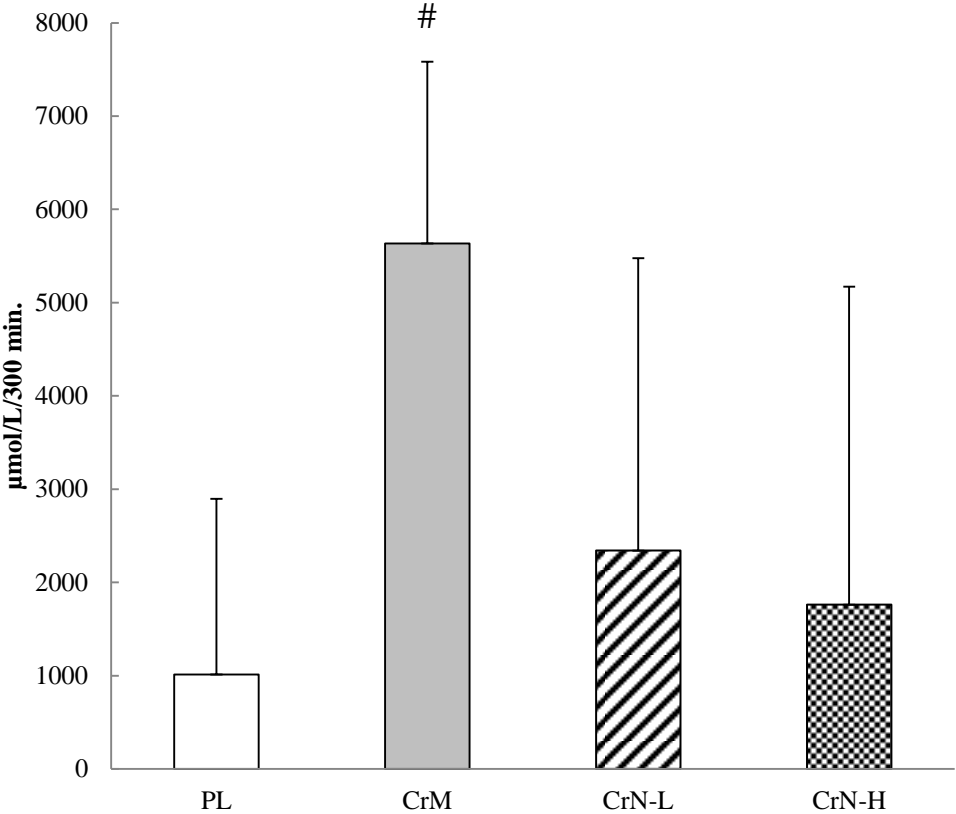
Figure 9. Plasma Creatine Area Under the Curve (AUC)
Values are means ± standard deviations. MANOVA analysis revealed overall Wilks' Lambda group (p=0.001). Greenhouse-Geisser group p-levels are reported. (#) denotes a significant difference (p<0.05) from PL, CrN-L, and CrN-H.

Plasma Nitrate Concentration
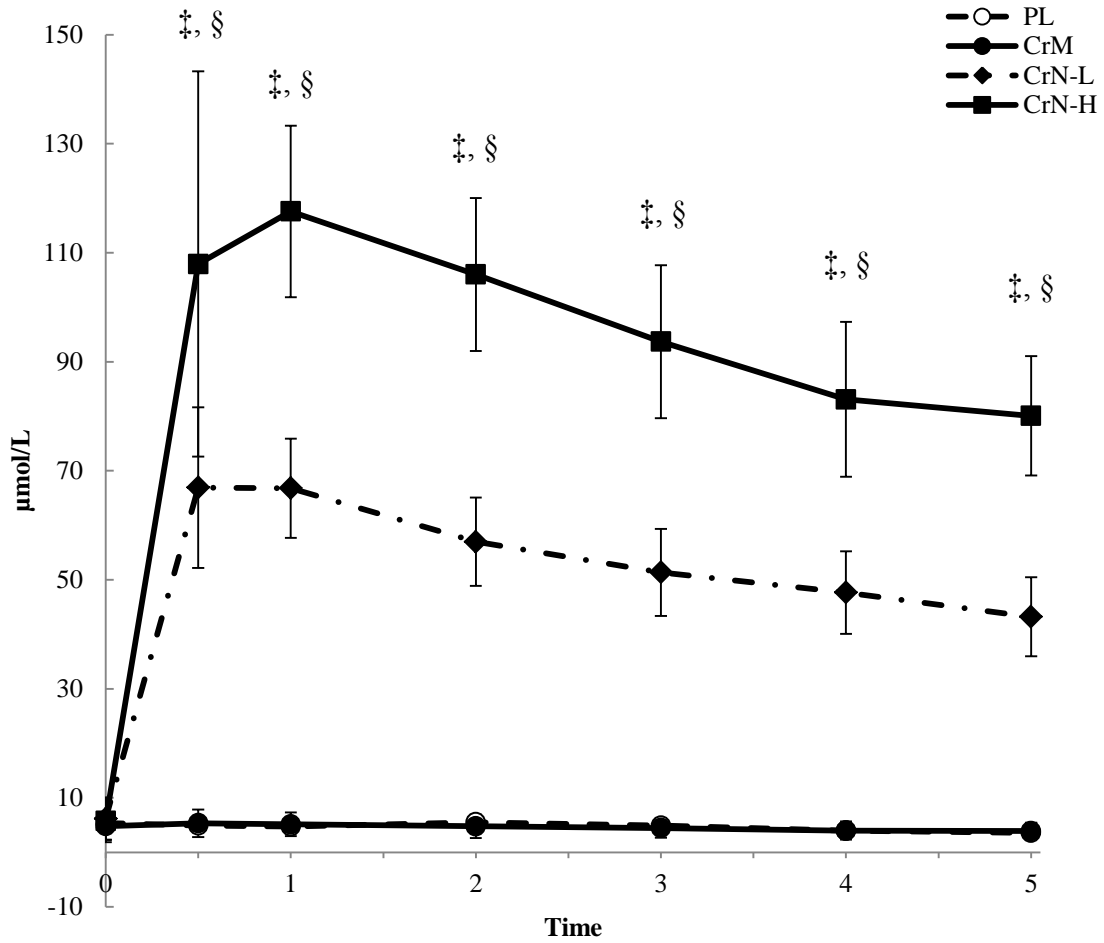
Figure 10. Comparison of Plasma Nitrate Concentration

Values reported are means ± standard deviation. MANOVA analysis revealed overall Wilks' Lambda group (p<0.001), time (p<0.001), and group x time (p<0.001). Greenhouse-Geisser group p-levels are reported. p<0.05 considered significant. (‡) denotes a significantly greater serum nitrate concentration with CrN-H compared to PL, CrM, and CrN-L. (§) denotes a significantly greater serum nitrate concentration with CrN-L compared to PL and CrM.

6.2±3.9 μM; CrN-H: 5.8±3.9 μM). Plasma nitrate concentrations with CrN-L and CrN-H significantly increase (all, $p < 0.001$) at all time-points (i.e., 0.5, 1, 2, 3, 4, 5 h) after supplementation compared to baseline. There were no significant changes across time in plasma nitrate concentrations with CrM ($p > 0.05$). In PL group, plasma nitrate concentrations significantly decrease at 0.5 ($p = 0.02$) and 1 h ($p = 0.045$) compared to baseline, increased to near baseline values at 2 h ($p = 0.85$) and 3 h ($p = 0.59$) post supplementation, then significantly decrease at 4 h ($p = 0.008$) and 5 h ($p = 0.003$) post supplementation.

Plasma nitrate concentration with CrN-H was significantly ($p < 0.001$) greater than all other groups (PL, CrM, and CrN-L) at all post-supplementation time-points − 0.5 h (PL: 4.9±2.8 μM; CrM: 5.3±2.5 μM; CrN-L: 66.9±14.7 μM; CrN-H: 107.9±35.3 μM), 1 h (PL: 4.7±2.5 μM; CrM: 5.3±2.5 μM; CrN-L: 66.9±14.7 μM; CrN-H: 107.9±35.3 μM), 2 h (PL: 5.5±3.1 μM; CrM: 4.8±2.1 μM; CrN-L: 57.0±8.1 μM; CrN-H: 106.0±14.0 μM), 3 h (PL: 4.9±3.0 μM; CrM: 4.5±1.8 μM; CrN-L: 51.4±8.0 μM; CrN-H: 83.1±14.2 μM), 4 h (PL: 3.9±1.8 μM; CrM: 4.0±1.6 μM; CrN-L: 47.7±7.8 μM; CrN-H: 83.1±14.2 μM), and 5 h (PL: 3.6±1.6 μM; CrM: 3.9±1.4 μM; CrN-L: 43.2±7.3 μM; CrN-H: 80.1±10.9 μM). Plasma nitrate concentration with CrN-L was significantly greater than CrM (all, $p < 0.001$) and PL (all, $p < 0.001$) at all post supplementation time-points. There were no significant differences between CrM and PL in plasma nitrate concentration at any post-supplementation time-point.

Figure 11 shows the plasma nitrate AUC after acute supplementation. Values reported are means ± standard deviation. MANOVA analysis revealed overall Wilks'

Lambda group (p=0.01). Plasma nitrate AUC for CrN-H (1988.2±1618.8 µmol/L) was

significantly greater (p<0.05) than CrM (48.0±73.1µmol/L, p=0.001) and PL (51.4±83.4

Plasma Nitrate AUC
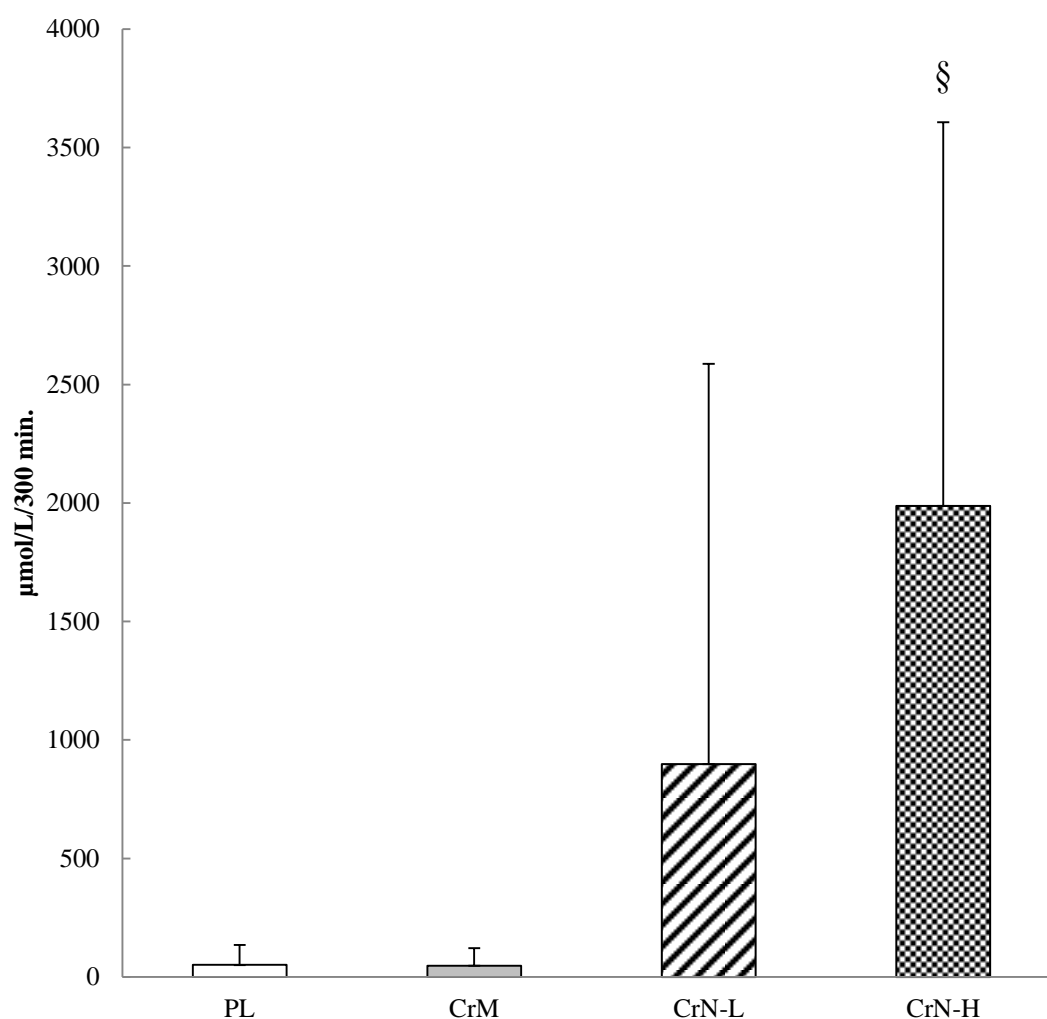
Figure 11. Plasma Nitrate Area Under the Curve (AUC)
Values are means ± standard deviations. MANOVA analysis revealed overall Wilks' Lambda group (p=0.01). Greenhouse-Geisser group p-levels are reported. (§) denotes a significant difference (p<0.05) from CrM and PL.

µmol/L, p=0.001), but not significantly different than CrN-L (898.8±1688.9 µmol/L, p=0.10). Test of within-subjects contrast indicates a quadratic (p=0.002) relationship between groups. Plasma nitrate AUC for CrN-L and CrN-H were 1649%, and 3771% greater, respectively, compared to plasma nitrate AUC for PL. Plasma nitrate AUC for CrM was 6% less than plasma nitrate AUC for PL; however, difference was not significant (p=0.91). Although there was a trend towards greater plasma nitrate AUC with CrN-L there was no significant difference compared to PL (p=0.10) or CrM (p=0.09). The results from the plasma nitrate analysis provides supporting evidence which fails to reject the null hypothesis of hypothesis 2 which stated that there will be significant differences among groups in plasma nitrate concentrations after acute supplementation.

Hematologic Profile

Table 6 presents plasma lipids. MANOVA analysis revealed overall Wilks' Lambda group (p=0.002), time (p<0.001), and group x time (p=0.10). Univariate analysis revealed no significant time effects were observed for TCHL (p=0.06), TCHL: HDL ratio (p=0.09), and LDL (p=0.06). Although changes from baseline were not statistically significant among groups across time (p=0.06), the Cohen's d effect size indicates a large effect size in the increase (1.2 – 3.7%) in TCHL at 0.5 h (1.2%, d=1.49), 1 h (2.6%, d=1.47), 2 h (1.9%, d=1.51), 3 h (3.7%, d=1.55), 4 h (3.7%, d=1.57), and 5 h (2.6%, d=1.54) post supplementation. Similarly, although change from baseline were not statistically significant among groups across time (p=0.09) the

Table 6. Plasma Lipids

Marker	N	Group	Time (hours)							Mean		p-level
			0	0.5	1	2	3	4	5	(SEM)		
TCHL	11	PL	153.8±28.4	156.2±31.1	161.0±31.8	162.5±32.9	164.5±31.2	164.6±36.7	165.0±36.1	161.1±8.9	Group	0.59
(mg/dl)	13	CrM	156.8±29.3	157.2±29.3	160.7±34.3	157.2±31.4	159.3±30.4	160.3±32.8	160.2±28.3	158.8±8.4	Time	0.06
	13	CrN-L	154.5±27.6	154.2±28.0	155.7±31.7	156.0±27.8	158.6±28.9	158.5±26.8	159.5±27.9	156.7±7.5	G x T	0.77
	11	CrN-H	158.3±30.0	163.2±27.0	162.3±32.6	159.9±26.9	163.9±32.6	162.9±26.8	163.5±27.9	162.0±7.5		
		Overall	155.9±28.1	157.7±28.2	159.9±31.8	158.9±29.1	161.6±31.0	161.6±30.2	160.0±29.4			
HDL	11	PL	55.8±15.3	58.2±15.6	58.8±17.4	59.9±16.1	61.0±15.7	61.6±17.0	62.2±17.9	59.6±4.5	Group	0.29
(mg/dl)	13	CrM	53.9±17.1	54.1±16.7	55.6±18.4	55.1±17.2	56.0±17.1	56.9±17.6	56.4±18.8	55.4±4.8	Time	0.001
	13	CrN-L	51.0±14.1	50.8±15.6	51.2±15.0	52.1±14.9	53.8±14.5	54.9±15.2	55.3±15.8	52.7±4.1	G x T	0.64
	11	CrN-H	52.9±17.5	54.7±17.0	54.2±16.8	54.2±17.3	55.4±16.9	55.2±16.3	55.4±17.7	54.6±4.6		
		Overall	53.4±15.7	54.5±16.0	55.0±16.7	55.4±16.2*	56.6±15.8*	57.1±16.3*	57.3±17.3*			
TCHL:HDL	11	PL	2.93±0.84	2.85±0.84	2.92±0.85	2.87±0.83	2.85±0.83	2.82±0.83	2.81±0.83	2.86±0.23	Group	0.40
Ratio	13	CrM	3.14±0.92	3.11±0.91	3.10±0.88	3.03±0.77	3.02±0.81	3.00±0.80	3.07±0.93	3.07±0.24	Time	0.09
(mg/dl)	13	CrN-L	3.27±1.16	3.30±1.15	3.26±1.02	3.21±1.03	3.16±1.02	3.11±1.04	3.10±1.02	3.20±0.29	G x T	0.62
	11	CrN-H	3.33±1.43	3.31±1.40	3.30±1.37	3.31±1.49	3.26±1.40	3.30±1.51	3.31±1.47	3.31±0.39		
		Overall	3.16±1.09	3.14±1.08	3.14±1.03	3.11±1.05	3.07±1.02	3.06±1.06	3.07±1.07			
LDL	11	PL	94.0±26.3	96.5±27.6	98.1±28.1	100.1±29.1	102.4±32.6	102.5±32.3	102.2±32.0	99.4±8.1	Group	0.72
(mg/dl)	13	CrM	99.1±25.6	99.0±24.1	102.5±27.7	101.1±24.7	102.0±23.7	103.8±26.5	102.5±22.9	101.4±6.8	Time	0.06
	13	CrN-L	97.2±23.0	97.1±22.4	98.8±24.0	100.0±22.4	102.4±23.1	102.6±21.6	104.4±22.5	100.4±6.1	G x T	0.67
	11	CrN-H	102.7±27.1	105.3±25.8	105.0±29.8	104.1±26.6	101.5±43.7	106.0±27.0	105.4±25.8	104.3±7.5		
		Overall	98.3±25.0	99.5±24.6	101.1±26.8	101.4±25.1	102.1±30.9	103.7±26.4	103.6±25.3			
TG	11	PL	79.2±26.2	82.1±27.9	78.8±24.8	73.9±23.5	72.6±21.0	71.8±22.1	71.1±22.6	75.6±6.2	Group	0.60
(mg/dl)	13	CrM	90.7±33.1	88.1±34.1	86.6±30.9	76.2±27.3	72.5±25.9	73.7±27.2	68.9±22.6	79.6±7.2	Time	0.01
	13	CrN-L	88.3±36.9	94.5±37.9	90.6±34.7	79.4±25.3	75.4±20.4	73.2±18.3	72.8±17.9	82.0±7.1	G x T	0.66
	11	CrN-H	79.4±25.6	86.1±34.1	81.8±29.1	73.0±18.3	70.9±19.5	71.9±19.4	70.2±20.3	76.2±5.4		
		Overall	84.45±30.37	87.7±33.0	84.5±29.5	75.6±23.3*	72.9±21.2*	72.6±21.4*	70.8±20.4*			

Values are means ± standard deviations. MANOVA analysis revealed overall Wilks' Lambda group (p=0.002), time (p<0.001), and group x time (p=0.10). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels. (*) denotes a significant (p<0.05) difference from baseline.

Cohen's d for TCHL:HDL ratio indicates a small effect size decrease (all, -2.2% from baseline across time at 0.5 h (d=0.06), 1 h (d=0.07), 2 h (d=0.06), 3 h (d=0.07), 4 h (d=0.06), and 5 h (d=0.06) h post supplementation. Furthermore, changes from baseline were not statistically significant among groups across time for LDL (p=0.06) the Cohen's d for LDL indicates a small effect size increase (1.3 – 5.5%) increase across time at 0.5 h (, 1.3%, d=0.05), 1 h (2.9%, d=0.11), 2 h (3.2%, d=0.13), 3 h (3.9%, d=0.14), 4 h (5.5%, d=0.21), and 5 h (5.4%, d=0.21) post-supplementation. There were significant time effects were observed for HDL (p=0.001) and TG (p=0.007). Test of within-subjects contrast indicates a linear increase (p<0.001) in HDL from baseline after 2 h post-supplementation, while TG linearly (p=0.01) decreased from baseline after 2 h post-supplementation. There were no significant group x time interaction for TCHL (p=0.77), HDL (p=0.64), TCHL: HDL ratio (p=0.62), LDL (p=0.67), and TG (p=0.66).

Table 7 presents clinical markers of health. There were significant overall group (p=0.002) and time effects (p<0.001), while there were no significant overall group x time effects (p=0.10) observed. Univariate analysis revealed no significant time effects for AST (p=0.12), CK (p=0.06), and LDH (p=0.40). While changes from baseline were not statistically significant among groups across time for CK (p=0.06), the Cohen's d indicates a small effect size change (-5.5 – 1.9%) at 0.5 h (1.9%, d=0.01), 1 h (1.7%, d=0.01), 2 h (-2.5%, d=0.02), 3 h (-2.4%, d=0.02), 4 h (-5.5%, d=0.04), and 5 h (-5.2%, d=0.03) post-supplementation. There were significant time effects for ALP (p<0.001), ALT (p=0.001), BUN (p<0.001), BUN:Creatinine ratio (p<0.001), and glucose (p=0.009). A test of within-subject contrast indicates a linear (p<0.01)

Table 7. Study 1: Health Markers

Marker	N	Group	Time (hours) 0	0.5	1	2	3	4	5	Mean (SEM)		p-level
ALP	11	PL	7.9±6.5	11.5±8.0	11.9±8.8	13.3±9.1	13.9±9.9	14.3±9.4	16.9±14.1	12.8±2.2	Group	0.23
(U/L)	13	CrM	14.7±9.1	15.9±11.7	15.9±10.4	16.8±12.9	20.5±14.4	25.4±15.3	24.6±14.9	19.1±2.9	Time	0.001
	13	CrN-L	12.3±9.3	16.3±10.3	20.3±10.8	18.7±11.5	21.7±10.9	20.6±10.2	22.8±10.5	18.9±2.0	G x T	0.65
	11	CrN-H	12.8±8.1	17.5±9.5	16.1±8.2	17.2±11.2	16.2±9.7	17.2±15.6	20.1±18.1	16.7±2.5		
		Overall	11.9±8.5	15.3±9.95*	16.0±9.8*	16.5±11.1*	18.1±11.5*	19.4±12.4*	21.1±14.5*			
ALT	11	PL	19±8.2	19.5±9.1	20.2±9.6	19.6±8.5	20.2±9.7	20.3±9.3	20.2±9.3	19.9±2.5	Group	0.70
(U/L)	13	CrM	20.3±7.3	20.5±7.7	21.4±7.8	21.1±8.4	21.1±8.4	21.0±7.9	21.2±8.7	20.9±2.2	Time	0.001
	13	CrN-L	20.1±7.7	20.63±8.4	20.7±8.5	20.8±8.1	21.3±8.2	21.0±8.3	21.1±8.0	20.8±2.3	G x T	0.66
	11	CrN-H	21.2±8.5	21.3±8.3	21.5±8.7	21.9±8.6	21.1±8.5	22.6±8.8	22.7±8.8	21.9±2.4		
		Overall	20.2±7.8	20.5±8.2	21.0±8.5*	20.8±8.2*	21.2±8.5*	21.2±8.4*	21.3±8.5*			
AST	11	PL	24.7±4.9	23.8±5.3	25.1±4.7	25.4±5.8	25.5±5.8	25.2±6.2	25.5±6.1	25.1±1.5	Group	0.40
(U/L)	13	CrM	25.9±7.9	26.5±8.8	27.1±8.9	26.0±9.1	26.3±8.8	26.5±8.6	27.0±9.5	26.5±2.4	Time	0.12
	13	CrN-L	24.1±6.1	24.6±6.3	23.9±5.9	24.7±6.1	24.8±5.3	24.3±5.6	24.6±5.4	24.4±1.6	G x T	0.64
	11	CrN-H	28.0±15.6	28.8±16.3	28.7±16.4	28.8±14.8	29.6±15.2	28.9±14.8	29.8±16.0	28.9±4.3		
		Overall	25.7±9.4	25.9±10.0	26.2±9.9	26.2±9.5	26.6±9.5	26.2±9.4	26.7±10.0			
CK	11	PL	260±222	264±231	266±219	262±210	259±211	253±200	247±193	2589±59	Group	0.40
(U/L)	13	CrM	160±220	262±222	263±212	240±207	249±195	242±189	237±185	251±56	Time	0.06
	13	CrN-L	265±275	270±273	272±287	267±271	266±273	256±250	255±252	264±74	G x T	0.51
	11	CrN-H	490±931	505±958	497±940	475±864	470±863	453±809	470±894	480±248		
		Overall	319±505	325±518	324±509	311±472	311±470	301±441	302±479			
LDH	11	PL	168±40	158±24	170±27	172±26	169±22	171±30	171±27	168±6	Group	0.08
(U/L)	13	CrM	168±21	164±31	168±31	162±23	164±28	165±31	172±29	166±7	Time	0.39
	13	CrN-L	146±25	165±31	155±19	161±23	160±18	158±22	159±18	158±5	G x T	0.40
	11	CrN-H	169±36	179±42	183±40	167±47	196±74	176±32	180±27	179±9		
		Overall	163±32	167±33	169±31	166±31	172±43	168±29	170±26			

Values are means ± standard deviations. MANOVA analysis revealed overall Wilks' Lambda group (p=0.002), time (p<0.001), and group x time (p=0.10). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels. (*) denotes a significant (p<0.05) difference from baseline.

Table 7. Continued

Marker	N	Group	Time (hours)							Mean		
			0	0.5	1	2	3	4	5	(SEM)		p-level
BUN	11	PL	14.4±4.8	14.0±4.2	14.0±4.3	13.4±4.5	13.2±4.2	12.8±4.1	12.3±3.8	13.4±1.2	Group	0.28
(mg/dl)	13	CrM	15.2±5.0	14.8±4.6	14.6±4.7	13.7±4.5	13.4±4.3	13.1±4.3	12.6±4.0	13.9±1.2	Time	0.001
	13	CrN-L	13.5±4.8	13.0±4.5	12.8±4.5	12.2±4.1	12.1±4.1	11.6±3.8	11.4±3.8	12.4±1.2	G x T	0.76
	11	CrN-H	14.6±5.1	14.5±5.22	14.1±4.9	13.7±5.1	13.2±4.6	12.9±4.3	12.7±4.3	13.7±1.3		
		Overall	14.4±4.8	14.1±4.6*	13.9±4.6*	13.6±4.5*	12.9±4.2*	12.6±4.1*	12.3±3.9*			
Creatinine	11	PL	1.00±0.15	0.99±0.16	0.99±0.15	0.98±0.15	0.98±0.15	0.98±0.17	0.95±0.14[d]	0.98±0.04[ψ]	Group	0.001
(mg/dl)	13	CrM	0.99±0.18	1.04±0.17	1.04±0.19	1.01±0.19	0.99±0.17	0.99±0.17	1.00±0.17	1.01±0.05[ɫ]	Time	0.001
	13	CrN-L	1.01±0.19	1.08±0.18[a]	1.09±0.17[a,b]	1.05±0.16[a]	1.05±0.17[a]	1.02±0.18[a]	1.01±1.16	1.05±0.05	G x T	0.001
	11	CrN-H	1.05±0.14[a]	1.11±0.15[a,b]	1.17±0.14[a,b,c]	1.12±0.16[a,b,c]	1.06±0.16[a]	1.03±0.13[a]	1.02±0.14	1.08±0.04		
		Overall	1.01±0.16	1.06±0.17*	1.07±0.17*	1.04±0.17*	1.02±0.16	1.00±0.16	0.99±0.15			
BUN:Creatinine	11	PL	14.4±4.2	14.2±4.1	14.3±4.1	13.7±3.9	13.5±3.6	13.1±3.4	12.9±3.2	13.8±1.0	Group	0.11
(mg/dl)	13	CrM	15.3±4.3	14.5±4.3	14.2±3.9	13.7±4.1	13.6±4.2	13.4±4.1	12.7±3.5	13.9±1.1	Time	0.001
	13	CrN-L	13.7±5.4	12.2±4.6	11.9±4.6	11.8±4.3	11.8±4.4	11.7±4.1	11.5±4.0	12.1±1.2	G x T	0.08
	11	CrN-H	14.0±4.8	13.2±4.6	12.0±3.9	12.3±4.0	12.6±3.9	12.5±3.8	12.5±3.9	12.7±1.1		
		Overall	14.4±4.6	13.5±4.4*	13.1±4.2*	12.9±4.1*	12.9±3.9*	12.7±3.8*	12.4±3.6*			
Glucose	11	PL	95.8±10.3	103.0±10.4	92.1±8.0	92.2±5.5	91.5±4.0	85.9±26.1	91.9±4.3	93.2±2.2	Group	0.33
(mg/dl)	13	CrM	96.3±9.9	85.3±27.0	91.4±6.0	89.8±5.1	91.4±5.4	91.6±5.6	91.2±4.9	91.0±1.8	Time	0.01
	13	CrN-L	96.3±5.5	102.6±8.1	94.1±5.9	91.8±6.5	91.2±6.8	92.9±6.7	92.1±6.2	94.4±1.6	G x T	0.12
	11	CrN-H	94.7±7.5	96.1±6.4	91.4±6.2	91.1±4.0	90.6±5.4	91.3±4.2	83.8±25.3	91.3±1.5		
		Overall	95.8±8.3	96.7±16.5	91.4±6.3*	91.2±5.3*	91.2±5.4*	90.4±13.8*	89.8±13.5*			

Values are means ± standard deviations. MANOVA analysis revealed overall Wilk's Lambda group (p=0.002), time (p<0.001), and group x time (p=0.10). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels. p<0.05 considered significant. (*) denotes a significant difference from baseline. (ɫ) denotes a significant difference from CrN-H. (ψ) denotes a significant difference from CrN-L and CrN-H. (a) denotes a significant difference from PL. (b) denotes a significant difference from CrM. (c) denotes a significant difference from CrN-L. (d) denotes a significant difference from CrM, CrN-L, and CrN-H.

relationship with ALP, ALT, BUN, BUN:Creatine ratio, and glucose across time. No significant group x time interactions were observed for ALP (p=0.65), ALT (p=0.66), AST (p=0.64), CK (p=0.51), LDH (p=0.40), BUN (p=0.76), BUN:Creatinine ratio (p=0.08), and glucose (p=0.12). Significant time (p=0.001) and group x time effects (p=0.001) were only observed for creatinine. A test of within-subject contrast indicates linear and quadratic time (p<0.01) and group x time (p<0.01) relationships for creatinine. Hematologic results provide supporting evidence which fails to reject the null hypothesis of hypothesis 3 which states that there will be no significant differences among groups in markers of clinical health after acute supplementation.

Hemodynamic Profile

Table 8 shows heart rate (HR), systolic blood pressure (SBP), and diastolic blood pressure (DBP) data to acute supplementation for each group. MANOVA analysis revealed overall Wilks' Lambda group (p=0.23), time (p=0.005), and group x time (p=0.26). Univariate analysis revealed were no significant time (p= 0.15) or group x time effects (p=0.56) for HR. Additionally, there were also no significant time (p=0.29) or group x time effects (p=0.11) for DBP. Significant differences in SBP were observed across time (p=0.02) with no significant group x time interactions (p=0.39). SBP at 0 h was significantly (p<0.05) greater than SBP at 0.5 h and 1 h post supplementation. SBP at 1 h was significantly (p<0.01) different than SBP at 0, 2, 3, 4, and 5 h post-supplementation. Tests of within-subjects contrasts indicates a cubic (p=0.02) relationship in changes in SBP across time. The results from the hemodynamic analysis provides supporting evidence which fails to reject the null hypothesis of hypothesis 4

Table 8. Heart Rate and Blood Pressure Response

| Variable | Group | Time (hours) | | | | | | | Mean | | |
		0	0.5	1	2	3	4	5	(SEM)		p-level
HR	PL	59.7±8.2	60.1±7.4	58.8±7.7	57.2±5.3	58.0±7.4	54.8±6.4	56.0±7.3	57.8±1.3	Group	0.23
	CrM	63.8±11.8	60.9±5.7	59.9±8.2	58.2±7.0	56.3±6.8	59.6±8.8	58.5±10.0	59.6±1.7	Time	0.15
	CrN-L	60.0±8.4	57.9±8.3	58.8±9.0	56.3±6.0	56.3±6.0	56.9±5.7	55.1±5.9	57.3±1.5	G x T	0.59
	CrN-H	59.1±9.0	56.7±7.4	57.9±5.8	58.6±3.8	58.0±4.1	56.8±4.7	57.5±3.8	58.2±1.1		
	Overall	60.7±9.4	59.6±7.1	58.8±7.6	57.6±5.6	57.2±6.1	57.0±6.6	56.8±7.0			
SBP	PL	114.8±6.2	113.9±5.6	112.6±4.9	116.5±6.7	116.0±4.8	117.2±8.5	115.5±5.2	115.2±1.4	Group	0.37
	CrM	114.6±5.1	112.6±4.4	112.2±4.2	114.9±5.3	112.9±6.1	113.1±5.8	114.9±3.8	113.6±1.1	Time	0.02
	CrN-L	115.9±6.1	113.1±4.1	111.9±5.2	113.5±6.9	114.6±6.9	114.62±5.0	112.5±3.5	113.7±1.1	G x T	0.39
	CrN-H	115.4±6.7	114.2±5.5	111.4±6.6	112.5±6.0	113.5±6.2	113.7±9.7	114.2±7.0	113.5±1.7		
	Overall	115.2±5.9	113.4±4.8*	112.0±5.1*	114.4±6.3	114.3±6.0	114.7±7.5	114.3±5.0			
DBP	PL	73.1±6.6	71.9±7.5	72.2±6.6	72.3±7.3	73.1±6.6	74.0±5.5	72.3±5.7	72.7±1.4	Group	0.66
	CrM	70.3±6.6	71.5±7.8	72.5±4.1	73.9±5.4	72.8±6.0	73.9±5.0	74.5±4.0	72.8±1.2	Time	0.29
	CrN-L	72.2±6.5	73.5±5.4	71.9±6.1	75.5±5.4	74.3±6.2	74.5±4.6	73.1±4.1	73.6±1.2	G x T	0.18
	CrN-H	73.1±7.9	77.1±4.8	73.9±6.5	74.5±5.2	73.7±6.1	72.2±7.9	72.9±4.8	73.9±1.4		
	Overall	72.2±6.8	73.5±6.7	72.6±5.8	74.0±5.8	73.5±6.0	73.6±5.8	73.2±4.6			

Values are means ± standard deviations. HR=Heart rate, SBP=systolic blood pressure, DBP=diastolic blood pressure. MANOVA analysis revealed overall Wilks' Lambda group (p=0.23), time (p=0.005), and group x time (p=0.26). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels. (*) denotes a significant (p<0.05) difference from baseline.

which stated there will be no significant difference among groups in heart rate after acute supplementation. Furthermore, there is also supporting evidence to reject hypothesis 5 which stated there will be significant differences among groups in blood pressure after acute supplementation.

Side Effects

Tables 9 and 10 present frequency and severity, respectively, of symptoms reported. A MANOVA analysis was run on side effects questionnaire to assess changes in symptoms which include frequency and severity of dizziness, headache, tachycardia, heart skipping or palpitations, shortness of breath, nervousness, blurred vision, and any other unusual or adverse effects. Participants were asked to rank their symptoms with 0 (none), 1 minimal: 1-2/wk), 2 (slight: 3-4/wk), 3 (occasional: 5-6/wk), 4 (frequent: 7-8/wk), or 5 (severe: 9 or more/wk). Some participants reported minimal to slight in side effects such as dizziness (PL: N=1 at 2 h; CrM: N=1 at 0.5 h, N=1 at 2 h, N=1 at 3 h; CrN-L: N=1 at 0.5 h; CrN-H: N=1 at 2 h, N=1 at 4 h), headaches (CrN-L: N=1 at 0.5 h, N=1 at 1 h, N=1 at 2 h, N=1 at 3 h, N=1 at 4 h, N=1 at 5 h; CrN-H: N=1 at 0.5 h), tachycardia (PL: N=1 at 2 h; CrM: N=1 at 1 h; CrN-L: N=0; CrN-H: N=0), nervousness (PL: N=1 at 0.5 h; CrM: N=1 at 0.5 h, N=1 at 1 h; CrN-L: N=0; CrN: N=0), and blurred vision (PL: N=0; CrM: N=1 at 0.5 h; CrN-L: N=1 at 0.5 h; CrN-H: N=1 at 2 h, N=1 at 3 h, N=1 at 4 h, N=1 at 5 h) during the supplementation period. MANOVA analysis revealed an overall Wilks' Lambda group (p=0.45), time (p=0.24), and group x time (p=0.47). Additionally, univariate analysis revealed no time (p=0.45) or group x time interaction (p=0.36) observed among treatment groups. The greatest ranking reported

84

Table 9. Study 1: Side Effects - Frequency of Symptoms

Symptoms	N	Group	Time (hours)						Mean (SEM)		p-level
			0.5	1	2	3	4	5			
Dizziness	11	PL	0.00±0.00	0.00±0.00	0.08±0.28	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.01	Group	0.12
	13	CrM	0.08±0.28	0.00±0.00	0.08±0.28	0.08±0.28	0.00±0.00	0.00±0.00	0.05±0.02	Time	0.14
	13	CrN-L	0.08±0.28	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.03±0.02	G x T	0.25
	11	CrN-H	0.00±0.00	0.00±0.00	0.08±0.28	0.00±0.00	0.08±0.28	0.00±0.00	0.01±0.01		
		Overall	0.04±0.19	0.04±0.19	0.00±0.00	0.00±0.00	0.00±0.24	0.08±0.24			
Headache	11	PL	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	Group	0.37
	13	CrM	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.03±0.03	Time	0.26
	13	CrN-L	0.08±0.28	0.08±0.28	0.08±0.28	0.08±0.28	0.08±0.28	0.08±0.28	0.04±0.04	G x T	0.58
	11	CrN-H	0.08±0.28	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00		
		Overall	0.04±0.19	0.00±0.14	0.04±0.14	0.02±0.14	0.00±0.14	0.00±0.28			
Tachycardia	11	PL	0.00±0.00	0.00±0.00	0.08±0.28	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.01	Group	0.10
	13	CrM	0.00±0.00	0.15±0.38	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.05±0.03	Time	0.28
	13	CrN-L	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.03±0.02	G x T	0.30
	11	CrN-H	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00		
		Overall	0.00±0.00	0.08±0.00	0.04±0.19	0.00±0.14	0.00±0.14	0.02±0.14			
Heart Skipping or Palpitations	11	PL	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	Group	0.08
	13	CrM	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.04±0.02	Time	0.26
	13	CrN-L	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	G x T	0.26
	11	CrN-H	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00		
		Overall	0.04±0.00	0.00±0.00	0.00±0.00	0.02±0.00	0.00±0.00	0.00±0.00			
Shortness of Breath	11	PL	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.03±0.02	Group	0.22
	13	CrM	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.01	Time	0.50
	13	CrN-L	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	G x T	0.41
	11	CrN-H	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00		
		Overall	0.00±0.00	0.02±0.00	0.02±0.00	0.00±0.00	0.00±0.00	0.02±0.00			
Nervousness	11	PL	0.08±0.28	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.03±0.03	Group	0.69
	13	CrM	0.23±0.44	0.08±0.28	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.01	Time	0.25
	13	CrN-L	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.01	G x T	0.46
	11	CrN-H	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.01		
		Overall	0.00±0.27	0.00±0.33	0.06±0.14	0.02±0.14	0.02±0.00	0.00±0.00			
Blurred Vision	11	PL	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.01	Group	0.73
	13	CrM	0.08±0.28	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.01	Time	0.33
	13	CrN-L	0.08±0.28	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.03±0.03	G x T	0.39
	11	CrN-H	0.00±0.00	0.00±0.00	0.08±0.00	0.08±0.28	0.08±0.28	0.08±0.28	0.03±0.03		
		Overall	0.00±0.19	0.00±19	0.06±0.00	0.02±0.00	0.02±0.14	0.00±0.14			
Any other unusual or adverse effects	11	PL	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.01	Group	0.34
	13	CrM	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	Time	0.34
	13	CrN-L	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	G x T	0.34
	11	CrN-H	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.01		
		Overall	0.02±0.00	0.00±00	0.00±0.00	0.00±0.00	0.02±0.00	0.00±0.00			

Values are mean ± standard deviation. MANOVA analysis revealed overall Wilks' Lambda group (p=0.45), time (p=0.24), and group x time (p=0.47). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels.

Table 10. Study 1: Side Effects - Severity of Symptoms

Symptoms	N	Group	0.5	1	2	3	4	5	Mean (SEM)		p-level
Dizziness	11	PL	0.00±0.00	0.00±0.00	0.08±0.28	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	Group	0.34
	13	CrM	0.08±0.28	0.00±0.00	0.08±0.28	0.08±0.28	0.00±0.00	0.00±0.00	0.01±0.01	Time	0.34
	13	CrN-L	0.08±0.28	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.01	G x T	0.34
	11	CrN-H	0.00±0.00	0.00±0.00	0.08±0.28	0.00±0.00	0.08±0.28	0.00±0.00	0.00±0.00		
		Overall	0.02±0.14	0.02±0.14	0.00±0.14	0.00±0.14	0.00±0.00	0.00±0.00			
Headache	11	PL	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	Group	0.51
	13	CrM	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.01	Time	0.44
	13	CrN-L	0.08±0.28	0.08±0.28	0.15±0.56	0.15±0.56	0.08±0.28	0.15±0.56	0.03±0.03	G x T	0.34
	11	CrN-H	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.01		
		Overall	0.02±0.14	0.00±0.28	0.02±0.14	0.04±0.14	0.00±0.14	0.00±0.28			
Tachycardia	11	PL	0.00±0.00	0.00±0.00	0.08±0.28	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	Group	0.45
	13	CrM	0.00±0.00	0.08±0.28	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	Time	0.50
	13	CrN-L	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.01	G x T	0.34
	11	CrN-H	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.03±0.03		
		Overall	0.00±0.00	0.00±0.00	0.02±0.00	0.00±0.00	0.02±0.00	0.02±0.00			
Heart Skipping or Palpitations	11	PL	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	Group	0.34
	13	CrM	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	Time	0.34
	13	CrN-L	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	G x T	0.34
	11	CrN-H	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.01		
		Overall	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.02±0.00	0.00±0.00			
Shortness of Breath	11	PL	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	Group	0.51
	13	CrM	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	Time	0.46
	13	CrN-L	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.01	G x T	0.36
	11	CrN-H	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.01		
		Overall	0.02±0.00	0.02±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00			
Nervousness	11	PL	0.08±0.28	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	Group	0.51
	13	CrM	0.00±0.60	0.08±0.28	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	Time	0.50
	13	CrN-L	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.01	G x T	0.36
	11	CrN-H	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.01		
		Overall	0.02±0.00	0.00±0.00	0.00±0.00	0.02±0.00	0.00±0.00	0.00±0.00			
Blurred Vision	11	PL	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	Group	0.45
	13	CrM	0.08±0.28	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	Time	0.42
	13	CrN-L	0.08±0.28	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.03±0.03	G x T	0.35
	11	CrN-H	0.00±0.00	0.00±0.00	0.08±0.28	0.08±0.28	0.08±0.28	0.08±0.28	0.01±0.01		
		Overall	0.00±0.14	0.00±0.14	0.02±0.14	0.00±0.14	0.00±0.14	0.04±0.14			
Any other unusual or adverse effects	11	PL	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	Group	0.34
	13	CrM	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	Time	0.34
	13	CrN-L	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	G x T	0.34
	11	CrN-H	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.01		
		Overall	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.02±0.00	0.00±0.00			

Values are mean ± standard deviation. MANOVA analysis revealed overall Wilks' Lambda group (p=0.45), time (p=0.24), and group x time (p=0.47). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels.

was 2, which was used by one participant to indicate the severity of his nervousness 0.5 h after ingesting CrM as well as to indicate the severity of his headache at 2, 3, and 5 h after consuming CrN-L. There were no symptoms ranked with 3, 4, or 5 in frequency or severity by any participant. Results of the side effects questionnaire analysis provide evidence which accepts the null hypothesis of hypothesis 5 which stated that there will be no significant difference among groups in side effect symptoms in frequency or severity after acute supplementation.

Study 2

Participant Demographics

Seventy-one participants were initially recruited for Study 2 and completed consent forms, and participated in the required familiarization session. However, of the original 71 participants, 48 completed (21.3 ± 3.4 y old, 176.8 ± 5.8 cm tall, 80.5 ± 13.8 kg, 25.8 ± 3.8 BMI, and a body fat percentage of 17.8 ± 5.9 %) the 28 d research study (Figure 12). Twenty-three participants dropped out after the familiarization, 22 of them due to time constraints and one due to apprehension of the muscle biopsy procedure. Fifty-three participants were randomized to the four treatment groups. Twelve were initially randomized to the placebo group, but one participant was dropped due to missed scheduled laboratory visits. Fifteen were initially randomized to the creatine monohydrate group. Four participants dropped out of this group, one due to missed scheduled laboratory visits, one developed abdominal pains after the second day of supplementation, one due to a family emergency, and one withdrew due to time
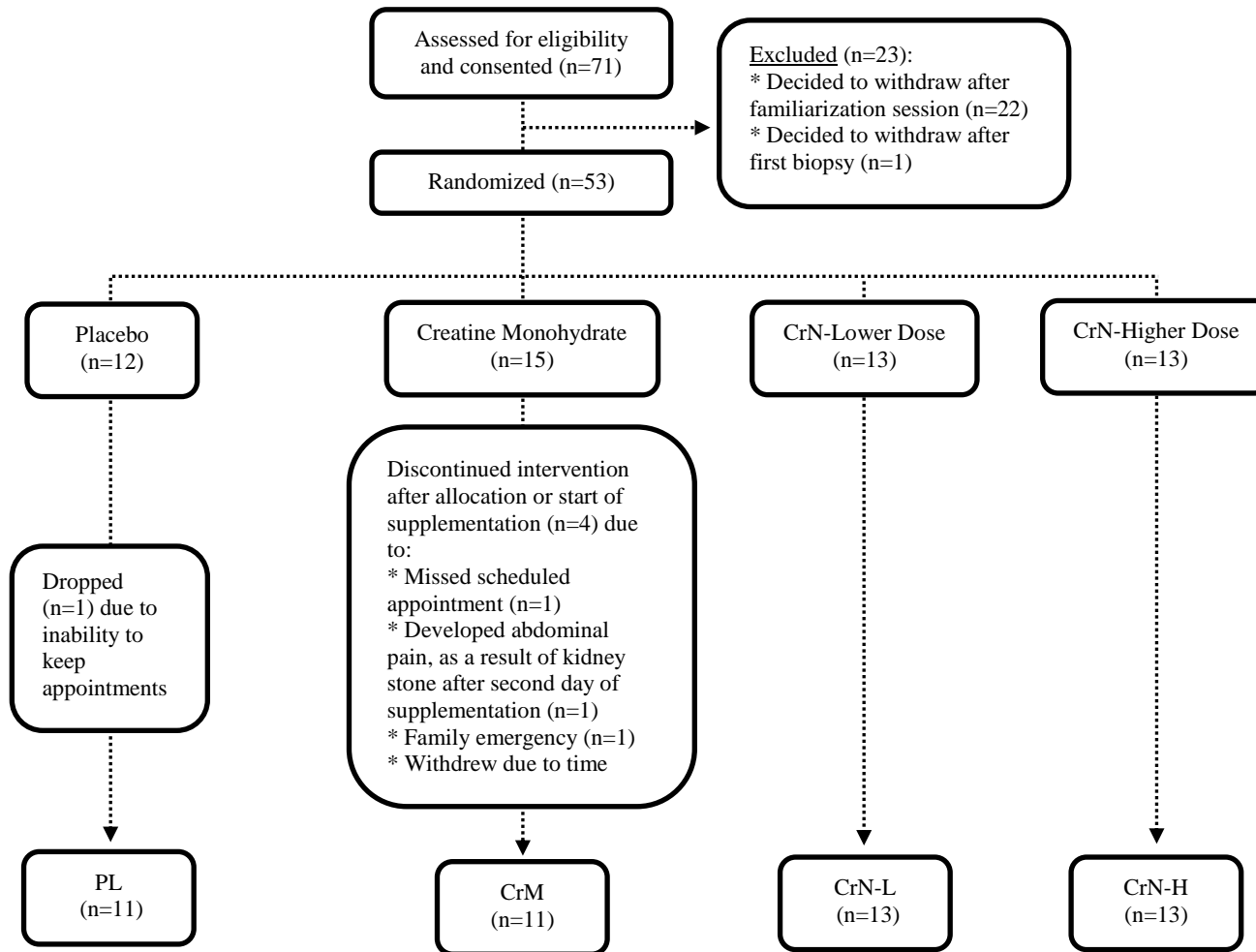
Figure 12. Consort Diagram for Study 2 Participation

constraints. There were no withdraws from either creatine nitrate group. Table 11 represents participant demographics.

Table 11. Study 2 - Participant Demographics

Group	N	PL	CrM	CrN-L	CrN-H	p-level
	N	11	11	13	13	
Age (yrs)		21.2±3.7	21.6±2.5	20.7±1.9	21.5±4.8	0.93
Height (cm)		177.8±7.2	177.3±4.7	175.0±7.1	177.5±3.9	0.63
Body Weight (kg)		77.3±11.8	81.7±13.2	71.9±9.7	90.8±13.4^	0.002
BMI		24.3±2.5	25.8±3.6	23.6±2.7	28.9±4.2^	0.001
Body Fat (%)		17.8±6.9	16.7±4.0	13.2±5.4	19.2±6.0	0.06

Values are means ± standard deviations (SD). Data were analyzed by one-way ANOVA. (^) denotes a significant difference ($p < 0.05$) from creatine nitrate (CrN-L) and placebo (PL).

Compliance, Training and Diet

Five of the forty-eight participants reported not supplementing on schedule based on the completed supplement checklist and verbal confirmation. 90% of the participants supplemented on schedule and exhibited 100% compliance based on the completed supplement checklist and verbal confirmation. The 10% that did not supplement on schedule reported taking the missed dose(s) within 1 -2 days. Two participants in CrN-H group each missed the ingestion of 6 g CrN-H (12 g CrN-H total) on schedule. Two participants in CrN-L group missed scheduled supplementation; one participant missed 3 g CrN-L, while the other participant missed 12 g CrN-L. In the CrM group one participant missed the ingestion of 3 g CrM on schedule.

Table 12 shows the training volumes for upper and lower body lifts throughout Study 2. A one-way ANOVA analysis was run to examine differences among the four groups. Results indicate no statistically significant differences among groups in total upper body training volume (p=0.78) or lower body training volume (p=0.49)

Table 12. Training Volume

Group	Upper Body (kg)	p-level
PL	117,048±21,951	0.78
CrM	104,017±33,926	
CrN-L	108,505±37,207	
CrN-H	108,596±23,708	
	Lower Body (kg)	
PL	121,285±24,295	0.49
CrM	101,966±27,299	
CrN-L	123,718±47,215	
CrN-H	117,038±38,939	

Values are means ± standard deviations. Training volume variables were analyzed by one-way ANOVA.

Tables 13 and 14 present absolute and relative, respectively, caloric macronutrient intake for each treatment group. MANOVA analysis revealed overall Wilks' Lambda group (0.38), time (p=0.24), and group x time (p=0.12). Univariate analysis showed no significant group x time effects in absolute and relative macronutrient intake.

Table 13. Absolute Caloric and Macronutrient Intake

Variable	Group	Day			Mean		
		0	7	28	(SEM)		p-level
Calories	PL	2,179±490	2,225±701	2,170±858	2,191±151	Group	0.98
(kcal/d)	CrM	2,258±400	2,254±473	2,303±405	2,272±151	Time	0.38
	CrN-L	2,282±689	2,149±656	2,395±575	2,275±139	G x T	0.89
	CrN-H	2,234.5±447	2,125±831	2,389±600	2,250±138		
	Overall	2,240±509	2,184±664	2,321±613			
Protein	PL	113.4±42.4	114.0±41.0	104.9±41.7	110.8±9.1	Group	0.96
(g/d)	CrM	112.3±31.0	114.9±24.5	113.3±24.9	113.5±9.1	Time	0.74
	CrN-L	101.8±33.46	106.1±44.5	123.5±41.7	110.5±8.3	G x T	0.40
	CrN-H	120.7±36.4	109.8±33.1	118.6±40.2	116.4±8.3		
	Overall	112.0±35.5	111.0±35.9	115.6±37.6			
Carbohydrate	PL	233.1±91.1	234.4±99.1	231.5±121.3	233.0±23.5	Group	0.94
(g/d)	CrM	215.3±46.0	236.2±83.5	220.6±74.5	224.1±23.5	Time	0.23
	CrN-L	273.4±94.3	224.5±104.5	235.9±67.8	224.6±21.6	G x T	0.31
	CrN-H	254.9±92.1	224.7±85.1	223.0±74.8	234.3±21.6		
	Overall	245.9±84.5	229.5±90.8	227.9±83.4			
Fat	PL	74.1±23.6	88.9±39.2	82.5±42.0	81.8±9.7	Group	0.70
(g/d)	CrM	97.2±17.8	90.1±24.9	92.3±21.4	93.2±9.7	Time	0.41
	CrN-L	83.1±40.5	87.9±38.6	101.5±38.2	90.8±8.9	G x T	0.64
	CrN-H	94.7±46.5	95.4±55.3	101.1±44.4	97.1±8.9		
	Overall	87.4±35.2	90.7±40.4	94.9±37.5			

Values are means ± standard deviations. MANOVA analysis revealed overall Wilks' Lambda group (0.38), time (p=0.24), and group x time (p=0.117). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels.

Plasma Creatine and Nitrate

Figure 13 presents plasma Cr concentrations at day 0, 7 and 28 d. A MANOVA analysis was run to assess plasma creatine concentrations. MANOVA analysis revealed overall Wilks' Lambda group (p=0.03), time (p<0.001), and group x time (p=0.01). Univariate analysis showed that mean plasma Cr concentrations significantly increased over time (140±223 μM, 75±197 μM, p<0.001) with significant

Table 14. Relative Caloric and Macronutrient Intake

Variable	Group	Day			Mean			p-level
		0	7	28	(SEM)			
Calories	PL	28.8±7.2	28.8±8.7	28.4±10.5	28.7±2.1	Group		0.13
(kcal/kg/d)	CrM	28.4±8.1	27.9±7.2	28.7±7.9	28.4±2.1	Time		0.37
	CrN-L	32.0±9.1	30.1±9.4	32.9±6.1	31.7±1.9	G x T		0.94
	CrN-H	25.3±7.4	23.4±8.7	26.5±7.8	25.1±1.9			
	Overall	28.6±8.1	27.5±8.7	29.2±8.3				
Protein	PL	1.53±0.71	1.48±0.54	1.38±0.52	1.46±0.13	Group		0.67
(g/kg/d)	CrM	1.40±0.43	1.44±0.46	1.42±0.51	1.42±0.13	Time		0.77
	CrN-L	1.43±0.46	1.46±0.53	1.69±0.55	1.53±0.12	G x T		0.39
	CrN-H	1.38±0.58	1.24±0.45	1.33±0.51	1.31±0.12			
	Overall	1.44±0.53	1.40±0.49	1.46±0.53				
Carbohydrate	PL	3.08±1.23	3.08±1.36	3.03±1.45	3.06±0.33	Group		0.25
(g/kg/d)	CrM	2.67±0.63	2.90±0.98	2.74±1.03	2.77±0.33	Time		0.15
	CrN-L	3.87±1.42	3.22±1.69	3.29±0.97	3.46±0.30	G x T		0.33
	CrN-H	2.93±1.31	2.52±1.01	2.49±0.96	2.65±0.30			
	Overall	3.16±1.26	2.93±1.29	2.89±1.12				
Fat	PL	0.975±0.32	1.13±0.48	1.08±0.56	1.07±0.13	Group		0.76
(g/kg/d)	CrM	1.23±0.36	1.11±0.35	1.15±0.35	1.17±0.13	Time		0.57
	CrN-L	1.14±0.50	1.20±0.47	1.37±0.38	1.24±0.12	G x T		0.54
	CrN-H	1.11±0.76	1.07±0.64	1.12±0.50	1.11±0.12			
	Overall	1.12±0.52	1.13±0.49	1.19±0.45				

Values are means ± standard deviations. MANOVA analysis revealed overall Wilks' Lambda group (0.38), time (p=0.24), and group x time (p=0.12). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels.

Plasma Creatine Concentration

Figure 13. Comparison of Plasma Creatine Concentration with Chronic Supplementation
Values are means ± standard deviations. MANOVA analysis revealed overall Wilks' Lambda group (p=0.03), time (p<0.001), and group x time (p=0.01). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels. p<0.05 considered significant. (¥) denotes a significant difference between creatine monohydrate (CrM) and creatine nitrate-lower dose (CrN-L) at day 0 (baseline). (†) denotes a significantly greater Cr concentration with CrM compared to CrN-L. CrN-H. and PL at day 7.

93

group x time interactions (PL: 12±73 μM, 19±97 μM; CrM: 340±354 μM, 66±66 μM; CrN-L: 97±129 μM, 75±69 μM; CrN-H: 121±125 μM, 132±360 μM, p<0.001) after 7 and 28 d of supplementation. Tests of within-subject contrasts indicates a linear (p=0.02) and quadratic (p=0.001) relationship over time, while only indicating quadratic (p=0.006) group x time relationship in plasma Cr concentration. Percent change in plasma Cr from baseline was greatest for CrM (205%) compared to the other groups (PL: 9.4%, CrN-L: 133%, CrN-H: 101%) after 7 d of supplementation. Although there were no significant difference in plasma Cr concentrations at 28 d of supplementation, concentrations were significantly greater than baseline (PL: 15%, CrM: 40%, CrN-L: 103%, CrN-H: 110%) values. The results of the plasma Cr analysis provide evidence which fails to reject hypothesis 7 which states there will be significant differences between groups in plasma Cr concentration after 7 and 28 d of supplementation.

Figure 14 presents plasma nitrate concentrations at day 0, 7 and 28 d. A MANOVA analysis was run to assess plasma nitrate concentrations. MANOVA analysis revealed an overall Wilks' Lambda group (p<0.001), time (p<0.001), and group x time (p<0.001). Univariate analysis showed a significant time effect (34.2±43.1 μM, 7.8±18.8 μM, p<0.001) with significant group x time interactions (PL: 0.6±3.6 μM, 4.4±15.9 μM; CrM: -1.1±2.6 μM, -0.4±2.8 μM; CrN-L: 59.2±29.8 μM, 8.3±13.7 μM; CrN-H: 67.7±47.8 μM, 17.3±28.4 μM, p<0.001) after 7 and 28 d of supplementation, respectively. There was a significant increase after 7 d of supplementation only with CrN-L and CrN-H. Tests of within-subject contrasts indicates a linear (p=0.007) and

quadratic (p<0.001) relationship across time, while only indicating a quadratic (p<0.001)

group x time relationship in plasma nitrate concentration. Plasma nitrate concentration

Figure 14. Serum Nitrate Concentration.
Values are means ± standard deviations. MANOVA analysis revealed an overall Wilks' Lambda group (p<0.001), time (p<0.001), and group x time (p<0.001). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels. p<0.05 considered significant. (§) denotes a significant difference with CrN-L and CrN-H compared to CrM and PL at day 7. (Ø) denotes a significantly greater concentration with CrN-H compared to CrM at day 28**.**

increase by 1057% and 1574% with CrN-L and CrN-H, respectively, after 7 d of supplementation. There was only a significant difference (p<0.001) between CrM and CrN-H after 28 d of supplementation. However, plasma nitrate concentrations were increased by 98%, 148% and 400% with PL, CrN-L, and CrN-H, respectively. The results of the plasma nitrate analysis provides evidence which fails to reject hypothesis 8 which states that there will be significant difference among groups in plasma nitrate concentration after 7 and 28 d of supplementation.

Muscle Creatine Concentration

Table 15 presents muscle free creatine (FCr) concentration data. An ANCOVA analysis with baseline muscle FCr as a covariate was run on all muscle FCr data. The covariate allows for the statistical model to consider the continuous independent variable (i.e., baseline muscle Cr concentration), which is not part of the experimental manipulation, into the statistical equation. Including this covariate also explains more within-group variance and improves the statistical power the analysis. Additionally, including a covariate in the statistical model helps reduce the occurrence of regression to the mean. The values listed in Table 16 are expressed in mmol • kg^{-1} DW and are presented as means ± standard deviations. ANCOVA analysis revealed overall Wilks' Lambda group (p<0.001), time (p<0.001), and group x time (p<0.001). Univariate analysis revealed a significant time effects (p=0.013) for mean muscle free Cr concentration for all groups over time 37.2±6.8, 38.5±9.0, 37.9±8.8 mmol • kg^{-1} DW for d 0, 7, and 28, respectively. Additionally, there was a significant group x time effects (p<0.001) in mean muscle FCr concentration after 7 and 28 d of supplementation. Tests

of within-subject contrasts indicates linear relationships for time (p=0.013) and group x time (p=<0.001).

Table 15. Muscle Free Creatine Concentration

Marker	N	Group	Day 0	Day 7	Day 28	Mean (SEM)		p-level
Creatine	11	PL	34.9±6.3	33.8±6.0	33.2±5.4	35.6±1.0[‡]	Group	0.001
(mmol/kg DW)	13	CrM	36.9±5.1	44.1±7.0[a,c]	45.8±5.6[a,d]	42.5±0.9[^]	Time	0.01
	13	CrN-L	35.8±6.6	32.5±4.2	32.3±5.6	34.5±0.9[‡]	G x T	0.001
	11	CrN-H	40.6±8.0	43.7±10.6[a,c]	40.9±9.9[c]	39.2±0.9		
		Overall	37.2±6.8	38.5±9.0*	37.9±8.8*			

Values are means ± standard deviations. ANCOVA analysis revealed overall Wilks' Lambda group (p<0.001), time (p<0.001), and group x time (p<0.001). Greenhouse-Geisser time and group x time (G x T) interaction p-leve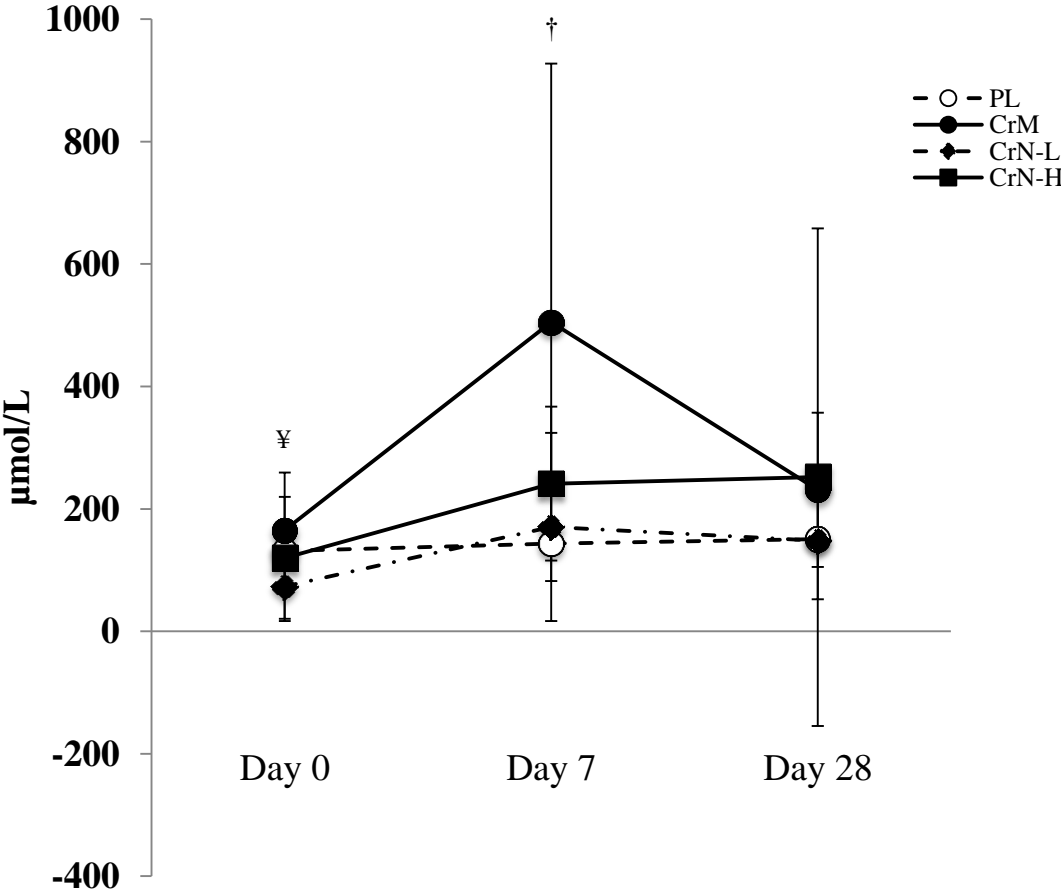ls are reported with univariate group p-levels. p<0.05 considered significant. (‡) denotes a significant difference from CrN-H. (^) denotes a significant difference from CrN-L and PL. (a) denotes a significant difference from PL. (c) denotes a significant difference from CrN-L. (d) denotes a significant difference from CrN-L and CrN-H. (*) denotes a significant difference from baseline.

Figure 15 presents percent changes in muscle FCr concentration. ANCOVA analysis revealed overall Wilks' Lambda group (p<0.001), time (p<0.001), and group x time (p<0.001). Univariate analysis showed that after d 7 of supplementation there was a significant percent increase in muscle FCr concentration in CrM (p<0.001) and CrN-H (p=0.003) by 20.2±16.4% and 8.5±18.5 %, respectively. However, percent change was not significantly different between CrM and CrN-H (p=0.40) at d 7. At d 7, percent change in muscle FCr was significantly greater with CrM compared to PL (-0.4±25.6%, p=0.02) and CrN-L (-6.8±18.9%, p=0.002). There were no significant differences in percent change in muscle FCr among PL, CrN-L, and CrN-H after 7 d of

supplementation. After 28 d of supplementation, muscle FCr concentrations increased by 25.7±19.3% with CrM, which was significantly greater when compared to CrN-L (-8.5±16.1 %, p<0.001), CrN-H (1.2±19.1 %, p=0.002), and PL (-4.2±8.9 %, p<0.001).

Muscle Creatine Concentration
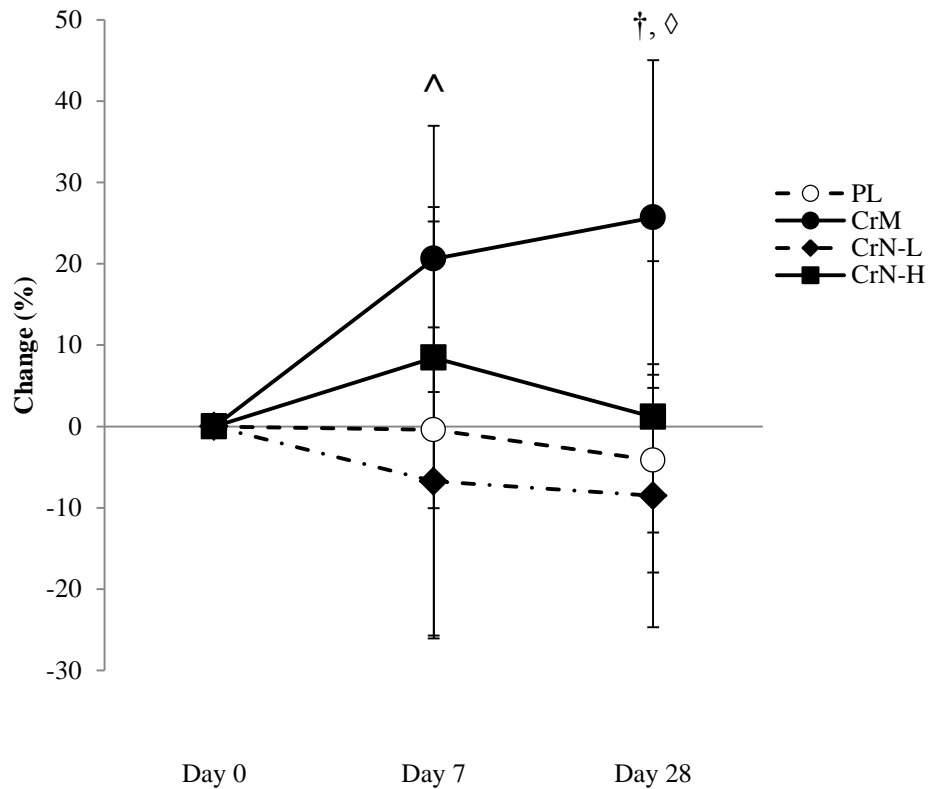
Figure 15. Percent Change in Muscle Creatine Concentration
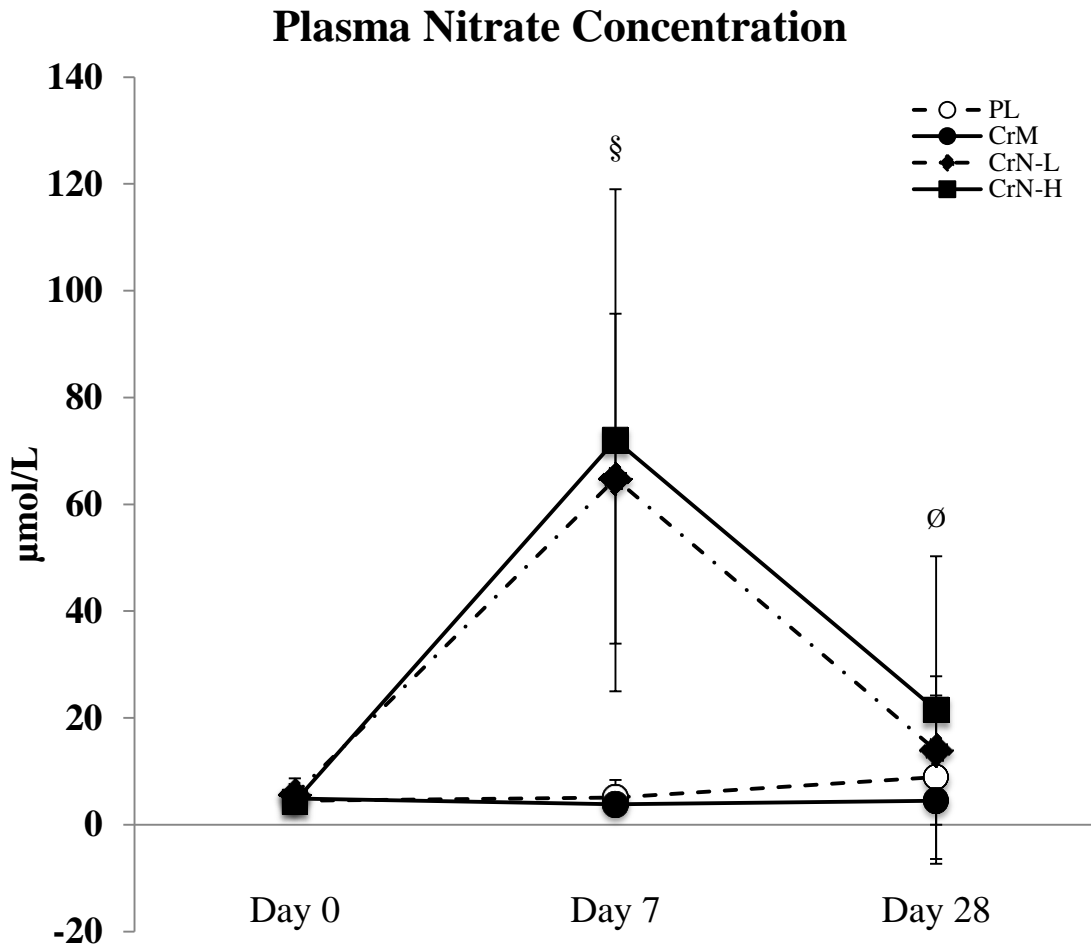Values are means ± standard deviations. ANCOVA analysis revealed overall Wilks' Lambda group (p<0.001), time (p<0.001), and group x time (p<0.001). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels. p<0.05 considered significant. (^) denotes a significantly greater Cr concentrations with CrM and CrN-H compared to CrN-L and PL at d 7. (†) denotes a significantly greater Cr concentration with CrM compared to CrN-L, CrN-H, and PL at d 28. (◊) denotes a significantly greater Cr concentration with CrN-H compared to CrN-L at d 28.

Percent change with CrN-H was significantly greater (p=0.02) at 28 days of supplementation when compared to CrN-L (p=0.002). There were no significant difference (p=0.52) in percent change in muscle FCr between CrN-L and PL. Tests of within-subjects contrast indicates a linear (p=0.02) and quadratic (p=0.01) relationship in percent change over time, while indicating a linear (p<0.001) group x time relationship.

Figure 16 represents mean muscle FCr concentration, 95% CI, and individual responses supplementation at d 7 (A) and d 28 (B). The values listed in this figure are expressed in mmol • kg^{-1} DW. ANCOVA analysis revealed overall Wilks' Lambda group (p<0.001), time (p<0.001), and group x time (p<0.001). Univariate analysis revealed a significant increase in muscle FCr concentration with CrM [mean±SEM] (7.1±1.9 mmol• kg^{-1} DW, 95% CI, 3.1, 11.1) and CrN-H (4.7±1.9 mmol • kg^{-1} DW, 95% CI, 0.9, 8.4) after 7 d of supplementation. There was a significant decrease in muscle FCr concentrations with CrN-L (-4.0±1.8 mmol • kg^{-1} DW, 95% CI, - 7.6, -0.3) and no significant change in muscle FCr with PL (-2.1±2.0 mmol • kg^{-1} DW, 95% CI, -6.2, 1.9) after 7 d of supplementation. After 28 d of supplementation, muscle FCr concentrations were only significantly elevated from baseline with CrM (8.9±1.7 mmol • kg^{-1} DW, 95% CI, 5.5, 12.2). There was no significant change from baseline with CrN-H (1.5±1.6 mmol • kg^{-1} DW, 95% CI, -1.7, 4.6) and PL (-2.4±1.7 mmol • kg^{-1} DW, 95% CI, -5.8, 1.0) after 28 d of supplementation. Muscle FCr concentration was significantly decreased from baseline with CrN-L (-4.0±1.5 mmol • kg^{-1} DW, 95% CI, -7.0, -0.9).
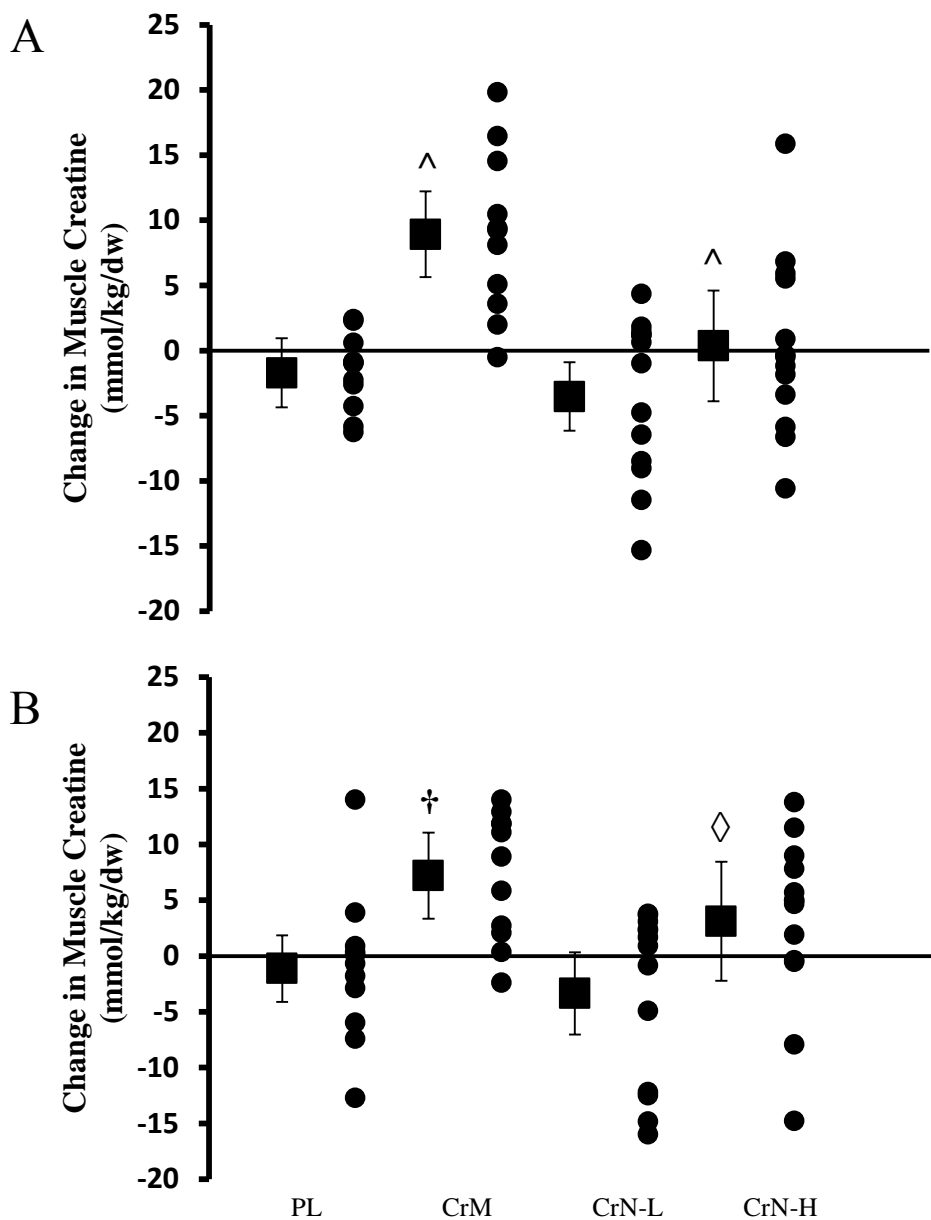
Figure 16. Mean, 95% CI, and Individual Responses to Treatment at Day 7 (A) and Day 28 (B).
ANCOVA analysis revealed overall Wilks' Lambda group (p<0.001), time (p<0.001), and group x time (p<0.001). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels. p<0.05 considered significant. (^) denotes a significantly greater change in Cr concentrations with CrM and CrN-H compared to CrN-L and PL at d 7. (†) denotes a significantly greater change in Cr concentration with CrM compared to CrN-L, CrN-H, and PL at d 28. (◊) denotes a significantly greater change in Cr concentration with CrN-H compared to CrN-L at d 28.

After 7 d of supplementation FCr concentrations with CrM were significantly greater than CrN-L (p=0.001) and PL (p=0.009), but not significantly different than CrN-H (p=0.17). Additionally, FCr concentrations with CrN-H were significantly greater than CrN-L (p=0.03), but not significantly different than PL (p=0.15) after 7 d of supplementation. There were no significant differences between CrN-L and PL (p=0.44). At 28 d of supplementation FCr concentrations with CrM were significantly greater than CrN-L (p<0.001), CrN-H (p=0.001), and PL (p<0.001). Although there was a trend towards a significant difference between CrN-H and CrN-L (p=0.096), there were no significant differences between other treatment groups after 28 d of supplementation. Tests of within-subject contrasts indicates linear relationships for time (p=0.013) and group x time (p=<0.001). The results of the muscle Cr analysis provide evidence which fails to reject hypothesis 9 which stated that there will be significant differences among groups in muscle Cr concentration after 7 and 28 d of supplementation.

Body Composition

Table 16 displays the results from all body composition analysis. A MANOVA analysis was run to examine changes in body in body composition variables which included body weight (kg), fat mass (kg), fat-free mass (kg), and percentage of body fat and total body water (BIA). MANOVA analysis revealed overall Wilk's Lambda group (p=0.20), time (p=0.15), and group x time (p=0.37). Univariate analysis showed a significant increase in body weight in all groups over time (0.29±1.04 kg, 0.74±1.33 kg, p=0.002) with no significant group x time interaction observed among groups (PL

Table 16. Body Composition

Variable	N	Group	Day 0	Day 7	Day 28	Mean (SEM)		p-level
Body Weight	11	PL	77.3±11.9	77.6±12.1	77.4±12.7	77.4±3.7[‡]	Group	0.003
(kg)	11	CrM	81.7±13.2	82.4±13.4	82.6±14.0	82.2±3.7	Time	0.002
	13	CrN-L	72.0±9.7	72.2±9.9	72.7±10.0	72.3±3.4[‡]	G x T	0.29
	13	CrN-H	90.8±13.4	90.8±13.2	92.0±14.3	91.2±3.4		
		Overall	80.5±13.8	80.8±13.8	81.3±14.5*			
Fat Mass	11	PL	12.7±6.3	12.6±6.3	12.7±6.3	12.7±1.8	Group	0.02
(kg)	11	CrM	12.9±5.3	13.0±5.5	13.2±5.2	13.0±1.8	Time	0.17
	13	CrN-L	8.9±4.7	8.7±4.6	9.1±4.7	8.9±1.6[‡]	G x T	0.90
	13	CrN-H	16.5±6.9	16.2±6.4	16.6±7.4	16.5±1.6		
		Overall	12.8±6.4	12.6±6.2	12.9±6.4			
Fat-Free Mass	11	PL	58.1±8.0	58.5±8.0	58.3±8.3	58.3±2.4[‡]	Group	0.01
(kg)	11	CrM	62.4±8.7	62.5±8.8	62.9±9.2	62.6±2.4	Time	0.02
	13	CrN-L	56.9±7.4	57.4±7.3	57.3±7.3	57.2±2.2[‡]	G x T	0.50
	13	CrN-H	67.4±8.1	67.7±8.2	68.5±8.2	67.9±2.2		
		Overall	61.3±8.9	61.6±8.9	61.9±9.2*			
Body Fat	11	PL	17.8±6.9	17.1±6.7	17.2±6.7	17.4±1.7	Group	0.07
(%)	11	CrM	16.7±4.0	16.8±4.3	16.8±3.7	16.8±1.7	Time	0.24
	13	CrN-L	13.1±5.4	12.8±5.2	13.2±5.2	13.1±1.5[‡]	G x T	0.78
	13	CrN-H	19.2±5.9	18.7±5.5	18.8±6.3	18.9±1.5		
		Overall	16.7±6.0	16.4±5.8	16.5±5.9			
Total Body Water	11	PL	51.3±4.5	50.6±5.1	52.5±6.9	51.5±1.3	Group	0.18
(%)	11	CrM	51.4±3.3	50.8±3.7	49.4±8.4	50.5±1.3	Time	0.64
	13	CrN-L	52.9±4.6	54.1±5.1	52.7±4.2	53.2±1.2	G x T	0.47
	13	CrN-H	50.6±6.5	48.8±4.1	49.0±4.7	49.5±1.2		
		Overall	51.6±4.8	51.1±4.8	50.9±6.2			

Values are means ± standard deviation. MANOVA analysis revealed overall Wilk's Lambda group (p=0.20), time (p=0.15), and group x time (p=0.37). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels. (*) denotes a significant (p<0.05) difference from baseline. (‡) denotes a significant (p<0.05 difference from CrN-H.

0.30±0.87 kg, 0.15±1.24 kg; CrM: 0.63±0.81 kg, 0.89±1.46 kg; CrN-L: 0.28±1.28 kg, 0.69±1.23 kg; CrN-H: 1.17±1.38 kg, p=0.29) after 7 and 28 d of supplementation, respectively. Tests of within-subjects contrast indicates a linear (p<0.001) change in body weight over time.

There were no significant differences over time for fat mass (-0.16±0.77, 0.10±0.86 kg, p=0.17) and no group x time interactions were observed (PL: -0.08±0.74

kg, 0.04±0.81 kg; CrM: 0.09±0.23 kg; CrN-L: -0.22±0.58 kg, 0.14±0.42 kg; CrN-H: -0.38±0.89 kg, 0.01±1.16 kg, p=0.90) after 7 and 28 d of supplementation, respectively. Fat-free mass significantly increased over time for all groups (0.30±1.22 kg, 0.56±1.30 kg, p=0.02) with no significant group x time interaction effects observed among groups (PL: 0.33±1.40 kg, 0.19±0.99 kg; CrM: 0.09±0.85, 0.53±1.50; CrN-L: 0.36±1.26 kg, 0.32±1.04 kg; CrN-H: 0.39±1.42 kg, 1.13±1.53 kg, p=0.49) after 7 and 28 d of supplementation, respectively. Tests of within-subjects contrast indicates a linear (p=0.006) change in fat-free mass over time.

No significant differences were observed over time in body fat percent for all groups (-0.33±1.38%, -0.19±1.36%, p=0.24) and no significant group x time interactions were observed among groups (PL: -0.62±2.28%, -0.54±2.02%; CrM: 0.09±1.15%, 0.11±1.22%; CrN-L: -0.37±0.91%, 0.07±0.61%; CrN-H: -0.41±0.96%, -0.42±1.37%, p=0.78) after 7 and 28 d of supplementation, respectively. Total body water expressed as a percentage of body weight was not significantly difference over time for any group (-0.45±4.11%, -0.66±5.65%, p=0.64) with no significantly group by time interactions observed among groups (PL: -0.69±2.60%, -1.98±8.70%; CrM: -0.57±1.82%, -0.25±2.95%; CrN-L: 1.15±5.42%, -1.57±5.40%; CrN-H: -1.75±4.88%, 1.25±4.62%, p=0.47) after 7 and 28 d of supplementation, respectively. The results of the body composition analysis provide evidence which fails to rejects the null hypothesis of hypothesis 10 which states there will be no significant differences among groups in body composition as measured by DXA after 7 and 28 d of supplementation.

Bench Press Performance and Anaerobic Sprint Test

Table 17 presents bench press performance and power output during the third bench press set. Participants performed three sets of bench press using a load equivalent to 70% of their 1 repetition max (1RM). One participant from the CrM group was omitted from the statistical analysis as he was unable to perform the bench press test at d 28 due to a shoulder injury that occurred outside the study. The first two sets consisted of ten repetitions each. The third set consisted of maximal repetitions performed. Workload was calculated by multiplying weight by repetitions completed during the third set. Peak power, average power, and average velocity were measured by a Tendo Fitrodyne.

MANOVA analysis revealed overall Wilks' Lambda group (p=0.24), time (p<0.001), and group x time (0.37). Univariate analysis showed a significant time effects for all variables (maximum repetitions, workload, peak power, average power, average velocity, all p<0.001) with no group x time interaction observed for any variable (maximum repetitions [p=0.55], workload [p=0.10], peak power [p=0.57], average power [p=0.82], average velocity [p=0.74]). The percent change in maximum repetitions with 70% of 1RM increased by 35.5%, 20.5%, 19.0%, and 26.9% with CrN-H, CrN-L, PL, and CrM, respectively after supplementation; however, there were no significant differences in maximum repetitions among groups after supplementation. After 28 of supplementation, workload with CrN-H was significantly greater than CrN-L (p=0.045) and PL (p=0.02), but not significantly greater than CrM (p=0.44). Percent change in workload was 35.4±27.7%, 20.5±31.0%, 19.0±23.6%, 26.9±19.4% for CrN-H, CrN-L,

Table 17. Bench Press (Set) - Maximum Repetition Performance

Variable	N	Group	Day		Mean (SEM)		p-level
			0	28			
Max Reps	11	PL	10.8±2.9	13.0±4.8	11.9±1.2	Group	0.83
	10	CrM	12.0±5.5	15.0±7.1	13.5±1.3	Time	0.001
	13	CrN-L	11.9±2.7	14.0±3.7	13.0±1.1	G x T	0.55
	13	CrN-H	11.5±3.6	14.8±3.7	13.2±1.1		
		Overall	11.6±3.7	14.2±4.7*			
Workload	11	PL	1474±374	1753±549	1614±216	Group	0.17
(wt x reps)	10	CrM	1827.±926	2255±1122	2041±227	Time	0.001
	13	CrN-L	1617±491	1877±535	1746±199	G x T	0.10
	13	CrN-H	1927±830	2517±867	2221±199		
		Overall	1714±688	2105±824*			
Peak Power	11	PL	426±101	443±107	434±27	Group	0.10
(W)	10	CrM	453±114	522±120	487±28	Time	0.001
	13	CrN-L	440±76	481±88	461±24	G x T	0.57
	13	CrN-H	492±95	553±98	523±24		
		Overall	454±96	501±108*			
Average Power	11	PL	358±88	382±93	370±27[‡]	Group	0.03
(W)	10	CrM	424±121	456±105	440±28	Time	0.001
	13	CrN-L	372±70	396±73	384±25[‡]	G x T	0.82
	13	CrN-H	451±92	489±90	470±25		
		Overall	402±98	432±97*			
Average Velocity	11	PL	0.50±0.09	0.53±0.11	0.52±0.03	Group	0.10
(m/s)	10	CrM	0.55±0.09	0.62±0.11	0.59±0.03	Time	0.001
	13	CrN-L	0.61±0.83	0.65±0.12	0.63±0.03	G x T	0.74
	13	CrN-H	0.54±0.11	0.61±0.15	0.58±0.03		
		Overall	0.56±0.10	0.61±0.13*			

Values are means ± standard deviations. MANOVA analysis revealed overall Wilks' Lambda group (p=0.24), time (p<0.001), and group x time (0.37). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels. (*) denotes a significant difference (p<0.05) from baseline. (‡) denotes a significant difference (p<0.05) from CrN-H.

PL, and CrM, respectively. Similarly, average power was significantly greater with CrN-H when compared to CrN-L (p=0.01) and PL (p=0.006), but not significantly greater than CrM (p=0.39). Percent change in average power was 9.3±10.1%, 7.7±13.8%, 7.2±12.2%, and 9.3±8.7% for CrN-H, CrN-L, PL, and CrM, respectively. Peak power was significantly greater with CrN-H after 28 d of supplementation compared to PL

(p=0.01). There were no significant differences in peak power between CrM and CrN-L (p=0.36) or PL (p=0.09) after 28 days of supplementation. Percent change in peak power after supplementation was 16.2±29.6%, 9.7±12.0%, 6.4±21.5%, and 16.7±16.7% for CrN-H, CrN-L, PL, and CrM, respectively. After 28 d of supplementation, average velocity was significantly greater with CrN-L when compared to PL (p=0.03), but not significantly greater than CrM (p=0.58) and CrN-H (p=0.46). Percent change in average velocity post-supplementation was 14.2±28.8%, 7.2±14.4%, 7.4±16.4%, and 12.9±11.9% for CrN-H, CrN-L, PL, and CrM, respectively. Tests of within-subjects contrasts indicates a linear (p<0.001) change in maximum repetition, workload, peak power, average power, and average velocity after supplementation. The results of the bench press performance analysis provide evidence which rejects hypothesis 11 which stated that there will be a significant difference among groups in bench press performance after 28 d of supplementation.

Table 18 shows the overall results for the anaerobic capacity observed for each group. A MANOVA analysis was run on order to assess changes in anaerobic capacity variables. MANOVA analysis revealed overall Wilks' Lambda group (p=0.25), time (p=0.41), and group x time (p=0.40). Univariate analysis showed no significant time or group x time effects for mean power, peak power and total work.

Table 18. Anaerobic Sprint Capacity

Variable	N	Group	Day 0	Day 28	Mean (SEM)		p-level
Mean Power (W)	10	PL	680±177	683±150	681±37[‡]	Group	0.04
	11	CrM	684±129	721±142	702±35	Time	0.19
	13	CrN-L	671±125	709±107	690±32[‡]	G x T	0.41
	13	CrN-H	808±91	798±61	803±32		
		Overall	714±140	731±121			
Peak Power (W)	10	PL	1,490±390	1,487±340	1,488±100	Group	0.17
	11	CrM	1,548±352	1,611±441	1,580±94	Time	0.16
	13	CrN-L	1,497±270	1,565±278	1,531±87	G x T	0.85
	13	CrN-H	1,739±276	1,784±307	1,761±87		
		Overall	1,575±326	1,617±349			
Total Work (J)	10	PL	6,006±967	6,232±919	6,119±373	Group	0.49
	11	CrM	6613±918	6,887±745	6,750±356	Time	0.17
	13	CrN-L	6,761±1618	6,818±1291	6,789±327	G x T	0.94
	13	CrN-H	6,716±1533	6,884±1495	6,800±327		
		Overall	6,553±1322	6,728±1172			

Values are means ± standard deviations. MANOVA analysis revealed overall Wilks' Lambda group (p=0.25), time (p=0.41), and group x time (p=0.40). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels. (‡) denotes a significant (p<0.05) difference from CrN-H.

Tables 19, 20, and 21 show peak power, mean power, and total work, respectively, during each sprint in the anaerobic sprint test. MANOVA analysis revealed overall Wilks' Lambda group (p=0.25), time (p=0.41), and group x time (p=0.40). Univariate analysis showed a significant time effects for mean power only at sprint 1 (p=0.03) and sprint 3 (both, p=0.02) after supplementation.

Table 19. Peak Power - Individual Sprints

Variable	N	Group	Day		Mean		p-level
			0	28	(SEM)		
Sprint: 1	10	PL	1,527±411	1,554±403	1,553±114	Group	0.05
(W)	11	CrM	1,589±484	1,670±542	1,629±114	Time	0.13
	13	CrN-L	1,609±310	1,718±341	1,663±105	G x T	0.94
	13	CrN-H	1,903±328	2,021±503	1,962±105		
		Overall	1,668±411	1756±472			
Sprint: 2	10	PL	1,787±594	1,677±451	1,736±123	Group	0.62
(W)	11	CrM	1,800±468	1,819±476	1,810±123	Time	0.98
	13	CrN-L	1,653±299	1,712±318	1,682±113	G x T	0.66
	13	CrN-H	1,869±455	1,907±500	1,888±113		
		Overall	1,772±444	1,783±435			
Sprint: 3	10	PL	1,458±399	1,605±471	1,529±112	Group	0.14
(W)	11	CrM	1,661±378	1,710±540	1,685±112	Time	0.12
	13	CrN-L	1,558±405	1,687±290	1,637±103	G x T	0.85
	13	CrN-H	1,880±365	1,906±453	1,893±103		
		Overall	1,658±405	1,735±442			
Sprint: 4	10	PL	1,568±426	1,461±304	1,502±95	Group	0.27
(W)	11	CrM	1,508±319	1,673±473	1,590±95	Time	0.56
	13	CrN-L	1,593±344	1,585±256	1,589±87	G x T	0.26
	13	CrN-H	1,737±301	1,797±399	1,767±87		
		Overall	1,608±346	1,638±375			
Sprint: 5	10	PL	1,415±368	1,380±305	1,382±95	Group	0.11
(W)	11	CrM	1,499±406	1,508±426	1,504±95	Time	0.37
	13	CrN-L	1,425±359	1,588±320	1,507±87	G x T	0.47
	13	CrN-H	1,703±344	1,735±304	1,719±87		
		Overall	1,517±376	1,566±354			
Sprint: 6	10	PL	1,320±349	1,356±374	1,315±91	Group	0.16
(W)	11	CrM	1,450±360	1,523±385	1,486±91	Time	0.21
	13	CrN-L	1,357±322	1,384±351	1,370±83	G x T	0.95
	13	CrN-H	1,552±263	1,639±304	1,596±83		
		Overall	1,425±325	1,481±360			
Wingate Test	10	PL	1,352±313	1,372±269	1,334±96	Group	0.32
(W)	11	CrM	1,329±427	1,374±482	1,351±96	Time	0.79
	13	CrN-L	1,253±285	1,279±296	1,266±88	G x T	0.86
	13	CrN-H	1,525±364	1,478±329	1,502±88		
		Overall	1,367±354	1376±349			

Values are means ± standard deviations. MANOVA analysis revealed overall Wilks' Lambda group (p=0.25), time (p=0.41), and group x time (p=0.40). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels.

Table 20. Mean Power - Individual Sprints

Variable	N	Group	Day 0	Day 28	Mean (SEM)		p-level
Sprint: 1	10	PL	793±186	822±194	803±44	Group	0.06
(W)	11	CrM	813±194	822±195	817±44	Time	0.03
	13	CrN-L	826±123	864±124	845±41	G x T	0.84
	13	CrN-H	949±103	974±114	961±41		
		Overall	850±160	876±165*			
Sprint: 2	10	PL	793±196	806±190	791±42	Group	0.18
(W)	11	CrM	811±145	851±203	831±42	Time	0.81
	13	CrN-L	803±144	813±105	808±39	G x T	0.32
	13	CrN-H	937±136	891±92	914±39		
		Overall	840±161	842±149			
Sprint: 3	10	PL	699±216	738±172	706±40‡	Group	0.05
(W)	11	CrM	706±135	798±153	752±40	Time	0.02
	13	CrN-L	693±184	765±118	729±37‡	G x T	0.20
	13	CrN-H	867±124	851±79	859±37		
		Overall	745±178	791±134*			
Sprint: 4	10	PL	690±198	660±162	660±38	Group	0.15
(W)	11	CrM	681±138	749±115	715±38	Time	0.81
	13	CrN-L	664±141	712±107	688±35	G x T	0.10
	13	CrN-H	818±118	753±177	785±35		
		Overall	716±157	721±143			
Sprint: 5	10	PL	655±181	633±138	627±36‡	Group	0.008
(W)	11	CrM	650±131	701±184	675±36‡	Time	0.41
	13	CrN-L	619±154	669±120	644±33‡	G x T	0.55
	13	CrN-H	797±108	792±101	795±33		
		Overall	683±157	703±145			
Sprint: 6	10	PL	640±188	635±149	624±39‡	Group	0.03
(W)	11	CrM	638±141	635±164	636±39‡	Time	0.45
	13	CrN-L	595±191	641±126	618±36‡	G x T	0.76
	13	CrN-H	755±99	777±102	766±36		
		Overall	659±165	676±145			
Wingate Test	10	PL	484±122	483±112	473±31	Group	0.56
(W)	11	CrM	487±116	485±130	486±31	Time	0.82
	13	CrN-L	493±109	496±99	494±28	G x T	0.97
	13	CrN-H	530±94	542±102	536±28		
		Overall	500±108	503±109			

Values are means ± standard deviations. MANOVA analysis revealed overall Wilks' Lambda group (p=0.25), time (p=0.41), and group x time (p=0.40). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels. (*) denotes a significant difference from baseline. (ɨ) denotes a significant difference (p<0.05) from CrN-H.

Table 21. Total Work - Individual Sprints

Variable	N	Group	Day 0	Day 28	Mean (SEM)		p-level
Sprint: 1	10	PL	5796±652	5,601±725	5,737±345	Group	0.66
(J)	11	CrM	6,148±831	6,360±889	6,254±345	Time	0.06
	13	CrN-L	6,075±1425	6,350±1225	6,212±317	G x T	0.08
	13	CrN-H	5,856±1478	6,068±1509	5,962±317		
		Overall	5,972±1163	6,115±1161*			
Sprint: 2	10	PL	5,677±580	5,565±880	5,672±318	Group	0.80
(J)	11	CrM	5,999±892	6,090±793	6,044±318	Time	0.73
	13	CrN-L	5,820±1374	5,905±1296	5,863±292	G x T	0.70
	13	CrN-H	6,108±1462	5,878±1222	5,993±292		
		Overall	5,911±1147	5,869±1071			
Sprint: 3	10	PL	4,694±986	5,265±811	5,056±312	Group	0.45
(J)	11	CrM	5,294±814	5,594±621	5,444±312	Time	0.04
	13	CrN-L	5,226±1514	5,392±1154	5,309±287	G x T	0.54
	13	CrN-H	5,659±1437	5,705±1161	5,682±287		
		Overall	5,249±1260	5,499±970*			
Sprint: 4	10	PL	4,574±760	4,719±843	4,722±283	Group	0.46
(J)	11	CrM	4,985±761	5,121±708	5,053±283	Time	0.84
	13	CrN-L	5,254±1305	4,809±1314	5,031±260	G x T	0.41
	13	CrN-H	5,263±1295	5,309±1068	5,286±260		
		Overall	5,049±1093	5,001±1027			
Sprint: 5	10	PL	4,352±952	4,731±1144	4,618±292	Group	0.74
(J)	11	CrM	4,856±716	4,877±536	4,867±292	Time	0.57
	13	CrN-L	5,014±1313	4,867±1133	4,941±269	G x T	0.64
	13	CrN-H	4,918±1215	4,985±1283	4,951±269		
		Overall	4,809±1089	4,873±1044			
Sprint: 6	10	PL	4,297±1024	4,252±1042	4,341±303	Group	0.57
(J)	11	CrM	4,749±761	4,850±513	4,800±303	Time	0.70
	13	CrN-L	4,776±1311	4,846±1105	4,811±279	G x T	0.98
	13	CrN-H	4,721±1228	4,774±1294	4,748±279		
		Overall	4,653±1099	4,701±1040			
Wingate Test	10	PL	12,650±3177	13,495±2775	13,466±968	Group	0.37
(J)	11	CrM	14,259±4680	15,315±2138	14,787±968	Time	0.16
	13	CrN-L	15,161±4171	15,553±3500	15,357±891	G x T	0.98
	13	CrN-H	14,490±4355	15,473±4079	14,981±891		
		Overall	14,230±4127	15,037±3271			

Values are means ± standard deviations. MANOVA analysis revealed overall Wilks' Lambda group (p=0.25), time (p=0.41), and group x time (p=0.40). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels. (*) denotes a significant difference from baseline.

There was also a time effects for total work at sprint 3 (p=0.04). Tests of within-subjects contrast indicated a linear change in mean power at sprint 1 (p=0.03) and sprint 3 (p=0.02) and total work at sprint 3 (p=0.04). No other time effects observed for any variable during the anaerobic sprint test. There was no group x time interactions for peak power, mean power, or total work during any sprint of the anaerobic sprint test. Although changes from baseline were not statistically significant between groups across time (p=0.06), the Cohen's d effect size indicates a small significant increase in total work at sprint 1 (d=0.12) after supplementation. The results of the anaerobic sprint test provide evidence which rejects hypothesis 12 which states there will be differences among groups in anaerobic capacity as measured by repeated sprints on a cycle ergometer after 28 d of supplementation.

Clinical Chemistry Panels

Table 22 presents whole blood markers assessed during Study 2. MANOVA analysis revealed overall Wilks' Lambda (p=0.32), time (p=0.03), and group x time (p=0.76). Univariate analysis revealed no significant time or group by time effect for any whole blood marker. While changes from baseline were not statistically significant between groups across time (p=0.09), the Cohen's d indicates a small to moderate effect size decrease across time in RBC count after supplementation.

Table 22. Study 2: Whole Blood Markers

Markers	N	Group	Day 0	Day 7	Day 28	Mean (SEM)		p-level
MCV	11	PL	92.2±3.1	91.6±3.7	91.6±3.8	91.8±1.3	Group	0.79
(fL)	13	CrM	92.9±3.9	93.6±3.9	93.6±4.7	93.4±1.3	Time	0.47
	13	CrN-L	91.6±3.7	91.7±4.0	92.2±4.2	91.9±1.2	G x T	0.49
	11	CrN-H	93.2±2.9	93.4±3.1	88.6±17.7	91.7±1.2		
		Overall	92.5±3.4	92.6±3.7	91.5±9.8			
MCH	11	PL	30.3±1.7	30.4±1.2	30.9±2.3	30.6±0.3	Group	0.45
(pg/cell)	13	CrM	30.5±1.3	31.1±1.4	30.9±2.6	30.9±0.3	Time	0.23
	13	CrN-L	30.5±1.6	31.5±2.0	31.3±2.2	31.1±0.3	G x T	0.92
	11	CrN-H	31.2±0.8	31.2±1.3	31.5±1.2	31.3±0.3		
		Overall	30.7±1.4	31.1±1.5	31.2±2.0			
MCHC	11	PL	32.9±1.1	33.2±1.7	33.7±1.8	33.3±0.4	Group	0.40
(g/dl)	13	CrM	32.8±0.7	33.2±1.5	33.1±1.9	33.1±0.4	Time	0.53
	13	CrN-L	33.3±0.7	34.4±3.3	33.9±1.7	33.9±0.3	G x T	0.66
	11	CrN-H	33.5±0.7	33.5±1.6	32.2±5.9	33.1±0.3		
		Overall	33.1±0.8	33.6±2.2	33.2±3.4			
RBCDW	11	PL	13.2±0.5	13.1±0.9	13.±0.3	13.1±0.1	Group	0.02
(%)	13	CrM	13.5±0.9	13.7±0.9	13.9±0.8	13.7±0.1[#]	Time	0.11
	13	CrN-L	13.3±0.6	13.4±0.6	13.5±0.7	13.3±0.1	G x T	0.66
	11	CrN-H	12.8±0.6	13.2±0.6	13.1±0.4	13.0±0.1		
		Overall	13.2±0.7	13.3±0.8	13.4±0.7			
Platelet Count	11	PL	206±36	188±32	208±69	200±4	Group	0.35
(x10³/µl)	13	CrM	234±56	225±91	255±50	238±4	Time	0.56
	13	CrN-L	218±69	183±50	194±68	198±3	G x T	0.39
	11	CrN-H	220±60	412±713	224±58	285±3		
		Overall	219±56	256±376	219±64			

Values are means ± standard deviations. MANOVA analysis revealed overall Wilks' Lambda (p=0.32), time (p=0.03), and group x time (p=0.76). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels. (#) denotes a significant difference (p<0.05) from CrN-H and PL.

Table 22. Continued

Markers	N	Group	Day 0	Day 7	Day 28	Mean (SEM)		p-level
WBC	11	PL	6.2±1.7	5.3±1.5	6.2±1.1	5.9±0.4	Group	0.72
(x10³/μl)	13	CrM	6.0±1.7	6.6±1.3	6.4±1.3	6.3±0.4	Time	0.58
	13	CrN-L	6.2±1.6	5.9±1.8	5.4±1.3	5.9±0.3	G x T	0.18
	11	CrN-H	5.9±1.4	5.5±1.6	6.1±1.6	5.8±0.3		
		Overall	6.1±1.6	5.8±1.6	6.0±1.4			
RBC	11	PL	5.2±0.8	5.0±0.8	4.8±0.6	5.0±0.1	Group	0.25
(x10⁶/μl)	13	CrM	5.3±0.6	5.6±1.0	5.1±0.8	5.3±0.1	Time	0.09
	13	CrN-L	5.0±0.5	5.1±01.0	4.9±0.7	5.0±0.1	G x T	0.65
	11	CrN-H	5.5±0.8	5.1±0.5	5.1±0.5	5.2±0.1		
		Overall	5.3±0.7	5.2±0.8	5.0±0.6			
Hematocrit	11	PL	47.7±7.1	46.0±8.2	44.0±4.5	45.9±1.8	Group	0.16
(%)	13	CrM	49.5±6.7	52.5±9.7	47.6±8.4	49.9±1.8	Time	0.51
	13	CrN-L	46.2±5.5	47.1±10.3	44.8±7.1	46.0±1.7	G x T	0.44
	11	CrN-H	51.2±7.8	47.5±5.2	51.7±15.4	50.1±1.7		
		Overall	48.7±6.9	48.2±8.6	47.1±10.1			
Hemoglobin	11	PL	15.7±2.5	15.3±2.7	14.8±1.2	15.3±0.9	Group	0.17
(g/dl)	13	CrM	16.3±2.1	20.1±10.2	15.7±2.8	17.3±0.9	Time	0.63
	13	CrN-L	15.4±2.1	16.1±3.1	15.2±2.3	15.6±0.8	G x T	0.19
	11	CrN-H	17.2±2.7	15.9±1.4	18.7±9.5	17.2±0.8		
		Overall	16.1±2.4	16.8±5.5	16.2±5.4			

Values are means ± standard deviations. MANOVA analysis revealed overall Wilks' Lambda (p=0.32), time (p=0.03), and group x time (p=0.76). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels.

Table 23 presents biological markers of health. MANOVA analysis revealed overall Wilks' Lambda (p=0.32), time (p=0.03), and group x time (p=0.76) Univariate analysis revealed a significant time effects for creatinine (p<0.001). Tests of within-subject contrasts indicated a linear (p=0.001) change over time. There was a trend towards a significant time effect for BUN:Creatinine ratio (p=0.07) and alanine aminotransferase (ALT) (p=0.06). However, there was no significant group x time

effects for any biological health marker. Although changes from baseline were not statistically significant between groups across time for BUN:Creatinine ratio ($p=0.07$) and ALT ($p=0.06$), the Cohen's d indicates a small effect change across time in BUN:Creatinine ratio and ALT after supplementation.

Table 23. Study 2: Health Markers

Marker	N	Group	Day 0	Day 7	Day 28	Mean (SEM)		p-level
ALP	11	PL	11.2±14.6	10.5±10.7	12.4±10.9	11.4±3.0	Group	0.44
(U/L)	13	CrM	11.4±9.5	12.0±10.4	11.0±7.8	11.5±3.0	Time	0.36
	13	CrN-L	15.5±11.4	16.2±11.9	19.5±15.9	17.1±2.7	G x T	0.85
	11	CrN-H	12.1±7.1	13.6±6.1	14.3±11.7	13.3±2.7		
		Overall	12.7±10.7	13.2±9.9	14.5±12.2			
ALT	11	PL	27.2±8.6	30.2±13.8	32.1±30.6	29.9±4.2	Group	0.24
(U/L)	13	CrM	28.9±16.6	25.1±10.0	32.7±22.9	21.2±3.9	Time	0.06
	13	CrN-L	21.3±6.7	19.9±4.4	22.4±6.7	21.2±3.9	G x T	0.70
	11	CrN-H	28.7±13.2	30.0±15.7	36.8±23.2	31.8±3.9		
		Overall	26.4±11.9	26.2±12.2	30.9±22.1			
AST	11	PL	31.2±13.3	30.1±11.6	29.5±13.4	30.3±2.7	Group	0.90
(U/L)	13	CrM	28.3±10.2	26.4±7.7	32.5±12.3	29.1±2.7	Time	0.56
	13	CrN-L	26.0±7.0	30.6±25.3	26.0±6.0	27.5±2.5	G x T	0.47
	11	CrN-H	26.4±5.9	28.7±7.0	32.0±7.6	29.0±2.5		
		Overall	27.8±9.2	29.0±14.8	29.9±10.1			
CK	11	PL	252±128	294±399	206±161	251±82	Group	0.79
(U/L)	13	CrM	288±169	345±319	409±373	346±82	Time	0.35
	13	CrN-L	283±169	480±987	243±136	335±75	G x T	0.56
	11	CrN-H	284±171	350±203	425±287	352±75		
		Overall	277±157	371±565	321±265			
LDH	11	PL	226±180	167±29	157±33	184±14	Group	0.93
(U/L)	13	CrM	185±62	172±38	184±48	180±14	Time	0.20
	13	CrN-L	175±50	173±41	166±28	171±12	G x T	0.23
	11	CrN-H	173±29	176±30	182±38	177±12		
		Overall	188±95	172±34	172±38			

Values are means ± standard deviations. MANOVA analysis revealed overall Wilks' Lambda ($p=0.32$), time ($p=0.03$), and group x time ($p=0.76$). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels.

Table 23. Continued

Marker	N	Group	Day			Mean		p-level
			0	7	28	(SEM)		
BUN	11	PL	16.3±3.6	15.6±5.2	15.7±4.9	15.8±1.2	Group	0.64
(mg/dl)	13	CrM	15.3±5.5	15.2±4.9	16.3±6.1	15.6±1.2	Time	0.48
	13	CrN-L	14.9±3.8	13.3±3.9	13.5±4.6	13.9±1.1	G x T	0.64
	11	CrN-H	14.6±3.2	14.8±2.7	15.7±5.8	15.0±1.1		
		Overall	15.2±4.0	14.7±4.2	15.2±5.3			
Creatinine	11	PL	1.18±0.26	1.19±0.33	1.15±0.27	1.18±0.09	Group	0.97
(mg/dl)	13	CrM	1.15±0.46	1.26±0.36	1.23±0.39	1.21±0.09	Time	0.001
	13	CrN-L	1.09±0.25	1.21±0.31	1.23±0.31	1.18±0.81	G x T	0.12
	11	CrN-H	1.12±0.18	1.15±0.23	1.20±0.23	1.16±0.08		
		Overall	1.13±0.29	1.20±0.30*	1.20±0.29*			
BUN:Creatinine	11	PL	14.2±3.7	13.9±6.8	13.6±3.1	13.9±1.2	Group	0.78
(mg/dl)	13	CrM	13.7±3.6	12.8±5.3	14.0±6.1	13.5±1.2	Time	0.07
	13	CrN-L	14.1±4.2	11.4±3.8	11.3±3.4	12.3±1.1	G x T	0.13
	11	CrN-H	13.3±3.8	13.3±3.3	13.4±4.8	13.3±1.1		
		Overall	13.84±3.73	12.82±4.82	13.01±4.45			
Glucose	11	PL	102.0±18.6	102.1±15.3	94.9±12.6	99.7±3.4	Group	0.74
	13	CrM	97.5±13.9	99.7±15.1	100.4±16.8	99.2±3.4	Time	0.70
	13	CrN-L	96.1±8.5	96.1±7.9	96.4±17.9	96.2±3.1	G x T	0.27
	11	CrN-H	97.1±9.4	93.2±5.2	96.0±9.4	95.4±3.1		
		Overall	98.0±12.7	97.5±11.5	96.8±14.2			

Values are means ± standard deviations. MANOVA analysis revealed overall Wilks' Lambda (p=0.32), time (p=0.03), and group x time (p=0.76). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels. (*) denotes a significant difference from baseline.

Table 24 presents plasma lipids. A MANOVA analysis was run to assess plasma

lipid concentrations. MANOVA analysis revealed overall Wilks' Lambda (p=0.32), time

(p=0.03), and group x time (p=0.76). Univariate analysis showed no significant time

effects or group x time interaction for any variable presented. The results of the markers

of clinical health and safety analysis provide evidence which fails to reject the null

hypothesis of hypothesis 13 which stated there will be no significant differences among

groups in markers of clinical health and safety after 7 and 28 d of supplementation.

Table 24. Plasma Lipids

Marker	N	Group	Day 0	Day 7	Day 28	Mean (SEM)		p-level
TCHL	11	PL	165.1±33.4	161.5±38.8	164.6±38.8	163.7±9.1	Group	0.19
(mg/dl)	13	CrM	174.4±25.5	175.4±27.8	183.4±41.1	177.7±9.1	Time	0.38
	13	CrN-L	151.9±35.8	149.7±29.5	150.0±37.5	150.5±8.4	G x T	0.83
	11	CrN-H	162.7±25.1	155.9±23.8	160.7±29.1	159.8±8.4		
		Overall	163.0±30.5	160.0±30.7	163.9±37.4			
HDL	11	PL	49.2±11.4	50.6±10.2	50.3±12.8	50.0±3.8	Group	0.62
(mg/dl)	13	CrM	51.8±15.2	54.7±15.5	55.2±17.4	53.9±3.8	Time	0.36
	13	CrN-L	48.6±15.7	48.3±15.1	47.9±12.8	48.3±3.5	G x T	0.85
	11	CrN-H	46.7±10.7	47.2±12.3	48.7±10.6	47.5±3.5		
		Overall	49.0±13.1	50.0±13.4	50.3±12.8			
TCHL:HDL	11	PL	3.5±0.7	3.2±0.6	3.3±0.6	3.3±0.3	Group	0.82
(mg/dl)	13	CrM	3.7±1.5	3.5±1.3	3.7±1.6	3.6±0.3	Time	0.24
	13	CrN-L	3.3±0.8	3.3±1.0	3.3±1.1	3.3±0.3	G x T	0.74
	11	CrN-H	3.6±0.8	3.5±0.9	3.4±0.9	3.5±0.3		
		Overall	3.5±1.0	3.4±1.0	3.4±1.1			
LDL	11	PL	93.4±23.4	91.7±29.6	95.8±34.6	93.6±8.6	Group	0.34
(mg/dl)	13	CrM	103.8±33.3	106.6±33.5	112.2±49.0	107.5±8.5	Time	0.36
	13	CrN-L	86.3±22.6	84.8±23.7	87.3±28.7	86.1±7.9	G x T	0.88
	11	CrN-H	95.2±25.0	93.4±22.9	94.3±28.4	94.3±7.9		
		Overall	94.4±26.1	93.7±27.7	96.8±35.5			
TG	11	PL	109.1±52.5	98.2±36.9	94.2±19.1	100.5±10.7	Group	0.63
(mg/dl)	13	CrM	114.5±60.6	93.1±30.9	112.0±42.6	106.5±10.7	Time	0.16
	13	CrN-L	87.5±43.2	90.7±49.1	85.8±30.7	88.0±9.8	G x T	0.69
	11	CrN-H	104.8±47.1	85.6±39.2	97.2±49.8	95.9±9.8		
		Overall	103.3±50.2	91.6±39.0	96.8±37.8			

Values are means ± standard deviations. MANOVA analysis revealed overall Wilks' Lambda (p=0.32), time (p=0.03), and group x time (p=0.76). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels.

Side Effects

Tables 25 and 26 present frequency and severity, respectively, of symptoms reported. MANOVA analysis was run on side effects questionnaire to assess changes in symptoms which include frequency and severity of dizziness, headache, tachycardia, heart skipping or palpitations, shortness of breath, nervousness, blurred vision, and any

Table 25. Study 2: Side Effects - Frequency of Symptoms

Symptoms	N	Group	Day				Mean (SEM)		p-level
			7	14	21	28			
Dizziness	11	PL	0.09±0.30	0.91±0.30	0.18±0.40	0.09±0.30	0.11±0.09	Group	0.32
	11	CrM	0.18±0.60	0.27±0.47	0.36±0.92	0.27±0.65	0.27±0.09	Time	0.37
	13	CrN-L	0.00±0.00	0.08±0.28	0.23±0.60	0.08±0.28	0.10±0.09	G x T	0.81
	13	CrN-H	0.08±0.28	0.08±0.28	0.00±0.00	0.00±0.00	0.04±0.09		
		Overall	0.08±0.35	0.13±0.33	0.19±0.57	0.10±0.37			
Headache	11	PL	0.27±0.47	0.18±0.40	0.27±0.47	0.18±0.40	0.23±0.14	Group	0.22
	11	CrM	0.36±0.67	0.46±0.69	0.46±0.93	0.36±0.67	0.41±0.14	Time	0.74
	13	CrN-L	0.23±0.43	0.31±0.85	0.23±0.60	0.23±0.60	0.25±0.13	G x T	0.94
	13	CrN-H	0.27±0.47	0.00±0.00	0.00±0.00	0.00±0.00	3.5E-17±0.13		
		Overall	0.21±0.46	0.23±0.59	0.23±0.59	0.19±0.49			
Fast or Racing Heart Rate	11	PL	0.00±0.00	0.09±0.30	0.27±0.65	0.36±0.92	0.18±0.22	Group	0.64
	11	CrM	0.27±0.65	0.36±0.81	0.46±1.0	0.36±0.81	0.36±0.22	Time	0.38
	13	CrN-L	0.31±0.48	0.23±0.44	0.15±0.38	0.15±0.38	0.21±0.20	G x T	0.31
	13	CrN-H	0.46±1.2	0.54±1.1	0.54±1.1	0.54±1.1	0.52±0.20		
		Overall	0.27±0.74	0.31±0.72	0.35±0.81	0.35±0.81			
Heart Skipping or Palpitations	11	PL	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.1E-17±0.13	Group	0.63
	11	CrM	0.09±0.30	0.18±0.60	0.27±0.90	0.18±0.60	0.18±0.13	Time	0.67
	13	CrN-L	0.00±0.00	0.08±0.28	0.00±0.00	0.00±0.00	0.02±0.12	G x T	0.29
	13	CrN-H	0.23±0.83	0.15±0.55	0.15±0.55	0.15±0.55	0.17±0.12		
		Overall	0.10±0.42	0.10±0.42	0.10±0.51	0.08±0.40			
Shortness of Breath	11	PL	0.00±0.00	0.00±0.00	0.09±0.30	0.00±0.00	0.02±0.15	Group	0.62
	11	CrM	0.09±0.30	0.09±0.30	0.09±0.30	0.09±0.30	0.09±0.15	Time	0.34
	13	CrN-L	0.08±0.28	0.00±0.00	0.00±0.00	0.00±0.00	0.02±0.14	G x T	0.49
	13	CrN-H	0.31±1.12	0.23±0.83	0.23±0.83	0.23±0.83	0.25±0.14		
		Overall	0.13±0.61	0.08±0.45	0.10±0.47	0.08±0.45			
Nervousness	11	PL	0.00±0.00	0.09±0.30	0.09±0.30	0.00±0.00	0.05±0.15	Group	0.72
	11	CrM	0.18±0.40	0.27±0.47	0.27±0.47	0.27±0.47	0.25±0.15	Time	0.35
	13	CrN-L	0.15±0.55	0.08±0.28	0.08±0.28	0.08±0.28	0.10±0.14	G x T	0.38
	13	CrN-H	0.15±0.55	0.23±0.83	0.23±0.83	0.23±0.83	0.21±0.14		
		Overall	0.13±0.44	0.17±0.52	0.17±0.52	0.15±0.50			
Blurred Vision	11	PL	0.00±0.00	0.00±0.00	0.09±0.30	0.00±0.00	0.02±0.06	Group	0.27
	11	CrM	0.18±0.40	0.18±0.60	0.18±0.40	0.09±0.30	0.16±0.06	Time	0.33
	13	CrN-L	0.08±0.28	0.08±0.28	0.08±0.28	0.00±0.00	0.06±0.06	G x T	0.92
	13	CrN-H	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-3.3E-18±0.06		
		Overall	0.06±0.24	0.06±0.32	0.08±0.28	0.02±0.14			
Any other unusual or adverse effects	11	PL	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-2.5E-17±0.14	Group	0.13
	11	CrM	0.55±1.51	0.46±1.21	0.46±1.21	0.18±0.40	0.41±0.14	Time	0.36
	13	CrN-L	0.15±0.55	0.00±0.00	0.00±0.00	0.00±0.00	0.04±0.13	G x T	0.72
	13	CrN-H	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-5.3E-19±0.13		
		Overall	0.17±0.78	0.10±0.59	0.10±0.59	0.04±0.20			

Values are means ± standard deviations. MANOVA analysis revealed an overall Wilks' Lambda group (p=0.11), time (p=0.35), and group x time (0.34). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels.

Table 26. Study 2: Side Effects - Severity of Symptoms

Variable	N	Group	Day 7	Day 14	Day 21	Day 28	Mean (SEM)		p-level
Dizziness	11	PL	0.09±0.30	0.09±0.57	0.18±0.40	0.09±0.30	0.11±0.11	Group	0.27
	11	CrM	0.09±0.30	0.64±1.02	0.18±0.40	0.18±0.40	0.27±0.11	Time	0.28
	13	CrN-L	0.00±0.00	0.08±0.28	0.39±0.96	0.30±1.11	0.19±0.10	G x T	0.16
	13	CrN-H	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.10		
		Overall	0.42±0.20	0.19±0.57	0.19±0.57	0.15±0.62			
Headache	11	PL	0.64±1.21	0.18±0.40	0.55±1.04	0.36±0.92	0.43±0.21	Group	0.30
	11	CrM	0.55±1.04	0.36±0.67	0.55±0.82	0.55±0.82	0.50±0.21	Time	0.23
	13	CrN-L	0.54±1.05	0.46±1.20	0.39±0.96	0.31±0.85	0.42±0.19	G x T	0.79
	13	CrN-H	0.08±0.28	0.00±0.00	0.00±0.00	0.00±0.00	0.02±0.19		
		Overall	0.44±0.94	0.25±0.73	0.35±0.81	0.29±0.74			
Fast or Racing Heart Rate	11	PL	0.00±0.00	0.09±0.30	0.09±0.30	0.09±0.30	0.07±0.17	Group	0.41
	11	CrM	0.09±0.30	0.09±0.30	0.18±0.60	0.09±0.30	0.11±0.17	Time	0.77
	13	CrN-L	0.39±0.65	0.23±0.43	0.15±0.38	0.15±0.37	0.23±0.16	G x T	0.17
	13	CrN-H	0.31±0.85	0.46±0.96	0.46±0.97	0.46±0.96	0.42±0.16		
		Overall	0.21±0.58	0.23±0.59	0.23±0.63	0.21±0.58			
Heart Skipping or Palpitations	11	PL	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.8E-17±0.10	Group	0.60
	11	CrM	0.18±0.40	0.09±0.30	0.18±0.60	0.09±0.30	0.14±0.10	Time	0.74
	13	CrN-L	0.00±0.00	0.08±0.27	0.00±0.00	0.00±0.00	0.02±0.10	G x T	0.49
	13	CrN-H	0.15±0.55	0.15±0.55	0.15±0.55	0.15±0.55	0.15±0.10		
		Overall	0.08±0.34	0.08±0.34	0.08±0.40	0.06±0.32			
Shortness of Breath	11	PL	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	5.6E-17±0.16	Group	0.61
	11	CrM	0.09±0.30	0.09±0.30	0.18±0.60	0.18±0.60	0.14±0.16	Time	0.46
	13	CrN-L	0.08±0.28	0.00±0.00	0.00±0.00	0.00±0.00	0.02±0.14	G x T	0.39
	13	CrN-H	0.31±1.11	0.23±0.83	0.23±0.83	0.23±0.83	0.25±0.14		
		Overall	0.13±0.61	0.08±0.45	0.10±0.51	0.10±0.51			
Nervousness	11	PL	0.00±0.00	0.27±0.90	0.27±0.90	0.00±0.00	0.14±0.17	Group	0.49
	11	CrM	0.18±0.40	0.55±1.04	0.46±0.93	0.55±1.04	0.43±0.17	Time	0.400
	13	CrN-L	0.23±0.83	0.15±0.55	0.08±0.28	0.08±0.28	0.12±0.15	G x T	0.30
	13	CrN-H	0.15±0.55	0.27±0.90	0.15±0.55	0.15±0.55	0.15±0.15		
		Overall	0.15±0.55	0.25±0.72	0.23±0.69	0.19±0.61			
Blurred Vision	11	PL	0.00±0.00	0.00±0.00	0.27±0.65	0.00±0.00	0.07±0.08	Group	0.31
	11	CrM	0.27±0.64	0.09±0.30	0.27±0.65	0.18±0.60	0.21±0.08	Time	0.13
	13	CrN-L	0.08±0.28	0.00±0.00	0.15±0.55	0.00±0.00	0.06±0.07	G x T	0.76
	13	CrN-H	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.3E-17±0.07		
		Overall	0.08±0.35	0.02±0.14	0.17±0.60	0.04±0.29			
Any other unusual or adverse effects	11	PL	0.09±0.30	0.00±0.00	0.00±0.00	0.00±0.00	0.02±0.10	Group	0.05
	11	CrM	0.18±0.40	0.27±0.65	0.46±1.21	0.45±0.93	0.34±0.10	Time	0.77
	13	CrN-L	0.23±0.83	0.00±0.00	0.00±0.00	0.00±0.00	0.06±0.09	G x T	0.57
	13	CrN-H	0.00±0.0	0.00±0.00	0.00±0.00	0.00±0.00	-2.2E18±0.09		
		Overall	0.13±0.49	0.06±0.32	0.10±0.59	0.10±0.47			

Values are means ± standard deviations. MANOVA analysis revealed an overall Wilks' Lambda group (p=0.11), time (p=0.35), and group x time (p=0.34). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels.

other unusual or adverse effects. MANOVA analysis revealed an overall Wilks' Lambda group (p=0.11), time (p=0.35), and group x time (0.34). Univariate analysis revealed no significant time (p=0.35) or group x time (p=0.34) effects for frequency or severity of reported symptoms.

Herein we report the weekly occurrence of a symptom ranking of 2 or greater on a $0 - 5$ ranking scale with each treatment group. In the PL group, participants reported headache/severity=3 (N=2) during wk 1, nervousness/severity=3 (N=1) during wk 2, tachycardia/frequency=2 (N=1), headache/severity=2 (N=1), nervousness/severity=3 (N=1), and blurred vision/severity=3 (N=1) during wk 3, and fast or racing heart rate/frequency=3 (N=1) during wk 4.

In CrM group, participants reported headache/frequency=2 (N=1), fast or racing heart rate/frequency=2 (N=1), adverse effect/frequency=5 (N=1), headache/severity=1 (N=1), blurred vision/severity=2 (N=1) during wk 1, headache/frequency=2 (N=1), tachycardia/frequency=2 (N=2), heart skipping or palpitations/frequency=2 (N=1), blurred vision/frequency=2 (N=1), adverse effect/severity=4 (N=1), headache/severity=2 (N=1), nervousness/severity=2 and 3 (N=2), adverse effect/severity=2 (N=1) during week 2, dizziness/frequency=3 (N=1), headache/frequency=3 (N=1), tachycardia/frequency=2 and 3 (N=2), heart skipping or palpitations/frequency (N=1), adverse effect/frequency=4 (N=1), headache/severity=2 (N=2), tachycardia/severity=2 (N=1), heart skipping or palpitations/severity=2 (N=1), shortness of breath/severity=2 (N=1), blurred vision/severity=2 (N=1), and adverse effect/severity=4 (N=1) during week 3, and dizziness/frequency=2 (N=1), headache/frequency=2 (N=1),

tachycardia/frequency=2 (N=1), headache/frequency=2 (N=1), tachycardia/frequency=2 (N=1), headache/severity=2 (N=2), shortness of breath/severity=3 (N=1), nervousness/severity=3 (N=1), blurred vision/severity=2 (N=1), and adverse effect/severity=3 (N=1) during wk 4.

In CrN-L group participants reported nervousness/frequency=2 (N=1), adverse effect/frequency=2 (N=1), headache/severity=2 (N=2), headache/severity=3 (N=3), tachycardia/severity=2 (N=1), nervousness/severity=3 (N=1) during week 1, headache/frequency=3 (N=1) and headache/severity=2 (N=1) and 4 (N=1) during week 2, dizziness/frequency=2 (N=1), headache/frequency=2 (N=1), dizziness/severity=2 (N=1) and 3 (N=1), headache/severity=2 (N=1) and 3 (N=1), and blurred vision/severity=2 (N=1) during wk 3, and headache/frequency=2 (N=1), dizziness/severity=4 (N=1), headache/severity=3 (N=1) and adverse effect/severity (N=1).

In CrN-H group participants reported fast or racing heart/frequency=2 (N=1) and 4 (N=1), heart skipping or palpitation/frequency=3 (N=1), shortness of breath/frequency=4 (N=1), tachycardia/severity=3 (N=1), heart skipping or palpitations/severity=2 (N=1), shortness of breath/severity=4 (N=1), nervousness/severity=2 (N=1) during wk 1, tachycardia/frequency=2 (N=2) and 3 (N=1), heart skipping or palpitations/frequency=2 (N=1), shortness of breath/frequency=3 (N=1), nervousness/frequency=3 (N=1), fast or racing heart/severity=2 (N=1) and 3 (N=1), shortness of breath/severity=3 (N=1), heart skipping or palpitations/severity=2 (N=1), and nervousness/severity=2 (N=1) during wk

2, tachycardia/frequency=2 (N=2) and 3 (N=1), heart skipping or palpitation/frequency=2 (N=1), shortness of breath/frequency=3 (N=1), nervousness/frequency=3 (N=1), tachycardia/severity=2 (N=1) and3 (N=1), heart skipping or palpitations/severity=2 (N=1), shortness of breath/severity=3 (N=1), and nervousness/severity=2 (N=1) during wk 3, and fast or racing heart/frequency=2 (N=2) and 3 (N=1), heart skipping/frequency=2 (N=1), shortness of breath/frequency=3 (N=1), nervousness/frequency=3 (N=1), fast or racing heart/severity=2 (N=1) and 3 (N=1), heart skipping or palpitation/severity=2 (N=1), shortness of breath/severity=3 (N=1), and nervousness/severity=2 (N=1) during wk 4. The results of the side effects questionnaire analysis provides evidence which accepts the null hypothesis of hypothesis 14 which states there will be no significant differences between groups in side effects symptoms after 7, 14, 21, and 28 d of supplementation.

CHAPTER V

DISCUSSION AND CONCLUSIONS

Creatine and nitrate are popular supplements amongst those wishing to improve exercise performance; yet have not been investigated together. We examined creatine nitrate (CrN) at a recommended dose (CrN-L) and twice the recommended (CrN-H) compared to creatine monohydrate (CrM) and a placebo (PL). The purpose of this study was to examine the acute safety and chronic efficacy of a lower dose of CrN (CrN-L: 6 g CrN-L/d for 7 d and 1.5 g CrN-L/d for 21 d), or a higher dose of CrN (CrN-H: 12 g CrN-H/d for 7 d and 3 g CrN-H/d for 21 d) compared to CrM (CrM: 20 g CrM/d for 7 d and 5 g CrM for 21 d) and PL (26 g dextrose/d for 7 d and 6.5 g/d for 21 d) on muscle creatine retention, body composition, strength, anaerobic capacity and markers of clinical health. Our findings refute the current marketing claims indicating that CrN is 10 times more effective than CrM, the gold standard form of creatine. The results of the present study show that creatine nitrate supplementation is safe; however, neither dose of CrN was more effective than CrM in enhancing muscle creatine storage, improving body composition, upper body strength, or anaerobic capacity.

Study 1

We observed significant changes in plasma creatine and nitrate after acute supplementation – CrM (5 g CrM, 1.5 g dextrose), CrN-L (1 g Cr, 0.5 g Nitrate, 5 g dextrose, CrN-H (2 g Cr, 1 g Nitrate, 3.5 g dextrose). We observed no significant differences among groups in baseline plasma creatine concentrations. Peak plasma Cr

concentrations were greatest with CrM, followed by CrN-H, then CrN-L. There were no significant changes in plasma Cr with PL. Peak plasma Cr concentrations occurred after 1 h of ingestion of one dose of supplementation and seemed unaffected by the form of Cr. Plasma Cr area under the curve (AUC) was significantly greater with CrM compared to all other groups, while no significant differences in plasma Cr AUC were observed among PL, CrN-L, and CrN-H. Other researchers have reported similar time-to-peak concentrations. Jager et al. (61), examined changes in plasma Cr concentrations for 8-hrs after supplementation with CrM, tri-creatine citrate (CrC), and creatine pyruvate (CrPyr). Plasma Cr concentrations peaked 1 h post supplementation regardless of the form of Cr. Peak plasma Cr concentrations at 1 h post supplementation were reported to be significantly greater with CrPyr when compared to CrC and CrM.

Changes in plasma nitrate concentration appear to be dose-dependent. Plasma nitrate concentrations with CrN-H were significantly greater than all groups at all time-points (0.5, 1, 2, 3, 4, 5-hr) during acute supplementation. Although, significantly lesser than CrN-H, plasma nitrate concentration with CrN-L was significantly greater than CrM and PL at all time-points. Peak plasma nitrate concentration was achieved 1 h post supplementation. Others have also noticed similar time-to-peak plasma nitrate concentrations. Cortas et al. (29), reported peak plasma nitrate concentration after 40-minutes of supplementation with 0.47 mmol nitrate • kg (~2 g nitrate for 70 kg person). Bartholomew et al. (9), also observed plasma nitrate concentrations peak after 30 – 60 minutes of supplementation with 25, 50, 100, and 170 mg potassium nitrate. AUC for plasma nitrate for CrN-H were significantly greater compared to CrM and PL, but not

significantly different than CrN-L. This suggests that CrN-L and CrN-H are bioequivalent; however, plasma nitrate concentrations were measured over a finite period of time. AUC calculations would need to be extrapolated infinitely to determine accurate bioequivalence.

Several studies have reported on the effects of nitrate supplementation on blood pressure (BP) and heart rate (79, 89, 123). We observed a significant decrease (~3 mm Hg) in systolic blood pressure (SBP) in all groups 1-hr after ingestion of supplementation with no significant differences observed among groups, while observing no significant changes in diastolic blood pressure (DBP) and heart rate (HR). Larsen et al. (79), reported no change in SBP after administering 0.1 mmol • kg • d^{-1} (~430 mg nitrate for a 70 kg person) for three days. Similarly, Murphy and colleagues (89) failed to observed a change in BP 1 h post-consumption of 200 g beetroot consumption ($\geq$ 500 mg nitrate). On the other hand, Webb et al. (123), observed a ~10 mm Hg decrease in systolic blood pressure 2.5 h after ingestion of 0.5 L (~1,400 mg nitrate) of beetroot juice (BRJ). Our findings for DBP changes in response to 0.5 g (CrN-L) and 1 g (CrN-H) of dietary nitrate are in agreement with some, but not all. Murphy et al. (89), who reported no significant change in DBP after the consumption of 200 g beetroot ($\geq$500 mg nitrate). Conversely, Larsen et al. (79) and Webb et al., (89, 123) both observed a decrease in DBP of 3.7 and 8.1 mm Hg after ~430 mg (70 kg body weight at 0.1 mmol • kg • d^{-1}) and 1,400 mg nitrate supplementation, respectively. Similarly, Larsen (79) and Webb (89, 123) failed to observe a significant change in HR after nitrate supplementation. In Study 1 we provided 0.5 g nitrate (CrN-L) and 1 g

124

(CrN-H) nitrate during Study 1 and found no evidence that CrN reduced blood pressure or affects HR in comparison to PL or CrM. Additionally, no participant had hypotensive response (SBP<90 mm Hg, DBP<60 mm Hg) to either dose of CrN studied. It is possible that doses greater than those prescribed in this study are required to affect blood pressure.

The nitrate doses provided in this study (0.5 − 1 g nitrate) are within the range recommended by national guidelines (25). Dietary nitrates are found primarily in plant-based foods such as fruits and vegetables (56). Therefore, the dietary approach to stop (DASH) diet, which recommends 4 − 5 servings of fruits and vegetables, is often prescribed as a nutritional therapy to reduce blood pressure. Celery, lettuce, red beetroot, and spinach are known to contain very high concentrations of nitrate (>250 mg nitrate/100 g food), while artichokes, asparagus, green beans, tomato, and watermelon are known to contain very low concentrations of nitrate (<20 mg nitrate/100 g food) (105). Researchers have reported that the daily nitrate concentration of the DASH diet can vary from ~175 mg to ~1220 mg nitrate by consuming low-nitrate or high-nitrate fruits and vegetables (56).

Plasma creatinine concentrations among groups were within normal physiological ranges for healthy, young males, although we observed significant interactions among groups. Creatinine significantly increased from baseline among all groups from 0.5 h to 2 h post-supplementation, then decrease back to baseline values thereafter. Creatinine concentration with CrN-L and CrN-H were significantly greater than PL at 0.5, 1, 2, 3, and 4 h, while only being significantly greater than CrM 0.5, 1,

and 2 h post-supplementation. Since creatinine is the end product of creatine metabolism via a spontaneous, nonenzymatic process it is reasonable to expect an increase in plasma creatinine after introducing exogenous creatine supplementation (129). With the exception of plasma creatinine, we observed no significant group x time interaction for any other blood marker in Study 1.

All blood markers were within normal physiologic ranges with the exception of ALP. At baseline, plasma ALP concentrations for all groups (11.9±8.5 U/L) were slightly below normal physiologic ranges of 30 – 100 (103). Although plasma ALP concentrations significantly increase over time among all groups, it remained below (15.5 – 21.1 U/L) normal physiologic ranges throughout the 5 h study period. Some may argue that the CK values for CrN-H are consistently above (453 – 504 U/L) normal clinical ranges of 50 – 398 U/L (112); however, athletic populations are known to regularly have greater CK concentrations than their sedentary counterparts. Mougios (87) examined the CK concentration of 483 male athletics from various sport backgrounds. A plasma CK reference interval of 82 - 1083 U/L was calculated for all male athletes, with football players (183 players) having an upper reference interval of 1492 U/L for plasma CK.

Although there were no significant group x time interactions for any blood lipid marker, we observed HDL significantly increased by 7 %, while TG significantly decreased by 16% at 5 h post-supplementation among all groups. Others have also reported change in blood lipids with Cr supplementation (33, 75). Kreider et al. (75) reported a ~15% increase in HDL in NCAA division IA football players after 28 d of Cr

126

supplementation (15.75 g Cr • d^{-1} • 28 d^{-1}). In the same study, Kreider and coworkers (75) also observed a 13% decrease in very low-density lipoprotein and 7% reduction in TCHL: HDL ratio. Earnest et al., also reported a significant reduction in TG by approximately 23% after 4 and wk of Cr supplementation (20 g Cr • d^{-1} • 5 d^{-1}; 10 g Cr • day^{-1} • 51 d^{-1}) (33). Conversely, Earnest and colleagues observed no significant changes in HDL cholesterol after the Cr supplementation period.

Creatine supplementation has repeatedly been shown to have minimally significant side-effects with no major impact on health markers (19), while a nitrate-rich diet is suggested to a beneficial influence on health (16). Although minor side-effects were reported in Study 1 there were no significant difference observed among groups or in the pattern and/or severity of the few symptoms reported. Symptom frequency and severity were evenly distributed among all groups, including PL group.

Study 2

CrN is a novel form of creatine-salt although many other forms of creatine salts already exist in the market place. Creatine-salts are typically developed due to the chemical properties of creatine (Cr). Cr, with a base dissociation constant (pKb) of 11.08, is a weak base and as a result can only form salt solutions with strong acids with an acid dissociation constant (pKa) of less than 3.98 (62). In the gastric environment CrN with a pKa of 3.8 would dissociate into its parent compounds, Cr and nitrate. As soon as CrN made contact with the gastric environment (up to 0.1 M hydrochloric acid, and sodium and potassium chloride), nitrate would be displaced by the excess of

chloride to yield creatine hydrochloride. The dissociation of Cr involves the hydrolysis of carboxyl groups (62), which is where the nitrate anion would be attached.

Pandit et al. (72), reported creatine nitrate (CrN) to be more soluble than creatine monohydrate (CrM) under various experimental conditions. They observed an increased solubility in CrN compared to CrM and buffered creatine (BC) in water at room temperature as well as in pH 2.5 buffer at room temperature and 37° C. CrN was approximately 10-fold more soluble than CrM and BC under all three conditions. Some claim that enhancing product solubility can enhance bioavailability and rate of absorption, which, in turn, results in increased creatine uptake and storage in the muscle (72). This claim of greater bioavailability is made despite previous research showing the absorption and bioavailability of CrM near 100% (32, 61, 62).

This is the first study to measure muscle Cr concentration after CrN supplementation. Our study failed to show greater Cr retention with CrN-L (6 g CrN-L/d for 7 d and 1.5 g CrN for 21 d) or CrN-H (12 g CrN-H/d for 7 d and 3.0 g/d for 21 d) compared to a standard loading and maintenance dose of CrM (12 g CrM/d for 7 d and 3 g CrM/d for 21 d). The present study showed a significantly greater increase in muscle creatine retention with CrM (20.2±16.4%) compared to CrN-L (-6.8±5.6%) and PL (-0.4±6.0%) after 7 d of supplementation. Although muscle creatine retention significantly increased with CrN-H by 8.5% there were no significant differences compared to CrM after 7 d of supplementation. After 28 d of supplementation muscle creatine retention was significantly greater with CrM (25.7±5.0%) compared to CrN-L (-8.5±4.6%), CrN-H (1.1±4.6%), and PL (-4.2±5.0%). Regarding the CrN-H group, our findings suggest

128

that the loading phase (12 g CrN • d^{-1} • 7 d^{-1}[providing 8 g/d of creatine) was sufficient to significantly increase muscle Cr concentrations after 7 d of supplementation, but the dose (3 g CrN • d^{-1} • 21 d^{-1}[providing 2g/d of Cr]) provided during the maintenance phase was not sufficient to maintain elevated muscle Cr stores. These finding contrast claims that there is no need to load when taking CrN due to greater solubility and retention.

Theoretically, 2 g of exogenous Cr should maintain elevated muscle Cr stores as the turn-over rate of endogenous Cr is approximately 2 g/d (122). However, some researcher have failed to observe a significant increase in muscle Cr when supplementing with 2 g Cr/d for 6 wk (114). Researchers have stated that 2 g of Cr supplementation may be insufficient to maintain muscle Cr store for larger, physically active individuals as high-intensity exercise training promotes greater Cr turnover (127). It is conceivable that participants in Study 2 required greater increment of CrM to maintained elevated muscle Cr stores as muscle Cr stores were not maintained with twice the recommended dose of CrN (3 g CrN) providing approximately 2 g of Cr. This suggests that CrN is not more efficacious than CrM at improving greater Cr uptake and storage. Other researchers have provided athletes with higher Cr doses during the maintenance phase of supplementation. For example, Kreider et al. (75), provided NCAA Division IA college football athletes with 15.75 g Cr during the loading phase and 5 – 10 g Cr during the maintenance phase of supplementation. Daily urinary Cr excretion (an estimate of whole body creatine retention) levels were low indicating that these athletes needed higher doses of Cr to maintain creatine stores.

129

Since CrN is approximately 66% Cr, the 3 g of CrN that were provided daily during the maintenance phase in CrN-H group provided approximately 1.98 g of exogenous Cr. Hultman et al., demonstrated that 0.03 g of Cr $\bullet$ kg body mass^{-1} $\bullet$ d^{-1} during the maintenance phase maintained elevated Cr stores (58). The participants in the CrN-H group weighed 90.8±13.8 kg at d 7 and therefore received approximately 0.02 g of Cr $\bullet$ kg body mass^{-1} $\bullet$ d^{-1} for 21 d. Others have based Cr dose on fat-free mass. Burke et al. (21), showed a significant increase in total Cr (TCr) after 8 wk of Cr supplementation. Participants ingested 0.25 g of Cr $\bullet$ kg fat-free mass^{-1} $\bullet$ d^{-1} for 7 d, followed by 0.0625 g of Cr $\bullet$ kg fat-free mass^{-1} $\bullet$ d^{-1} for 49 d. Using fat-free mass as a determinant for dosing guidelines, the participants in the CrN-H group received 0.11 g of Cr $\bullet$ kg fat-free mass^{-1} $\bullet$ d^{-1} for 7 d, followed by 0.0292 g of Cr $\bullet$ kg fat-free mass^{-1} $\bullet$ d^{-1} for 21 d; approximately 50% less than what Burke et al. prescribed according to fat-free mass. The inability to maintain elevated stores with 3 g of CrN (CrN-H) may be due to an insufficient dosage of creatine in these athletes.

Results of the present study indicated that there was a significant increase in body weight in all groups as has been reported by others. Volek et al. (120), have reported an increase in total body weight of 1.5 kg and 5.2 kg after one and 12 wk of CrM supplementation, respectively. The greatest changes in body weight were seen in the CrM group after 7-days of supplementation (0.63±0.81 kg) and with CrN-H after 28-days of supplementation (1.17±1.38 kg). It is important to note that Volek et al. (120), supplemented their subjects with 25 g of CrM for 5 d followed by 5 g of supplementation for the remained of the 12 wk study. During our loading phase, the

130

CrM and CrN-H group received 12 g of CrM and 12 g of creatine nitrate (CrN) per d, respectively. During the loading phase, the CrM and CrN-H group received 3 g of CrM and 3 g of CrN per d, respectively.

We also observed a significant increase in fat-free mass in all groups with an average increase of 0.30 ± 1.22 and 0.56 ± 1.30 kg after 7 and 28 d of supplementation, respectively. These increases in fat-free mass are lesser than those seen in previous Cr supplementation studies (11, 47, 120). The lack of significant change in total body water among all groups suggests that the change in fat-free mass were not attributed to an increase in water retention that has been reported to follow Cr supplementation (99). We failed to observe any significant differences in caloric and macronutrient intake and training volume suggesting that the significant change in body weight and fat-free mass observed results from the supplemental treatments provided.

Cr supplementation is well known for improving muscular strength and power (34, 94, 117, 121). In the present study, there was a significant improvement over time in all groups in maximum repetitions, workload (weight [kg] x repetitions), peak power, average power, and average velocity during the bench press test. On average the greatest percent increase in workload, peak power, average power, and average velocity were observed with CrN-H (35.4%, 16.2%, 9.3%, and 14.2%) and CrM (26.9%, 16.7%, 9.3%, and 12.9%). Despite the greater percent increase with CrN-H and CrM, there were no significant differences between groups in percent change in workload, peak power, average power, and average velocity after 28 d of supplementation. Maximum bench press repetitions increased by19.0%, 26.9%, 20.5%, and 35.5% with PL, CrM, CrN-L,

and CrN-H, respectively, after 28 d of supplementation; however, there were no significant difference in percent change among groups.

Similar increases in maximum bench press repetitions using 70% of 1RM were observed by Earnest et al (34). Experienced resistance-trained males were examined on strength indices before and after Cr supplementation (20 g Cr • d^{-1} • 14 d^{-1}). The Cr group significantly increased 1RM bench press and bench press repetition using 70% of 1RM by 35%. Others have reported similar changes in bench press power with a shorter supplementation period. Izquierdo et al., observed a significant increase (20%) in bench press power after five days of Cr (20 g Cr • d^{-1} • 5 d^{-1}) supplementation in handball players (60).

We observed no significant difference among groups in overall peak power, mean power, and total work during anaerobic sprint capacity test. On average, peak power increase by 0.4%, 4.0%, 5.3%, and 3.0% and with PL, CrM, CrN-L, and CrN-H, respectively. Mean power increased only with CrN-L and CrM by 7.6% and 5.5%, respectively; while mean power slightly decreased by 0.6% and 2.1% with CrN-H and PL. Changes in mean power during anaerobic sprint capacity test were not significant among groups. Total work increase by 3.9%, 3.0%, 7.3%, and 0.3% with CrN-H, CrN-L, PL, and CrN, respectively, but there was no significant difference among groups. Similar improvements were reported by others. Dawson et al. (30), observed a 4.5% and 4.6% increase in total work and peak power, respectively, on a cycle ergometer after Cr supplementation (20 g Cr • d^{-1} • 5 d^{-1}). Jagim et al. (63), reported a 3% improvement in

total work during a 30-second cycle ergometer test after 28-days of CrM and two doses of a buffered form of Cr supplementation.

The lack of significant difference during the anaerobic sprint capacity may be explained by several factors. It is possible that a learning effect was present, although we attempted to reduce a learning effect by having participants practice the anaerobic capacity test during the familiarization session. A normal intra-personal variation in performance could have also influence cycle ergometer performance before and after the supplementation period. The magnitude of change in muscle Cr concentration may have also played a role in performance outcomes. We observed an approximate -4%, 26%, 8%, and 1% change with PL, CrM, CrN-L, and CrN-H, respectively, in muscle Cr concentration after 28 d of supplementation. Finn et al. (37), examined cycle ergometer performance (4 x 20-sec sprints with 20-sec recovery between sprints) in triathletes after Cr supplementation (20 g Cr/d) for 5 d. They reported no significant changes in peak power and mean power performance during the cycle ergometer test as well as no significant increases in muscle Cr concentration, although muscle Cr increased by ~20% after the supplementation period. It is also possible that some participants in our study did not respond to the Cr supplementation protocol. Approximately $20 - 30\%$ of those ingesting Cr supplementation show little to no response as evidence by muscle Cr concentrations (43). Greenhaff et al. (42), characterized non-responders as those with less than a 10 mmol/kg dw response in muscle total creatine after loading with Cr supplementation. Based on muscle Cr analysis we would have expected greater a significantly greater power output with CrM (42.5 ± 0.9 mmol • kg^{-1} DW) as Cr stores

were significantly greater than CrN-L (32.3 ± 5.6 mmol • kg^{-1} DW), CrN-H (40.9 ± 9.9 mmol • kg^{-1} DW), and PL (33.2 ± 5.4 mmol • kg^{-1} DW), but that was not the case. This further demonstrates that CrN-L and CrN-H are not more efficacious than CrM during repeated anaerobic sprints on a cycle ergometer.

Similar to Study 1, we observed ALP values ($10.48 – 19.48$ U/L) slightly below normal ($30 – 100$ U/L) physiologic values at baseline, prior to supplementation (103). However, there were no significant differences among groups in changes in ALP after 7 and 28 days of supplementation. With the exception of ALP values, all other markers of whole blood, clinical health, and blood lipids were within normal ranges.

There is supporting evidence that shows Cr supplementation to have minimal to no negative effects on markers of clinical health and safety (19, 63, 75, 76). In the present study, neither dose of CrN or CrM resulted in significantly different side-effects or health outcomes compared to PL. These findings suggest that both doses of CrN are well tolerated and are just as safe to consume as CrM and PL.

Summary and Conclusions

Results of Study 1 indicate that acute supplementation with a lower dose (CrN-L: 1 g Cr, 0.5 g nitrate, 5 g dextrose) and higher dose (CrN-H: 2 g Cr, 1 g nitrate, 3.5 g dextrose) of CrN is as safe as CrM (5 g CrM, 1.5 g dextrose). In Study 1, all blood markers were within normal physiology range and lacked a clinically significant change during the 5 h treatment period. Although minimal side effects were infrequently reported, they were distributed among all groups including PL (6.5 g dextrose).

Results of the Study 2 indicate that dietary supplementation with a lower dose (1.5 g CrN-L • 4 x/d^{-1} • 7 d^{-1}; 1.5 g CrN-L • d^{-1} • 21-d^{-1}) and higher dose (3.0 g CrN-H • 4 x/day^{-1} • 7 d^{-1}; 3.0 g CrN-H • d^{-1} • 21-d^{-1}) of CrN is safe for chronic consumption up to 28 d. We found CrN-L and CrN-H to significantly increase plasma nitrate concentration after 7 days of supplementation; however, plasma nitrate concentrations did not remain significantly elevated, compared to baseline, after 28 d of supplementation. During Study 2 we observed a significant increase in plasma creatine only with CrM after the 7 d loading phase (12 g CrM • d^{-1} • 7 d^{-1}), while observing no significant increase from baseline or differences between CrN-L (6 g CrN • d^{-1} • 7 d^{-1}) CrN-H (12 g CrN • d^{-1} • 7 d^{-1}) and PL (26 g dextrose • d^{-1} • 7 d^{-1}) after the 7 d loading period. Furthermore, muscle Cr concentration only significantly increase after the loading period with CrM and CrN-H and only remained significantly elevated with CrM after 28 d of supplementation. Our findings refute claims that an increase in solubility leads to an increase in absorption and storage.

We observed significant increase in bench press workload (weight [kg] x max reps) in all groups (but no group x time interaction) after supplementation, however, only CrN-H group demonstrated significance compared to PL group. Similar findings were observed for bench press peak and average power, with only CrN-H demonstrated significance compared to PL and CrN-L, but not significantly different than CrM. We failed to observe a significant treatment effect for the anaerobic sprint capacity test. Body mass also increased over time in all groups with significantly greater improvements in fat-free mass only observed with CrM and CrN-H. Furthermore, only

changes in fat-free mass with CrN-H were significantly greater than CrN-L, but not significantly different than CrM and PL. The exercise performance and slight changes in body composition suggests little to no evidence that either dose of CrN is more efficacious than CrM. Overall, CrN was well tolerated, demonstrated similar performance benefits to loading and maintenance doses of CrM, and void of significant alterations in hemodynamics or blood enzymes denoting hepatorenal and muscle enzyme safety. However, we found no evidence that CrN at recommended or twice recommended doses is more efficacious than CrM at the doses studied.

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APPENDIX A

Consent Form

Project Title: **Pharmacokinetic Assessment of Acute Ingestion of Different Forms of Creatine**

You are invited to take part in a research study being conducted by Dr. Richard Kreider, a researcher from Texas A&M University and funded by Woodbolt International. The information in this form is provided to help you decide whether or not to take part. If you decide to take part in the study, you will be asked to sign this consent form. If you decide you do not want to participate, there will be no penalty to you, and you will not lose any benefits you normally would have.

Why Is This Study Being Done?
The purpose of this study is to examine acute pharmacokinetics of different forms of creatine compared to placebo on serum creatine/creatinine, serum nitrate/nitrite, serum glucose, blood pressure, heart rate, endothelial function and self-reported side effects.

Why Am I Being Asked To Be In This Study?
You are being asked to be in this study because you are an apparently healthy and recreationally active man between the ages of 18 and 40. You will not be allowed to participate if; you have a history of treatment for metabolic disease (i.e., diabetes), hypertension, hypotension, thyroid disease, arrhythmias and/or cardiovascular disease; you are currently using any prescription medications; you have a history of smoking; you drink excessively (12 drinks per week or more); or you have a recent history of creatine supplementation within six weeks of the start of supplementation. If you do not qualify for this study we will keep your contact information (phone number and/or e-mail) and contact you at a later date for potential entry into a similar study with your permission.

How Many People Will Be Asked To Be In This Study?
Approximately 12 people (participants) will be invited to participate in this study locally.

What Are the Alternatives to being in this study?
The alternative to being in the study is not to participate.

What Will I Be Asked To Do In This Study?
You will be asked to not exercise, consume no alcohol and refrain from non-steroidal anti-inflammatory drugs (NSAIDS) for 48 hours nor eat or drink calorie containing foods or drinks 12 hours before each testing session/visit. Your participation in this study will last up to approximately four weeks and include five visits (visit 1 ~ 1 hour/visits 2, 3, 4 & 5 ~ 6 hours/visit). These visits are detailed below and in the protocol overview table below.

149

Protocol Overview

Familiarization	T1	T2	T3	T4
Phone Screening	4 Day Food Record	4 Day Food Record	4 Day Food Record	4 Day Food Record
Familiarization	48 hour prior: no exercise, alcohol, and NSAIDS	48 hour prior: no exercise, alcohol, and NSAIDS	48 hour prior: no exercise, alcohol, and NSAIDS	48 hour prior: no exercise, alcohol, and NSAIDS
Physical Exam				
Body Weight	8 hour fast	8 hour fast	8 hour fast	8 hour fast
Schedule Testing	Body Weight	Body Weight	Body Weight	Body Weight
Randomized, Double Blind, Crossover Administration of Supplements with at least a 1 week washout period:	DEXA Body Composition	DEXA Body Composition	DEXA Body Composition	DEXA Body Composition
1. CrM (5 gm CrM with 1.5 gm dextrose)	Flow-mediated dilation (FMD) 0-h 1-h 2.5h	Flow-mediated dilation (FMD) 0-h 1-h 2.5h	Flow-mediated dilation (FMD) 0-h 1-h 2.5h	Flow-mediated dilation (FMD) 0-h 1-h 2.5h
2. CrN-1 (1 gm CrM, 0.5 gm N with 5 gm dextrose) 3. CrN-2 (2 gm CrM, 1 gm N with 3.5 gm dextrose) 4. Placebo (6.5 gm dextrose)	Fasting Blood Samples (7 total) 0-h 0.5-h 1-h 2-h 3-h 4-h 5-h	Fasting Blood Samples (7 total) 0-h 0.5-h 1-h 2-h 3-h 4-h 5-h	Fasting Blood Samples (7 total) 0-h 0.5-h 1-h 2-h 3-h 4-h 5-h	Fasting Blood Samples (7 total) 0-h 0.5-h 1-h 2-h 3-h 4-h 5-h
	Blood pressure, heart rate and side effect questionnaire at each data collection point.	Blood pressure, heart rate and side effect questionnaire at each data collection point.	Blood pressure, heart rate and side effect questionnaire at each data collection point.	Blood pressure, heart rate and side effect questionnaire at each data collection point.

Visit 1 (week 1) – Familiarization
This visit will last about one hour. During this visit the details of the study will be explained, human subject consent forms will be signed, personal and medical history information will be completed and you will have a general physical that may include measurement of fasting blood to determine if you can participate in the study. You will donate approximately 5 ml (about 1 teaspoon) of fasting blood from a vein in your arm according to standard procedures. Next you will have your height and weight measured. Next you will be given four food logs and asked to record all calorie containing foods and drinks for a total of four days (including one weekend day) prior to your second, third, fourth and fifth visits.

Visit 2, 3, 4 & 5 (week 2, 3, 4 & 5) – (T1, T2, T3 & T4)
These visits will last about six hours. You will first have your body weight measured. You will then have your total body composition measured. Next flow-mediated dilation (FMD) will be measured using a noninvasive ultrasound probe and a blood pressure cuff. You will then be asked to donate approximately 20 ml (about 4 teaspoons) of fasting blood from a vein in your arm according to standard procedures using either a venipuncture needle or a blood collection catheter set. Next you will be randomized and counterbalanced to ingest either: 1.) creatine monohydrate (5 gm creatine + 1.5 gm dextrose); 2.) creatine nitrate – 1 (1 gm creatine + 0.5 gm nitrate + 5 gm dextrose): 3.) creatine nitrate – 2 (2 gm creatine + 1 gm nitrate + 3.5 gm dextrose); 4.) placebo (6.5 gram dextrose). After supplementation blood samples, blood pressure, heart rate and self-reported side effects will be taken at 0.5, 1, 2, 3, 4 and 5 hours post ingestion of the supplement. FMD will be measured at 1 and 2.5 hours post ingestion. You will be asked to repeat these procedures three additional times using an alternate supplement following a one week washout after each testing session.

You may be removed from the study by the investigator for these reasons:
- You do not show up for your scheduled testing sessions/visits and the investigators are unable to contact you to reschedule.
- You do not follow your assigned supplemental protocol.

Are There Any Risks To Me?
The things that you will be doing are greater than risks that you would come across in everyday life. Although the researchers have tried to avoid risks, you may feel that some questions/procedures that are asked of you will be stressful or upsetting. You do not have to answer anything you do not want to. You will be exposed to a low level of radiation during the body composition test, which is similar to the amount of natural background radiation you would receive in one month while living in College Station Texas. The use of the body composition scanner has been shown to be a safe method of measuring body composition and is approved by the FDA. During the non-invasive flow-mediated dilation (FMD) procedure, your hand might become slightly numb during the five minutes of blood pressure cuff inflation around the forearm. This slight tingling

is normal and resolves quickly following cuff release. You will donate approximately 5 ml (about 1 teaspoon) of fasting blood during the initial familiarization/screening visit and then approximately 20 ml (about 4 teaspoons) of blood seven times at each of the four testing sessions throughout the study using standard procedures. These procedures may cause a small amount of pain when the needle/catheter is inserted into the vein as well as some bleeding and bruising. You may also experience some dizziness and/or faint if you are unaccustomed to having blood drawn.

Are There Any Benefits To Me?
The direct benefit to you by being in this study is to know more about your health and fitness status from the tests to be performed. However, even if no individual benefit is obtained, you will be paid for your participation.

Will There Be Any Costs To Me?
Aside from your time, there are no costs for taking part in the study.

Will I Have To Pay Anything If I Get Hurt In This Study?
If you suffer any injury as a result of taking part in this research study, please understand that nothing has been arranged to provide free treatment of the injury or any other type of payment. However, all needed facilities, emergency treatment and professional services will be available to you, just as they are to the community in general. You should report any injury to Dr. Richard Kreider at 979-845-1333. You will not give up any of your legal rights by signing this consent form.

Side effects (injury) can happen in any research study. These effects may not be your fault or the fault of the researcher involved. Known side effects have been described in the "Are there any risks to me?" section of this consent form. However, side effects that are not currently known may happen and require care. You do not give up any of your legal rights by signing this form.

Will I Be Paid To Be In This Study?
You will receive a total of $125 ($25 for each visit) in one check at the end of the study. Payment will occur after finishing all five sessions and after all study materials (questionnaires, food records, etc.) have been turned in to the study staff. You will be paid on a prorated basis if you are unable to complete the entire study.

Will Information From This Study Be Kept Private?
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 Exercise & Sport Nutrition Laboratory staff will have access to the records.

Information about you will be stored in locked file cabinets in a locked file room in an ID card swipe access controlled laboratory. Computer files will be protected with a password. This consent form will be filed securely in an official area.

People who have access to your information include the Principal Investigator and research study personnel. Representatives of regulatory agencies such as the Office of Human Research Protections (OHRP) and entities such as the Texas A&M University Human Subjects Protection Program may access your records to make sure the study is being run correctly and that information is collected properly.

The agency that is funding this study (Woodbolt International) and the institutions(s) where study procedures are being performed (Texas A&M University) may also see your information. However, any information that is sent to them will be coded with a number so that they cannot tell who you are. Representatives from these entities can see information that has your name on it if they come to the study site to view records. If there are any reports about this study, your name will not be in them.

Information about you and related to this study will be kept confidential to the extent permitted or required by law.

Who may I Contact for More Information?
You may contact the Principal Investigator, Richard Kreider, PhD, to tell him about a concern or complaint about this research at 979-845-1333 or rkreider@hlkn.tamu.edu. You may also contact the Protocol Director/Laboratory Research Associate, Chris Rasmussen, at 979-458-1741 or crasmussen@hlkn.tamu.edu.

For questions about your rights as a research participant; or if you have questions, complaints, or concerns about the research, you may call the Texas A&M University Human Subjects Protection Program office at (979) 458-4067 or irb@tamu.edu.

What if I Change My Mind About Participating?
This research is voluntary and you have the choice whether or not to be in this research study. You may decide to not begin or to stop participating at any time. If you choose not to be in this study or stop being in the study, there will be no effect on your student status, medical care, employment, evaluation, relationship with Texas A&M University, etc. Any new information discovered about the research will be provided to you. This information could affect your willingness to continue your participation.

STATEMENT OF CONSENT
I agree to be in this study and know that I am not giving up any legal rights by signing this form. The procedures, risks, and benefits have been explained to me, and my questions have been answered. I know that new information about this research study will be provided to me as it becomes available and that the researcher will tell me if I must be removed from the study. I can ask more questions if I want. A copy of this entire consent form will be given to me.

_____   _____
Participant's Signature   Date

_____   _____
Printed Name   Date

INVESTIGATOR'S AFFIDAVIT:
Either I have or my agent has carefully explained to the participant the nature of the above project. I hereby certify that to the best of my knowledge the person who signed this consent form was informed of the nature, demands, benefits, and risks involved in his/her participation.

_____   _____
Signature of Presenter   Date

_____   _____
Printed Name   Date

Consent Form

Project Title: **Effects of 28 Days of Different Forms of Creatine Supplementation on Muscle Creatine, Body Composition, and Exercise Performance in Recreationally Active Males**

You are invited to take part in a research study being conducted by Dr. Richard Kreider a researcher from Texas A&M University and funded by Woodbolt International. The information in this form is provided to help you decide whether or not to take part. If you decide to take part in the study, you will be asked to sign this consent form. If you decide you do not want to participate, there will be no penalty to you, and you will not lose any benefits you normally would have.

Why Is This Study Being Done?
The purpose of this study is to determine whether ingesting creatine nitrate will enhance muscle creatine stores and/or promote greater training adaptations in comparison to a placebo. This study will examine the effects of different forms of creatine nitrate on muscle creatine content, body composition, exercise capacity, bench press performance and blood panels in recreationally active males.

Why Am I Being Asked To Be In This Study?
You are being asked to be in this study because you are an apparently healthy and recreationally active male between the ages of 18 and 40. You will need to have at least six months immediate prior history of resistance training on the bench press and leg press or squat. You will not be allowed to participate if; you have a history of treatment for metabolic disease (i.e., diabetes), hypertension, hypotension, thyroid disease, arrhythmias and/or cardiovascular disease; you are currently using any prescription medications; you have a history of smoking; you drink excessively (12 drinks per week or more); or you have a recent history of creatine supplementation within six weeks of the start of supplementation. If you do not qualify for this study we will keep your contact information (phone number and/or e-mail) and contact you at a later date for potential entry into a similar study with your permission.

How Many People Will Be Asked To Be In This Study?
Approximately 48 people (participants) will be invited to participate in this study locally.

What Are the Alternatives to being in this study?
The alternative to being in the study is not to participate.

What Will I Be Asked To Do In This Study?
You will be asked to not exercise for 48 hours nor eat or drink calorie containing foods or drinks 12 hours before each testing session/visit. Your participation in this study will

last approximately six weeks and include six visits (visit 1 ~ 1 hour/visit 2&5 ~ 30 minutes/visit 3&6 ~ 2 hours/visit 4 ~ 1 hour). You will be asked to provide three muscle biopsies during this study. These visits are detailed below in the table below.

Protocol Overview

Familiarization(T1)	T2 (Day 1 & 2)		T3 (Day 9)	T4 (Day 30 & 31)	
	Biopsy ~ 1 day prior	Performance testing	Biopsy & Resting Measures	Biopsy ~ 1 day prior	Performance testing
Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6
Phone Screening Familiarization Physical Exam Body Weight Anaerobic Sprint Practice Test (load corresponding to 0.075 kp/kg body mass) 1 Repetition Maximum Bench Press Test Schedule Testing Refrain from exercise, alcohol and NSAIDS 48 hours prior to each testing session Instructions for standardized resistance training program (start 2 weeks prior to T2) **1** – (1.5 g CN, 0.5 g F, 3.5 g D) – 5.5 g **2** – (3 g CN, 0.5 g F, 2 g D) – 5.5 g **3** – (5 g D, 0.5 g F) – 5.5 g (placebo) **4** – (3 g C, 0.5 g F, 2 g D) – 5.5 g CN – Creatine Nitrate F – Flavoring D – Dextrose C - Creatine	Muscle Biopsy	4 Day Food Record 12 Hour Fasting Blood Sample Body Weight Body Water Body Composition Bench Press Warm-Up Bench Press (3 sets of 10 repetitions @ 70% of 1 Repetition 2 minutes rest recovery between sets with total repetitions to failure on last set) 3 minute warm-up cycle ergometer 6 x 6 sprints with 30 second rest between sprints 3 minute rest 30 sec. Anaerobic Sprint Test	Muscle Biopsy 4 Day Food Record 12 Hour Fasting Blood Sample Body Weight Body Water Body Composition Self-Reported Side Effects	Muscle Biopsy	4 Day Food Record 12 Hour Fasting Blood Sample Body Weight Body Water Body Composition Bench Press Warm-Up Bench Press (3 sets of 10 repetitions @ 70% of 1 Repetition 2 minutes rest recovery between sets with total repetitions to failure on last set) 3 minute warm-up cycle ergometer 6 x 6 sprints with 30 second rest between sprints 3 minute rest 30 sec Anaerobic Sprint Test Self-Reported Side Effects

Visit 1 – Familiarization (T1)

This visit will last about one hour. During this visit the details of the study will be explained, human subject consent forms will be signed, personal and medical history information will be completed and you will have a general physical that will include measurement of blood to determine if you can participate in the study. You will donate approximately 5 ml (about 1 teaspoon) of blood from a vein in your arm according to standard procedures. Next you will have your height and weight measured. You will then be asked to perform a warm-up and one repetition maximum test on the bench press with a two minute recovery between attempts. Next you will be familiarized to the sprint bike test. You will then be asked to follow a standardized weight lifting program for a total of six weeks and record the amount of weight lifted and repetitions performed on training logs. The first two weeks will be performed without supplementation. The final four weeks will be performed with supplementation. Finally you will be given three food logs and asked to record all calorie containing foods and drinks for a total of four days (including one weekend day) prior to your third, fourth and sixth visits.

Visit 2 & 5 (day 1 and 30) – (T2 & T4 Biopsy)

These visits will last about 30 minutes and will take place approximately one day prior to the T2 and T4 performance testing sessions. Biopsies will be obtained from the middle portion of the thigh muscle between the knee and the upper leg using the Bergstrom biopsy (muscle tissue sample) technique. All biopsies will be taken by trained and experienced personnel in the Human Countermeasures Laboratory or the Exercise & Sport Nutrition Laboratory at Texas A&M University. The muscle biopsy procedure involves the following: First, you will be asked to lie down or assume a comfortable reclining position on an exam table. The biopsy technician will identify the point where the biopsy will be obtained. The area will be shaved clean of any leg hair, washed with a sterilizing soap, cleaned with rubbing alcohol, and further cleansed by swabbing the area with a fluid sterilizing soap and then draped with sterile padding. A small area of the thigh approximately 2 cm in diameter will be anesthetized with a 1.0 mL injection of 2% Xylocaine/Lidocaine (a numbing agent). Once the local anesthesia has taken effect (approximately 2-5 minutes) each biopsy procedure will take approximately 15-20 minutes. A scalpel point will be used to produce the initial biopsy site by making an incision approximately 1 cm. in length through the skin, subcutaneous fat, and fascia. Due to the localized effects of the anesthetic, you will feel little to no pain during this process.   The biopsy needle will be advanced into the incision approximately ½ inch. During this part of the procedure you may feel pressure to the thigh area. The biopsy procedure will obtain approximately 100 – 200 micro-grams of muscle tissue (smaller than a pencil eraser). Once the muscle sample has been obtained, direct pressure will be immediately applied. The site will be subsequently closed, and the wound dressed with a pressure bandage. Due to the small incision site, only minimal bleeding is expected.   Afterwards, written instructions for post-biopsy care will be reviewed and issued. You will be instructed to leave the bandages on for 24 hours (unless unexpected bleeding or pain occurs) and asked to report back to the lab within 24 hours to have the old bandages removed, the incision inspected and new bandages

applied. These suggestions will minimize pain and possible bleeding of the area. If needed, you may take non-prescription analgesic medication such as Tylenol to relieve pain. Soreness of the area may occur for about 24 hours post-biopsy.

Visit 3 & 6 (day 2 and 31) – (T2 & T4 – Performance Testing)
These visits will last about two hours. You will first donate approximately 20 ml (about 4 teaspoons) of fasting blood from a vein in your arm according to standard procedures. Next body weight, body water and body composition will be measured. You will then be asked to perform a warm-up and three sets of 70% of your one repetition maximum on the bench press with two minutes recover between sets with total repetitions to failure on the last set. Next you will be asked to perform a three minute warm-up at 100 W on a cycle ergometer. You will then perform six, six second sprints on the cycle ergometer with 30 seconds rest between sprints. After a three minute rest period you will be asked to perform one 30 second Wingate sprint test. Next you will be assigned to ingest either: 1.) 1.5 g. creatine nitrate, 0.5 g. flavoring and 3.5 g. dextrose; 2.) 3 g. creatine nitrate, 0.5 g. flavoring, 2 g. dextrose; 3.) 5 g. dextrose, 0.5 g. flavoring (placebo); or 4.) 3 g. creatine, 0.5 g. flavoring, 2 g. dextrose. You will be asked to consume your assigned supplement with eight ounces of water between approximately 7:00 to 8:00 a.m., 2:00 to 3:00 p.m., 9:00 to 10:00 p.m., and 20 – 30 minutes prior to your workout (total of four doses per day) on days one through seven. You will be asked to consume your assigned supplement with eight ounces of water 20 – 30 minutes prior to your workout (one dose per day) for the remainder of the study (days eight through 28). You will also be asked to record supplement intake and complete weekly medical side effects questionnaires throughout the supplementation period. After six weeks of training (four weeks of supplementation), you will be asked to repeat baseline testing.

Visit 4 (day 9) – (T3 Biopsy and Resting Measures)
This visit will last about one hour and will include everything as the normal biopsy visit along with a fasting blood draw. In addition body weight, body water and body composition will also be measured and self-reported side effects will be recorded.

You may be removed from the study by the investigator for these reasons:
- You do not show up for your scheduled testing sessions/visits and the investigators are unable to contact you to reschedule
- You do not follow your assigned supplemental protocol
- You do not follow your assigned exercise protocol

Are There Any Risks To Me?
The things that you will be doing are greater than risks that you would come across in everyday life. Although the researchers have tried to avoid risks, you may feel that some questions/procedures that are asked of you will be stressful or upsetting. You do not have to answer anything you do not want to. You will be exposed to a low level of radiation three times during the body composition exam, which is similar to the amount of natural background radiation you would receive in one month while living in College Station Texas. In addition, a very low level of electrical current will be passed through your body using a bioelectrical impedance analyzer. 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 the body composition scanner and bioelectrical impedance analyzer have been shown to be safe methods of assessing body composition and total body water and are approved by the FDA. You will donate approximately 5 ml (about 1 teaspoon) of fasting blood during the initial familiarization/screening visit and then approximately 20 ml (about 4 teaspoons) of blood three additional times at each of the three testing sessions throughout the study using standard procedures. The procedures 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 and/or faint if you are unaccustomed to having blood drawn. Similar risks as well as minimal bleeding, soreness and bruising may be involved with the three muscle biopsy procedures. There is a slight risk of contracting an infection. However, only a trained phlebotomist will be performing blood sampling using previously approved sterile procedures. The biopsy procedure may also carry a risk of soreness (100%), infection (<1%), and permanent numbness (<<1%). Additional risks include discomfort, bleeding and possible scarring at the biopsy site. The exercise tests that will be performed may cause symptoms of fatigue, shortness of breath and/or muscular fatigue/discomfort. The exercise tests may 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.

Are There Any Benefits To Me?
The direct benefit to you by being in this study is to know more about your health and fitness status from the tests to be performed. However, even if no individual benefit is obtained, you will be paid for your participation.

Will There Be Any Costs To Me?
Aside from your time, there are no costs for taking part in the study.

Will I Have To Pay Anything If I Get Hurt In This Study?
If you suffer any injury as a result of taking part in this research study, please understand that nothing has been arranged to provide free treatment of the injury or any other type of payment. However, all needed facilities, emergency treatment and professional

services will be available to you, just as they are to the community in general. You should report any injury to Dr. Richard Kreider at 979-845-1333. You will not give up any of your legal rights by signing this consent form.

Side effects (injury) can happen in any research study. These effects may not be your fault or the fault of the researcher involved. Known side effects have been described in the "Are there any risks to me?" section of this consent form. However, side effects that are not currently known may happen and require care. In the event you experience side effects, particularly "unusual or adverse effects" you will be referred to or given the option of speaking with the Principle Investigator, Dr. Richard Kreider, the ESNL Research Nurse, Cassi Walkoviak, the ESNL Protocol Director/Laboratory Research Associate, Mr. Chris Rasmussen and/or the ESNL Supervising Physician Dr. J.P. Bramhall. If you are not comfortable with these options you are encouraged to discuss these side effects with your personal physician. You do not give up any of your legal rights by signing this form.

Will I Be Paid To Be In This Study?
You will receive a total of $225 ($25 for each visit plus $50 for each biopsy visit) in one check at the end of the study. Payment will occur after finishing all testing sessions and after all study materials (questionnaires, food logs, etc.) have been turned in to the study staff. You will be paid on a prorated basis if you are unable to complete the entire study.

Will Information From This Study Be Kept Private?
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 Exercise & Sport Nutrition Laboratory staff will have access to the records.

Information about you will be stored in locked file cabinets in a locked file room in an ID card swipe access controlled laboratory. Computer files will be protected with a password. This consent form will be filed securely in an official area.

People who have access to your information include the Principal Investigator and research study personnel. Representatives of regulatory agencies such as the Office of Human Research Protections (OHRP) and entities such as the Texas A&M University Human Subjects Protection Program may access your records to make sure the study is being run correctly and that information is collected properly.

The agency that is funding this study (Woodbolt International) and the institutions(s) where study procedures are being performed (Texas A&M University) may also see your information. However, any information that is sent to them will be coded with a number so that they cannot tell who you are. Representatives from these entities can see information that has your name on it if they come to the study site to view records. If there are any reports about this study, your name will not be in them.

Information about you and related to this study will be kept confidential to the extent permitted or required by law.

Who may I Contact for More Information?
You may contact the Principal Investigator, Richard Kreider, PhD, to tell him/her about a concern or complaint about this research at 979-845-1333 or rkreider@hlkn.tamu.edu. You may also contact the Protocol Director/Laboratory Research Associate, Chris Rasmussen, at 979-458-1741 or crasmussen@hlkn.tamu.edu.

For questions about your rights as a research participant; or if you have questions, complaints, or concerns about the research, you may call the Texas A&M University Human Subjects Protection Program office at (979) 458-4067 or irb@tamu.edu.

What if I Change My Mind About Participating?
This research is voluntary and you have the choice whether or not to be in this research study. You may decide to not begin or to stop participating at any time.   If you choose not to be in this study or stop being in the study, there will be no effect on your student status, medical care, employment, evaluation, relationship with Texas A&M University, etc. Any new information discovered about the research will be provided to you. This information could affect your willingness to continue your participation.

STATEMENT OF CONSENT
I agree to be in this study and know that I am not giving up any legal rights by signing this form. The procedures, risks, and benefits have been explained to me, and my questions have been answered. I know that new information about this research study will be provided to me as it becomes available and that the researcher will tell me if I must be removed from the study.   I can ask more questions if I want. A copy of this entire consent form will be given to me.

_____   _____
Participant's Signature   Date

_____   _____
Printed Name   Date

INVESTIGATOR'S AFFIDAVIT:

Either I have or my agent has carefully explained to the participant the nature of the above project. I hereby certify that to the best of my knowledge the person who signed this consent form was informed of the nature, demands, benefits, and risks involved in his/her participation.

_____   _____
Signature of Presenter   Date

_____   _____
Printed Name   Date

Texas A&M University: Exercise & Sport Nutrition Laboratory
Trial: Pharmacokinetic Assessment of Acute Ingestion of Different Forms of Creatine

Demographics   ESNL Staff Initials:_____
Name: _____
D.O.B.: _____
ID: _____

FAM:			**FMD Settings:**			
Consent Form:	_____		AC Dist:	_____	Gain:	_____
Gen Scr Form:	_____		PW Freq:	_____	B Freq:	_____
Height:	_____in _____cm		SV:	_____	Depth:	_____
Weight:	_____lbs _____kg					
T1 scheduled:	_____					

T1:
Date: _____
Group: _____   FMD:
Last Wkout: _____   Disk: _____
Last Meal: _____ pm   0-h: _____am/pm
Hrs Fasted: _____   1-h: _____am/pm
Weight: _____ lbs _____kg 2.5-h: _____am/pm
DEXA Consent: _____
DEXA: _____

Heart Rate and Blood Pressure: Blood Samples (3) SST/(1) EDT .
0-h: _____bpm BP: _____/_____ mmHg 0-h: _____am/pm
[Complete side effects questionnaire at 0, 0.5, 1, 2, 3, 4, and 5-h] Supplement: _____am/pm
0.5-h: _____bpm BP: _____/_____ mmHg 0.5-h: _____am/pm
1-h: _____bpm BP: _____/_____ mmHg 1-h: _____am/pm
2-h: _____bpm BP: _____/_____ mmHg 2-h: _____am/pm
3-h: _____bpm BP: _____/_____ mmHg 3-h: _____am/pm
4-h: _____bpm BP: _____/_____ mmHg 4-h: _____am/pm
5-h: _____bpm BP: _____/_____ mmHg 5-h: _____am/pm

T2:
Date: _____
Group: _____   FMD:
Last Wkout: _____   Disk: _____
Last Meal: _____ pm   0-h: _____am/pm
Hrs Fasted: _____   1-h: _____am/pm
Weight: _____ lbs _____kg 2.5-h: _____am/pm
DEXA Consent: _____
DEXA: _____

Heart Rate and Blood Pressure: Blood Samples (3) SST/(1) EDTA:

0-h: _____bpm BP: _____/_____ mmHg   0-h: _____am/pm
[Complete side effects questionnaire at 0, 0.5, 1, 2, 3, 4, and 5-h]   Supplement: _____am/pm
0.5-h: _____bpm BP: _____/_____ mmHg   0.5-h: _____am/pm
1-h: _____bpm BP: _____/_____ mmHg   1-h: _____am/pm
2-h: _____bpm BP: _____/_____ mmHg   2-h: _____am/pm
3-h: _____bpm BP: _____/_____ mmHg   3-h: _____am/pm
4-h: _____bpm BP: _____/_____ mmHg   4-h: _____am/pm
5-h: _____bpm BP: _____/_____ mmHg   5-h: _____am/pm

T3:

Date: _____
Group: _____   FMD:
Last Wkout: _____   Disk: _____
Last Meal: _____ pm   0-h: _____am/pm
Hrs Fasted: _____   1-h: _____am/pm
Weight: _____ lbs _____kg   2.5-h: _____am/pm
DEXA Consent: _____
DEXA: _____

Heart Rate and Blood Pressure: Blood Samples (3)
SST/(1) EDTA:

0-h: _____bpm BP: _____/_____ mmHg   0-h: _____am/pm
[Complete side effects questionnaire at 0, 0.5, 1, 2, 3, 4, and 5-h]   Ingest _____am/pm
                                                       Supplement:
0.5-h: _____bpm BP: _____/_____ mmHg   0.5-h: _____am/pm
1-h: _____bpm BP: _____/_____ mmHg   1-h: _____am/pm
2-h: _____bpm BP: _____/_____ mmHg   2-h: _____am/pm
3-h: _____bpm BP: _____/_____ mmHg   3-h: _____am/pm
4-h: _____bpm BP: _____/_____ mmHg   4-h: _____am/pm
5-h: _____bpm BP: _____/_____ mmHg   5-h: _____am/pm

Financial Paperwork: _____

Notes:_____
_____
_____
_____
_____

Texas A&M University: Exercise & Sport Nutrition Laboratory
Trial: Effects of 28 Days of Different Forms of Creatine Supplementation on Muscle Creatine, Body Composition, and Exercise Performance in Recreationally Active Males

Demographics   ESNL Staff Initials:_____

Name: _____

D.O.B.:   _____

ID: _____

FAM: **Wingate Settings:**

Consent Form: _____   Seat ht: _____

Gen Screening Form: _____   Seat position: _____

Height: _____in   _____cm   Bar ht: _____

Weight: _____lbs   _____kg   Bar position: _____

[1RM Test before Practice Wingate]   Practice Wingate: _____

1RM Bench Press Test: [0.075 kp/kg body mass]

Estimated 1RM: _____lbs

50% of estimated 1RM, 10 reps, 2 sets   _____lbs

70% of estimated 1RM, 5 reps, 1 set   _____lbs   RT Program Instructions: _____

Actual 1 RM: determine in 3 – 5 sets   _____lbs   Schedule T1:   _____

T1: Day 0

Date: _____   ESNL Staff Initials:_____

-24 h biopsy: _____   BIA:

Group: _____   FFM (kg):   _____   _____

Last Wkout: _____   FM (kg):   _____   _____

Last Meal: _____pm   TBW (L):   _____   _____

Hrs Fasted: _____   ICW (L):   _____   _____

Weight: _____lbs   _____kg   ECW (L):   _____   _____

DEXA Consent: _____

DEXA: _____

Blood: _____

[Bench Press before Wingate Protocol]

Bench Press: Wingate Protocol:

50% of actual 1RM: _____lbs   [start 5 min after Bench Press]

70% of actual 1RM: _____lbs

   1.   50% of 1RM, 10 reps, 1 set: _____   1.   3-min warm-up: _____

   2.   70% of 1RM, 10 reps, 1 set: _____   2.   6 x 6-sec sprints: _____

   3.   70% of 1RM, 10 reps, 1 set: _____   3.   3-minute rest: _____

   4.   70% of 1RM, max reps,1set: _____ # of reps   4.   30-sec sprint: _____

[must remain seated during sprints]

Supplementation Instructions: _____

T2: Day 7

.Date:	_____		ESNL Staff Initials:_____
-24 h biopsy:	_____		
Last Wkout:	_____		BIA:
Last Meal:	_____pm		FFM (kg): _____ _____
Hrs Fasted:	_____		FM (kg): _____ _____
Weight:	_____lbs	_____kg	TBW (L): _____ _____
DEXA Consent:	_____		ICW (L): _____ _____
DEXA:	_____		ECW (L): _____ _____
Blood:	_____		Side effects _____
			questionnaire:

T3: Day 28

Date:	_____		ESNL Staff Initials:_____
-24 h biopsy:	_____		
Last Wkout:	_____		BIA:
Last Meal:	_____pm		FFM (kg): _____ _____
Hrs Fasted:	_____		FM (kg): _____ _____
Weight:	_____lbs	_____kg	TBW (L): _____ _____
DEXA Consent:	_____		ICW (L): _____ _____
DEXA:	_____		ECW (L): _____ _____
Blood:	_____		

[Bench Press before Wingate Protocol]

Bench Press: Wingate Protocol:
50% of actual 1RM: _____lbs   [start 5 min after Bench Press]
70% of actual 1RM: _____lbs

1. 50% of 1RM, 10 reps, 1 set: _____   1. 3-min warm-up: _____
2. 70% of 1RM, 10 reps, 1 set: _____   2. 6 x 6-sec sprints: _____
3. 70% of 1RM, 10 reps, 1 set: _____   3. 3-minute rest: _____
4. 70% of 1RM, max reps,1set: _____ # of reps   4. 30-sec sprint   _____

[must remain seated during sprints]

Notes:_____
_____
_____
_____
_____

#32033

THERMO-LIFE INTERNATIONAL
1334 E. Chandler Blvd. #5-D76, Phoenix, Arizona, 85048 Tel: (480) 704-7536 Fax: (480) 704-7537

March 5, 2014
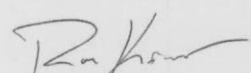
CERTIFICATE OF ANALYSIS

Product Name		Creatine Nitrate	
Batch No.	131239	Quantity	1000kg
Packaging	25kg/Drum	Production Date	Dec 11 2013
Test Standard	Enterprise Standard	Expiry Date	Dec 10 2015

ITEM	SPECIFICATION	RESULT	Method
Appearance	Almost white crystalline powder	Almost white crystalline powder	Visual
Loss on drying	≤1.0%	0.4%	ChP 2010
Residue on ignition	≤0.1%	0.2%	ChP 2010
Heavy Metals	≤10ppm	<10ppm	ChP 2010
Arsenic (As)	≤4.0ppm	<4.0ppm	ChP 2010
Cadmium (Cd)	≤1.0ppm	<1.0ppm	ChP 2010
Lead (Pb)	≤0.2ppm	<0.2ppm	ChP 2010
Mercury (hg)	≤0.1ppm	<0.1ppm	ChP 2010
Melting Point	125~144°C	138~139°C	ChP 2010
Bulk Density	415-795g/L	467g/L	USP
Tapped Bulk Density	660-997g/L	636g/L	USP
Total Plate Count	≤1000cfu/g	10cfu/g	ChP 2010
E. Coli	≤10cfu/g	<10cfu/g	ChP 2010
Yeast & Mold	≤100cfu/g	<10cfu/g	ChP 2010
Coliform Bacteria	≤100cfu/g	<10cfu/g	ChP 2010
Salmonella	Negative/25g	Negative	ChP 2010
Staph Aureus	≤10cfu/g	<10cfu/g	ChP 2010
Assay	97.0~102.0%	100.6%	Titration

Kind Regards,

Ron Kramer