SHORT-TERM SAFETY AND DOSE EFFECTS OF DIFFERENT FORMS OF CREATINE INGESTION

A Dissertation

by

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ABSTRACT

Creatine and nitrates are popular dietary supplements, but little is known regarding their co-ingestion relative to performance, side effects and safety. The purpose of this study was to examine the safety and efficacy of a creatine nitrate dietary supplement. In a double-blind, crossover, randomized and placebo-controlled manner; 28 apparently healthy and recreationally active men and women ingested daily supplements for 7 days consisting of a dextrose flavored placebo; a low dose of creatine nitrate and a high dose of creatine nitrate. Participants repeated the experiment with the alternate supplements with a 7 day washout period between each. Blood pressure, heart rate, blood samples, body weight, body composition, side effects questionnaires, bench press, leg press, and cycle ergometry performance were measured during each supplement period. No differences among treatments were found for any of the hemodynamic responses. No blood measurements exceeded normal clinical limits among treatments. No significant differences were observed in body composition or reported side effects among treatments. Pairwise comparisons found a significant difference between CNH and PLA, but not CNL at day 5 pre supplementation (PLA: 0.3 [-0.8, 1.5], CNL: 0.9 [-0.3, 2.1], CNH: 2.7 [1.6, 3.9], p=0.01) and a significant decrease in PLA and CNL, but not CNH, at day 5 post supplementation (PLA: -4.2 [-5.7, -2.7], CNL: -4.2 [-5.7, -2.7], CNH: -1.8 [-3.3, -0.3], p=0.01) in bench press 1RM and in leg press 1RM (PLA: -13.9 [-23.1, -4.7], CNL: -13.2 [-22.3, -4.0], CNH: -6.0 [-15.2, 3.1], p=0.01). No other changes were noticed in any of the performance variables. Creatine

nitrate supplementation appears to be safe and enhance performance at the doses and for the duration studied.

DEDICATION

I would like to dedicate this work to my parents, grandparents, aunts and uncles, my sister, my girlfriend Esmeralda, and all my other friends throughout the years.

Without each of you I would not be who I am today and without your support I would not have been able to achieve what I have. Thank you.

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Contributors

This work was supervised by a dissertation committee consisting of Dr. Richard Kreider [Chair of Committee], Dr. Christopher Woodman [Committee Member], and Dr. James Fluckey [Committee Member] of the Department of Health and Kinesiology and Dr. Nancy Turner [Committee Member] of the Department of Nutrition and Food Science.

Ryan Dalton served as study coordinator and assisted with data collection, sample analysis, data analysis, and manuscript preparation. Ryan Sowinski, Tyler Grubic, Blaise Collins, Brittany Sanchez, Aimee Reyes, Adriana Coletta, Majid Koozehchain, and Peter Jung assisted in data collection and sample analysis. Christopher Rasmussen serves as coordinator of the Exercise and Sport Nutrition Lab and project manager. Dr. Mike Greenwood assisted in research design and consultation. Dr. Peter Murano served as quality assurance manager. Dr. Conrad Earnest served as scientific liaison to the sponsor and assisted in study design, data analysis, and interpretation. However, Dr. Conrad Earnest was not involved in data collection or data entry and there were no restrictions on publication of the data or preparation of this paper. Dr. Richard Kreider obtained the grant, served as study PI, and assisted in the design of the study, data analysis, and manuscript preparation.

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NOMENCLATURE

CNH	6 gram dose of creatine nitrate group
CNL	3 gram dose of creatine nitrate group
PLA	Placebo group
AGAT	L-arginine:glycine amidinotransferase
GAA	Guanidinoacetic acid
SAM	S-adenosyl-L-methionine
GAMT	S-adenosyl-L-methionine:N-guanidinoacetate methelytransferase
ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
$NADP^+$	Nicotinamide adenine dinucleotide phosphate
H^2O	Water
O_2	Oxygen
H^+	Hydrogen
TNF-α	Tumor necrosis factor alpha
IL-6	Interleukin 6
Il-8	Interleukin 8
VO_2	Oxygen consumption
VO_{2peak}	Peak oxygen consumption
MVC	Maximal voluntary contraction

FAM	Familiarization Session
HR	Heart rate
SBP	Systolic blood pressure
DBP	Diastolic blood pressure
1RM	One repetition maximum
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
BUN	Blood urea nitrogen
CRE	Creatinine
CK	Creatine kinase
LDH	Lactate dehydrogenase
LDL	Low density lipoprotein cholesterol
VLDL	Very low density lipoprotein cholesterol
HDL	High density lipoprotein cholesterol
RBC	Red blood cells
RDW	Red blood cell distribution width
MCV	Mean corpuscular volume
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MPV	Mean platelet volume
WBC	White blood cells

DXA	Dual-energy x-ray absorptiometer
BIA	Bioelectrical impedance analysis
g	Gram
kg	Kilogram
mg	Milligram
km	Kilometer
J	Joule
W	Watt
mmol	millimole
L	Liter
mL	Milliliter
min	Minute

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CHAPTER I

INTRODUCTION AND RATIONALE

Background

Creatine and nitrates are two popular dietary supplements. Individually, creatine has been shown to improve muscular strength, power, and endurance, increase muscle mass, and decrease fat mass more that exercise alone [1-7]. Whereas, nitrate supplementation has been shown to increase time to exhaustion, improve time trial performance, and increase average power output during endurance exercise in moderately trained endurance athletes [8-10].

Studies have found no adverse effects of long term creatine supplementation among markers from blood and urine, reported side effects, or reported injury rates [11-13]. In fact, recent publications have examined the potential medical benefits of creatine supplementation in various diseases, such as, fibromyalgia, Huntington's, ALS, and Parkinson's disease, glycemic control and insulin resistance, and protect against muscle mass and muscular strength and endurance loss from disuse [14-30].

Studies show long-term dietary nitrate consumption from whole foods is safe (1.2 grams per day) [31-38], and short-term studies find nitrate supplementation apparently safe (300 milligrams per day) [10,39]. Nitrate supplements have also been studied for health benefits. Research has shown nitrate supplementation can help improve vasodilatation, angiogenesis, mitochondrial function and synthesis, time to claudication pain, and glucose uptake in healthy individuals [8-10,40,41].

While creatine and nitrate supplementation has been studied individually, little research has looked at their concurrent consumption. Galvan et al. examined a 5-hour acute supplementation of 1.5 and 3 grams of creatine nitrate compared to creatine monohydrate and a dextrose placebo and a 28 day resistance training program using the same doses of supplements on performance and blood biomarkers [42]. Joy et al. also examined blood biomarkers after 28 days of 1 or 2 grams of creatine nitrate supplementation taken daily [43]. Jung et al. studied 2 grams of creatine nitrate as part of a multi-ingredient supplement acutely [44] and over an 8 week training period [45]. Each of these researchers concluded creatine nitrate supplementation appears safe for the doses and duration of supplementation used with no adverse effects reported.

The current study examines if higher doses of creatine nitrate may have adverse effects on health or performance. We compared a 3 g (CNL, 2 g creatine, 1 g nitrate) and 6 g (CNH, 4 g creatine, 2 g nitrate) dose of creatine nitrate to a dextrose placebo (PLA, 6 g dextrose) over a 7-day acute supplementation period. We hypothesized no adverse effects would be found in either creatine nitrate group compared to placebo for hemodynamic reactivity, blood markers, or reported side effects.

Statement of the Problem

Is 7 days of acute supplementation with a creatine nitrate supplement apparently safe? Will acute supplementation with creatine nitrate improve performance?

Purpose of the Study

The primary purpose of this study was to examine the 7 day acute effects of ingesting either a 6 gram per day or a 3 gram per day dose of creatine nitrate on

hemodynamic reactivity and changes in hepatorenal and muscle enzyme levels. The secondary purpose of this study was to examine the affect creatine nitrate may have on bench press, leg press, and cycle ergometry performance.

General Study Overview

This study was carried out in a randomized, crossover, double blind, placebo controlled manner. Participants ingested a 6 gram creatine nitrate, 3 gram creatine nitrate, or dextrose placebo over a seven day period with a seven day washout period between each supplement. On days 0, 1, 5, and 6 clinical blood markers were measured and side effects questionnaires were collected. On days 0 and 5 hemodynamic and strength testing occurred. On days 1 and 6 cycle ergometer tests were performed.

Hypotheses

$H_0$1: There will be no significant differences among treatments in systolic, diastolic, or mean arterial pressure, pulse pressure, heart rate, and rate pressure product.

$H_0$2: There will be no significant differences among treatments in any of the clinical markers of health.

$H_0$3: There will be no significant differences among treatments in hydration status.

$H_0$4: There will be no significant differences among treatments in reported side effects.

$H_0$5: There will be no significant differences among treatments in bench press or leg press performance.

$H_0$6: There will be no significant differences among treatments in cycle ergometer performance.

Delimitations

1. Thirty eight (n=38) male (n=20) and female (n=18) subjects were recruited for this study.
2. This study included males and females ages 18-40 with at least 6 months of previous resistance training in bench press and leg press or squats.
3. Participants had not consumed nutritional supplements containing creatine or nitrates at least 3 months prior to the study.
4. Eligible participants took part in a familiarization session during which they were informed of the study protocol, filled out necessary forms including the informed consent and health history screening, scheduled all future testing sessions, and underwent practice testing on the bench press, leg press, and cycle ergometer.
5. Subjects refrained from the consumption of NSAIDs, alcohol, and strenuous exercise at least 48 hours prior to each testing session.
6. Subjects were advised to maintain a consistent diet and exercise regimen over the study duration (on permitted days).
7. Subjects fasted for at least 8-h prior to each testing session.
8. Subjects were instructed to consume all supplements according to directions provided, specifically ingesting one supplement package per day in the morning with breakfast.
9. Subjects performed to their maximal ability on all exercise performance measures.

Limitations

1. The subjects 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. While there may have been 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. Subjects were instructed to maintain a consistent diet throughout the duration of the study.
3. Subject motivation and effort during the exercise performance testing may not have been 100% at each testing session.
4. Subjects may not have followed the supplement instructions as defined during the familiarization session or during supplement distribution.
5. All subjects were instructed to maintain their normal training program on permitted days as defined by the study protocol. However, exercise habits during the duration of the study may have changed and therefore changes in performance may have been influenced by individual differences in training rather than the assigned supplement.
6. All equipment was calibrated according to manufacturer guidelines and all samples were run in duplicate to reduce likelihood of error. However, there may have been some innate limitations of the laboratory equipment used for data collection and analysis.

Assumptions

1. Subjects followed the protocol that was explained to them during the familiarization session.
2. Subjects answered the entrance questionnaires accurately and honestly prior to being accepted into the study.
3. Subjects adhered to the 7 day supplementation protocol and the 7 day washout protocol each cycle as explained to them during the familiarization session.
4. Subjects refrained from NSAIDs, alcohol, and strenuous exercise 48 hours prior to each testing sessions.
5. Subjects accurately and honestly answered all side effects questionnaires.
6. All laboratory equipment was calibrated and functioning properly prior to all testing sessions.
7. The population, which the sample was drawn from, was normally distributed.
8. The variance among the population sample was approximately equal.
9. The sample was randomly assigned to the different supplement groups. Subjects and researchers remained blinded to their assigned supplement throughout the study.
10. Subjects maintained a consistent dietary intake and exercise regimen (when permitted) throughout the duration of their respective studies.
11. Subjects exerted 100% effort at each exercise test.
12. Subjects fasted for 8 hours prior to each testing session and maintained a consistent hydration status across all testing sessions within the study protocol.

CHAPTER II

REVIEW OF LITERATURE

Introduction

Creatine monohydrate is one of the most studied nutritional supplements in modern literature. A search on Google Scholar for creatine monohydrate returns over 10,000 results and a simple Google search returns over 1.6 million results. Creatine monohydrate is probably the most popular ergogenic aid available today, with over 300 separate items available on Google Shopping. A recent study among NCAA athletes found an average creatine reported use of 14% across all athletes, ranging from 0.2% to 3.8% for female athletes and 11.1% to 29.4% for male athletes [46]. Past research has estimated creatine usage rates ranging from 25-78% in collegiate athletes [47].

With any widely available product, safety and efficacy concerns have arisen over creatine supplements. Popular sources, such as, Wikipedia warn of potential muscle cramps, strains, and pulls, diarrhea, dizziness, high blood pressure, and weight gain [48] and the Mayo Clinic goes even further with a list of more than 50 side effects creatine "may cause" ranging from abnormal heart rate, aggression, and anorexia to ischemic stroke, metabolic acidosis, pressure to the shins, and seizures [49]. On the other hand, peer reviewed research has shown creatine supplementation to have no adverse effects and may be of health benefit to both normal and diseased populations [2,7].

Creatine Metabolism

Creatine ($C_4H_9N_3O_2$) is a nitrogenous compound synthesized from arginine, glycine, and methionine in the liver, kidney, and pancreas [50-52]. Arginine and glycine are catalyzed by L-arginine:glycine amidinotransferase (AGAT) to L-ornithine and guanidinoacetic acid (GAA) (Figure 1). GAA and S-adenosyl-L-methionine (SAM) is then catalyzed by S-adenosyl-L-methionine:N-guanidinoacetate methelytransferase (GAMT) to S-adenosyl-L-homocysteine and creatine. Through a reversible reaction, creatine and adenosine triphosphate (ATP) can be catalyzed by creatine kinase into adenosine diphosphate (ADP) and creatine phosphate. This reaction allows creatine phosphate to quickly provide ATP to working muscle for short durations. When working muscles use available ATP, converting it into ADP, creatine phosphate can quickly convert ADP back into ATP to continue working. This mechanism is thought to be creatine's primary benefit to exercise performance enhancement. Creatine is also thought to influence gene expression [50], and possibly IGF-1 [53,54], myostatin [54,55], and testosterone [56] levels in the blood. Both creatine and creatine phosphate can be degraded through hydrolysis into creatinine, which is then excreted as a waste product through urine [57].

In humans, the liver, kidney, and pancreas all have high concentrations of AGAT to form GAA. After which, GAMT, found in high concentrations in the liver and pancreas is used to form creatine. In humans, the liver is thought to be the most important organ for synthesizing both GAA and creatine, but is lacking in the CK concentrations necessary for conversion into creatine phosphate. On the other hand, high

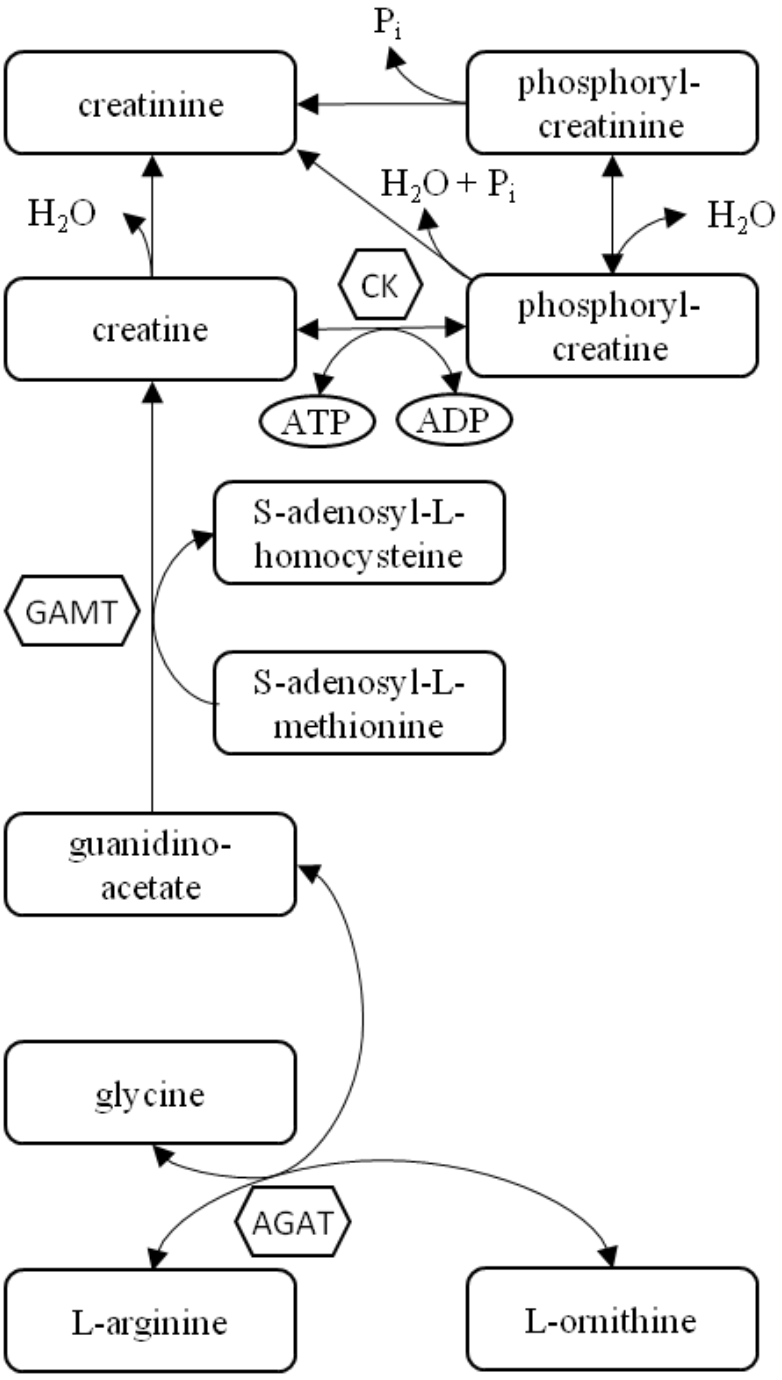

Figure 1. Creatine metabolism. Adapted from Wyss et al [57].

CK concentrations are found in cardiac and skeletal muscle tissue. In fact, strong correlations exist for most tissues, except the liver, between the CK activity of a tissue and the total creatine content of the tissue. The highest levels of creatine and creatine phosphate are found in skeletal muscle, heart, spermatozoa, and photoreceptor cells of the retina, followed by the brain, brown adipose tissue, intestine, seminal vesicles, seminal vesicle fluid, endothelial cells, and macrophages, with low levels found in the lungs, spleen, kidney, liver, white adipose tissue, blood cells, and serum [57]. An average 70 kg human stores around 120-140 g of total creatine in their body, approximately 35-40% as free creatine and 60-65% as creatine phosphate, with more than 90% found within skeletal muscle [50,58].

Through this process humans can synthesize around 1 g of creatine per day [52,58]. Humans also consume creatine through dietary sources. An 8 oz serving of meat typically has between 1.5-2.5 grams of creatine [50] and with cooking can lose between 20-50% of the creatine content depending on temperature, cooking time, and the type and cut of meat [59-62]. Based on these data a typical American receives another 1 g of creatine per day through dietary sources [47,52,58,63]. The average conversion of creatine to creatinine in the human body is about 2 grams per day [52,57,58,63], which equals the endogenous and exogenous contributions resulting in homeostasis.

Creatine Supplementation and Exercise Performance

In a landmark study, Harris et al [64], demonstrated supplementing with 5 grams of creatine monohydrate 4 or 6 times per day for 2 or more days resulted in increased creatine content of the quadriceps femoris muscle, from 126.8 mmol/kg to 148.6

mmol/kg. When 1 hour of hard cycling exercise for one leg was added to the protocol creatine content increased further, from 118.1 mmol/kg at baseline to 148.5 mmol/kg in the control leg to 162.2 mmol/kg in the exercised leg. This was the first study to demonstrate adding supplemental creatine monohydrate to the diet could increase the creatine and phosphocreatine content of human skeletal muscle. No ill effects or changes in blood profiles were noted from this supplementation protocol.

Soon after this study many researchers were testing if supplementing creatine monohydrate to the diet could enhance athletic performance. Greenhaff et al. [65] compared the effect of 20 grams of creatine monohydrate and 4 grams of glucose to a 24 gram glucose placebo taken for 5 days on maximal isokinetic knee extension torque during 5 sets of 30 repetitions. The group taking creatine monohydrate had a 5% increase in total peak torque produced compared to baseline, whereas the placebo group had a nonsignificant decrease in performance.

Harris et al. [66] examined the effect 30 grams of creatine monohydrate plus 30 grams of glucose versus a 60 gram glucose placebo, taken each day for 6 days, could have on running performance of trained middle distance runners. Before and after the supplementation protocol, participants ran 300 meters and 1000 meters 4 times with 4 and 3 minute rest intervals, respectively, between each. The participants in the creatine monohydrate group experienced a significant improvement in best 300 and 1000 meters times, -0.3 and -2.1 seconds, in final 300 and 1000 meters times, -0.7 and -5.5 seconds, and in total 4 x 1000 meter time, -13.0 seconds, with no changes in the control group.

During this time, a number of researchers found no significant differences between creatine supplemented groups and placebo for endurance performance [67-70], but researchers continued to find positive results for short duration high intensity activities [71-73]. Afterwards, research examining if creatine monohydrate could be used to enhance the benefit of training soon appeared.

Vandenberghe et al. [74] studied the effect creatine monohydrate supplementation could have on resistance training in college age females. The participants were divided between two groups; one test group received 20 grams of creatine monohydrate per day for 4 days and 5 grams of creatine monohydrate per day for 10 weeks, the placebo group received maltodextrine in the same fashion. During the 10 week training period all participants resistance trained 1 hour three times per week. Supervised exercise sessions consisted of 5 sets of 12 repetitions at 70% of one repetition maximum on seven different exercises: leg press, leg extension, leg curl, squat, bench press, shoulder press, and sit ups. After the initial 4 day supplementation period the phosphocreatine content of muscle increased by 6%, and remained at this level over the 10 week period, in the test group compared to placebo. After the 10 week training period both groups significantly increased 1 repetition maximum strength in all exercises, with the test group increasing 20-25% more than placebo in the leg press, leg extension and squat. Isokinetic arm flexion torque over 30 maximal repetitions for 5 sets was 11-25% higher across each set for the creatine group compared to placebo. At the end of the 10 weeks fat free mass increased significantly more for the creatine group

versus placebo, 5.8% vs. 3.7%. There were no significant differences found in diet or reported side effects between either group.

Kreider et al. [5] investigated the effect 15.75 grams of creatine monohydrate per day, compared to placebo, would have on NCAA Division 1A football players over a 28 day offseason training period. Participants were divided between the creatine and placebo groups, where both groups would consume, roughly, the same diet and engage in the same exercise protocol. The supervised exercise program consisted of 1-3 sets of 2-8 repetitions at 60-95% one repetition maximum for 12 different exercises 5 hours per week and high intensity sprint and football agility drills 3 hours per week. No significant differences between groups were found in dietary intake nor blood parameters: plasma glucose, carbon dioxide, urea nitrogen, uric acid, total protein, albumin, alkaline phosphatase, sodium, potassium, chloride, calcium, ionized calcium, phosphorus, leukocytes, neutrophils, lymphocytes, monocytes, eosonophils, basophils, hemoglobin, hematocrit, total bilirubin, total iron, platelets, red blood cells, red blood cell distribution width, mean corpuscular volume, or mean platelet volume. Other the other hand, creatinine, globulin, the ratio of albumin/globulin, creatine kinase, lactate dehydrogenase, alanine aminotransferase, were higher after creatine supplementation compared to placebo. There was also a 13% increase in HDL cholesterol and a 13% decrease in VLDL cholesterol in the creatine group. Both groups experienced an increase in total mass and muscle mass, with the creatine group gaining more than placebo. No changes in total body water or fat mass were found. In the cycle sprint test, maximal sprinting for 6 seconds repeated 12 times, the creatine group performed

significantly more work during each of the first 5 sprints with no significant differences found in the remaining 7 sprints compared to the placebo group.

Volek et al. [75] examined creatine monohydrate supplementation versus a placebo over a 12 week resistance training period on changes in strength and body composition. Both groups underwent the same supervised periodized resistance training program. The creatine monohydrate group consumed 25 grams of creatine for 7 days, followed by 5 grams for the remaining 11 weeks. The placebo group consumed an equivalent amount of cellulose. After 1 week and at 12 weeks the change in fat free mass was significantly greater for the creatine group than placebo. Additionally, the change in muscle fiber cross sectional area was significantly greater for the creatine group compared to placebo for Type I, Type IIA, and Type IIAB muscle fibers.

Willoughby et al. [76] demonstrated the effect supplementing with creatine monohydrate compared to placebo can have on muscle protein content over 12 weeks. Participants were divided into a non-exercise control group, and two resistance training groups: a 5 gram per day creatine monohydrate groups and a 5 gram per day dextrose placebo group. Resistance exercise for both training groups consisted of 3 sets of 6-8 repetitions at 85-90% 1RM 3 days per week on leg press, knee extension, and knee curl. After 12 weeks serum creatine was significantly elevated for the creatine monohydrate group by 58.93% compared to control at 4.27% and placebo at 4.43%. The change in total body mass, fat free mass, thigh volume, and lower body relative strength were significantly greater for both training groups compared to control and for the creatine group compared to placebo. The change in myofibrillar protein content and myosin

heavy chain mRNA for Type I, Type IIA, and Type IIX expression was significantly greater for both training groups compared to control and for the creatine group compared to placebo.

Many other researchers found similar results over the years. The research, on average, showed a 5-15% improvement in strength and maximal work capacity after supplementing with creatine compared to placebo [2,6,77,78]. In a meta analysis, Nissen et al. [79] showed creatine supplementation resulted in a 1.09%/week increase in strength and a 0.36%/week increase in lean muscle mass compared to placebo. Multiple reviews have found around 70% of acute studies and 90% of training studies yield positive results due to creatine supplementation, with no studies finding negative results [2,6]. While most studies found few or no side effects due to creatine supplementation, health concerns still remained.

Creatine Supplementation Side Effects

A number of long term studies arose to assess the risks associated with creatine supplementation. Kreider et al. [13] examined the effects of consuming creatine monohydrate over a 21 month period on clinical health markers in NCAA division 1 football players undergoing training. Forty four individuals who did not take creatine during this period served as controls. Twelve individuals consumed creatine monohydrate for 0-6 months, 25 for 7-12 months, and 17 for 12-21 months. Blood and urine samples were taken throughout the study and analyzed for muscle and liver enzymes, metabolic and hematological markers, lipid profiles, electrolytes, and lymphocytes. Among the 54 quantitative and 15 qualitative markers assessed for each

group at each time point no clinically significant interactions were found. In fact, the only differences between the controls and any group were in sodium, chloride, and hematocrit, but all values were within normal ranges and appear to be of no physiological or clinical significance.

Greenwood et al. [11] measured the effect supplementing with creatine monohydrate could have on NCAA division 1 football players during the season on injury rates. Thirty eight participants consumed creatine and 34 participants not consuming creatine served as controls. During the season athletic trainers recorded and categorized injury rates. Over the season rates of cramping, heat illness/dehydration, muscle tightness, muscle strains, and total injuries were significantly lower for creatine users compared to controls. There were no differences between groups for the other measured variables: noncontact joint injuries, contact injuries, illness, missed practices, or players lost for the season.

One of the most common concerns with creatine supplementation is increased levels of creatinine, which is used to diagnose kidney problems by the medical community, indicating creatine may cause kidney damage. Gualano et al. [80] studied the effects of 10 grams per day of creatine monohydrate compared to dextrose placebo on kidney function in healthy sedentary males over a 3 month period. All participants ran on a treadmill at 70% VO_2 for 40 minutes bouts, three times per week, for the duration of the study. At weeks 4 and 12, creatinine levels decreased for placebo, but remained unchanged for the creatine supplementation group. On the other hand, cystatin C decreased for both groups and for every individual, except one in the placebo group,

with no significant difference between the groups. Whereas, both creatinine and cystatin C can be used to estimate glomelular filtration rate of the kidneys, and hence kidney function, creatinine is a byproduct of creatine metabolism and cystatin C is not. This study indicates 1) exercise training can possibly improve kidney function and 2) creatine supplementation does not negatively impact kidney function.

In a case study, Gualano et al. [81] measured kidney function of a man, with 1 kidney and a mildly decreased glomelular filtration rate, supplementing with creatine for a 35 day period. The first 5 days he consumed 20 grams of creatine monohydrate per day and the following 30 days he consumed 5 grams per day. Before and after the 35 day supplementation period chromium-EDTA measurements, the gold standard of kidney function measurement, found no difference between before and after creatine monohydrate supplementation. While serum creatinine was higher and estimated creatinine clearance was lower, these measures falsely indicated kidney function decrements.

Creatine Supplementation Health Benefits

Recently, publications have examined the potential health benefits of creatine supplementation. Due to the performance enhancing benefits of creatine, supplementation with exercise tends to be better than exercise alone for preventing muscle and bone loss and fat accumulation found in conditions, such as osteoporosis, congestive heart failure, COPD, and leukemia [22,82-84]. Other health conditions seem to benefit from creatine supplementation directly. Muscle wasting conditions, such as certain muscular dystrophies and neuropathy disorders, have been shown to benefit with

creatine supplementation [29,30,85]. Creatine supplementation has also demonstrated a potential benefit for a number of brain and nervous system disorders, fibromyalgia, Huntington's, ALS, and Parkinson's disease [14,15,17,18,24,27,28,86]. Creatine helps improve brain function in healthy individuals during a mentally fatiguing task [86,87] and may help with glycemic control and insulin resistance [20,21]. Other studies examined short duration cast-induced immobilization and found creatine supplementation to have a protective effect against muscle mass and muscular strength and endurance loss [25] and enhance rehabilitative strength training [88].

Santos et al. [89] demonstrated creatine supplementation prior to running a 30 kilometer race in experienced marathon runners reduces inflammatory markers. Thirty four experienced runners (2.5-3 hour marathon times) volunteered to participate in the study. The experimental group ingested 20 grams of creatine monohydrate and 60 grams of maltodextrine and the placebo group ingested 60 grams of maltodextrine for 5 days before running a 30 kilometer race. Blood samples were taken pre and 24 hours post race. Compared with placebo, the creatine group had 60.9% less PGE_2, 33.7% less TNF-α, and 100% less LDH change from pre to 24 hours post race. No participants experienced any side effects during the supplementation period or during the race.

Lawler et al. [90] demonstrated a direct antioxidant effect of creatine in situ, Sestili et al. [91] showed mouse and human cells treated with creatine had increased survivability compared to controls when exposed to hydrogen peroxide, and Guidi et al. [92] found creatine protected against reactive oxygen species mediated damage to DNA compared to control cells. Bender et al. [18] studied the effect of creatine

supplementation of the health and survival of ageing mice. Starting at 12 months of age 81 mice were fed a standard rodent diet plus 1% creatine and 81 mice were fed an equicaloric standard diet. At 2 years of age mice were checked daily for health status. Creatine fed mice had a mean "healthy" life span 9% greater and a maximum life span 3.5% greater than control mice. No differences in neoplasm, renal damage, or dermatitis were different between groups. Creatine treated mice also showed better object recognition memory and a lower latency to initiate exploration in a novel environment.

Creatine Variations

With the success of creatine monohydrate many supplement companies have attempted to develop a more effective form of creatine. With the exception of anhydrous creatine, all other forms of creatine: creatine ethyl ester, creatine malate, creatine methyl ester HCL, creatine citrate, creatine malate, creatine pyruvate, creatine α-amino butyrate, creatine α-ketoglutarate, sodium creatine phosphate, creatine taurinate, creatine pyroglutamate, creatine ketoisocaproate, creatine orotate, carnitine creatinate, creatine decanoate, and creatine gluconate, all have less creatine content than creatine monohydrate, ranging from 6.3-54.3% less creatine. Even when creatine content is matched no alternate forms of creatine have better uptake into the muscle or performance enhancing benefits superior to creatine monohydrate, the only exceptions being when creatine monohydrate is ingested with carbohydrate, protein, or D-pinitol [93]. Creatine pyruvate, creatine citrate, creatine malate, creatine taurinate, creatine pyloglutamate, and creatine gluconate are unlikely to pose health risks to healthy individuals at recommended doses. Creatine phosphate, magnesium creatine chelates,

and creatine ethyl ester pose minimal health concerns, but creatine orotate may pose significant safety concerns in humans due to tumor risk [94].

Unlike creatine monohydrate, none of the alternate forms of creatine have been studied as extensively and no long term studies have been conducted to assess their safety. Creatine nitrate is another novel form of creatine marketed as a sports enhancing supplement. Only two studies have been conducted to measure the effectiveness and safety of creatine nitrate, but extensive data exists on the effects of nitrates in the human body.

Nitrate Metabolism

Nitrate metabolism, for our purposes, centers around the formation of nitric oxide. The classical model of nitric oxide formation begins with L-arginine. Nitric oxide synthase catalyzes the two step reaction: 2 L-arginine + 3 NADPH + 1 H^+ + 4 O_2 ⇌ 2 citrulline + 2 nitric oxide + 4 H_2O + 3 $NADP^+$. Nitric oxide can then be converted, through a number of processes, into nitrite and nitrate. Nitrates, through nitric oxide metabolism or though the diet, are absorbed into the blood stream, where about 25% is taken up by the salivary glands. Oral bacteria convert the nitrates into nitrites. Some of these nitrites will be converted into nitric oxide in the stomach, but a large percentage will enter systemic circulation where a number of proteins and enzymes, including globins, cytochrome c, mitochondrial proteins, polyphenols, and vitamins C and E, catalyze the reduction of nitrite into nitric oxide [40,95-97] (Figure 2).

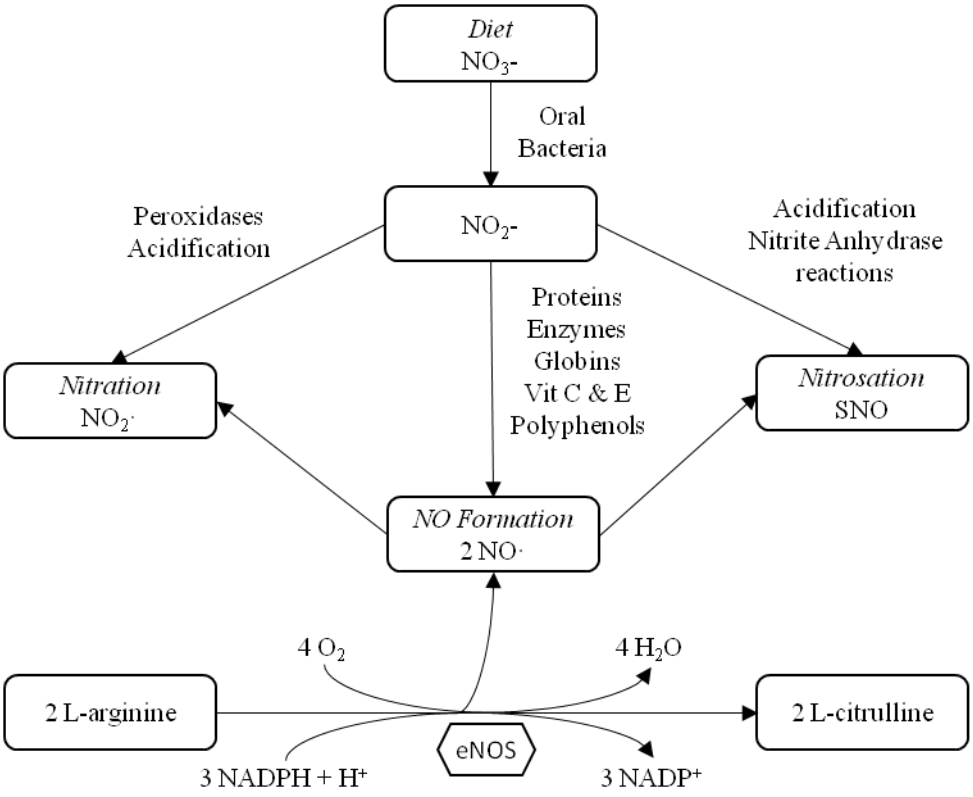

Figure 2. Nitrate metabolism. Adapted from Weitzberg et al [97].

Nitric oxide acts directly on endothelial cells causing a relaxation in vascular smooth muscle, inducing vasodilatation, which leads to an increased blood flow [98,99]. Nitric oxide may also enhance muscle contractility [100] and improve mitochondrial function [101]. The nitrate to nitric oxide reaction is oxygen independent and may help improve exercise performance in a hypoxic state, more so than the oxygen dependant L-arginine pathway [97].

Nitrate Supplementation and Exercise Performance

Humans regularly ingest nitrates in the diet through vegetable and meat sources, but research has found ergogenic benefits by supplementing with additional nitrate

sources. Larsen et al. [102] had participants ingest 0.1 mmol sodium nitrate per kilogram of bodyweight per day (~0.5 grams per day) for 3 days, while avoiding all foods with high nitrate levels. An equal amount of sodium chloride was used as the placebo control. Participants exercised on an electronically braked cycle ergometer before and after the supplementation period. When compared to the placebo, the nitrate group's VO_2 at 60-80% VO_{2peak} was on average 0.16 L/min lower. Gross efficiency was 1.4% higher during nitrate supplementation and both systolic and diastolic blood pressure was significantly lower, 112 versus 120 and 68 versus 74 mmHg. There were no differences found in heart rate, lactate, ventilation, respiratory exchange ratio, or VO_{2peak}.

Another study conducted by Larsen et al. [103] using the same supplementation protocol found a 0.1 L/min decrease in VO_{2max} after nitrate supplementation, with no change in the placebo group. There was a nonsignificant trend for time to exhaustion to increase with the nitrate supplementation and a significant correlation between the change in VO_{2max} and the change in time to exhaustion. No changes in heart rate, ventilation, blood lactate, respiratory exchange ratio, cyclic guanosine monophosphate, plasma renin, plasma aldosterone, or plasma amino acids were found between groups. Submaximal VO_2 was found to decrease after nitrate supplementation, but not with placebo. Blood pressure was unchanged before exercise, but 2 minutes post exercise diastolic blood pressure was significantly lower in the nitrate group [104]. Other studies have found similar results with decreased VO_2 at sub maximal workloads indicating improved exercise efficiency [105-108], lower resting blood pressure [106-108], and equal [105] or better times to exhaustion [106,108,109].

In an interesting study, Bailey et al. [110] had participants ingest either 500 mL of beetroot juice (5.1 mmol nitrate) or black currant juice (negligible nitrate content) for 6 days. Pre and post supplementation participants provided blood samples and engaged in low (15% MVC) and high (30% MVC) knee extension exercise. Resting blood pressure was lower for the nitrate group and under both exercise conditions the nitrate group had a lower VO_2 compared to placebo. Time to exhaustion was increased 25% for the nitrate group compared to placebo, with no difference in heart rate or VO_2 at exhaustion. Magnetic resonance spectroscopy was used to measure muscle metabolism in vivo. Phosphocreatine and ATP turnover were unchanged at rest, but phosphocreatine levels were higher and ATP turnover rates were lower in the nitrate group, compared to placebo, during exercise. No changes in pH were noticed between groups at any condition. This study demonstrated the reduced cost of exercise from nitrate supplementation may be due to a reduction in the ATP cost of muscle force production.

Data demonstrating a beneficial effect of nitrate supplementation for strength and power oriented exercise has also begun to present itself in the literature. Clifford et al. [111] compared a high (250 mg nitrate), low (125 mg nitrate), and placebo supplement on muscle soreness and recovery after 100 drop jumps conducted over 5 sets of 20 repetitions. Pre, immediately post, 24, 48, and 72 hours post participants had muscle soreness, blood, counter movement jump, and maximal isometric voluntary contraction measured. Three doses of the assigned supplement were taken after exercise on the first day, with two doses taken each of the following two days. At 72 hours post exercise muscle soreness ratings for both treatment groups had returned to baseline, while

placebo was still reduced by 20% from their initial value. Counter movement jump recovered faster with the high dose treatment, compared to placebo, at 48 and 72 hours post exercise by 16.4% and 7.3% respectively. No differences between groups were found for maximal isometric voluntary contraction, creatine kinase, TNF-α, IL-6, or IL-8. A couple of other studies have found similar benefits showing an improvement in recovery time [112,113] and work performed [114] from nitrate supplementation.

Nitrate Supplementation Side Effects

Nitrates are commonly found in the diet naturally through vegetables and added to meats as a preservative. Nitrates are generally thought to be safe with low intake levels. The Joint Food and Agriculture Organization / WHO Expert Committee on Food Additives and the European Food Safety Authority recommend ADI limits of 3.7 mg/kg/day for nitrate (~250 mg for a 70 kg person) [32,97]. On the other hand, the American Heart Association recommends the Dietary Approaches to Stop Hypertension (DASH) diet for cardio protective benefits [115]. The DASH diet, being high in fruits and vegetables, can exceed 1.2 grams of nitrate per day, ~5 times the recommended ADI of nitrate [37,38]. Acute studies examining the performance enhancing benefits of nitrate supplementation have found few side effects [9,101,116], primarily a red coloring of urine and stool from beetroot juice supplementation [110,111]. Most experts agree a diet high in vegetables and reasonable amounts of nitrate supplementation through vegetable sources appears to be safe [10,39,117,118]. On the other hand, it should be noted the LD_{50} of nitrite is around 100-200 mg/kg, comparable to cyanide [117].

While the acute effects of nitrate supplementation appear safe, the chronic effects of nitrates are more debated. Carcinogenic nitrosamine compounds can form through the metabolism of nitrates and nitrites [32,97,119] potentially leading to various forms of cancer in humans. In the Shanghai Women's Health Study, DellaValle et al. [120] evaluated the association between dietary nitrate consumption and colorectal cancer risk in over 73,000 women. Dietary nitrate consumption was estimated at baseline and over a mean 11 year follow-up period with vitamin C and red meat intake for colorectal cancer hazard ratios. No association was found between overall nitrate consumption, animal, plant, or preserved food source nitrate and colorectal cancer risk. When nitrate consumption was analyzed by vitamin C intake a strong association was found. The lowest quintile of nitrate intake (97.1 mg per day) having a hazard ratio of 1.00 to the highest quintile of nitrate intake (291.5 mg per day) having a hazard ratio of 2.45 in people with low vitamin C (<83.9 mg per day) intake. In people with high vitamin C intake (>83.9 mg per day) the association was abolished. The general consensus from experts is there is not enough evidence in humans to conclude an increased risk for cancer with increasing nitrate intake [32,97].

Nitrate Supplementation Health Benefits

Nitric oxide is a well known mediator of endothelium-dependant vasodilatation, and endothelium dysfunction is linked to many disease processes [121,122]. Recently, nitrates have been studied for their potential health benefits. As in many of the exercise studies mentioned previously, other studies have found nitrates reduce resting blood pressure by an average of 5-10 mmHg [41,103,123,124]. Studies have found nitrates, by

their conversion to nitric oxide, are an important antibacterial agent in the gastrointestinal tract [125-129]. Endothelial function, and potentially atherosclerosis, diabetes, and cardiovascular disease risk, improves with nitrate supplementation [124,130,131]. Studies have also noticed decreased platelet aggregation [124], increased cerebral blood flow [132], and an 18% increase in time to claudication pain in peripheral artery disease [41].

Recently, Kina-Tanada et al. [133] examined the effects of long-term nitrate deficiency on mice. Mice were fed nutrient identical chow, except for nitrate content. At 3 months the low nitrate mice began to experience visceral obesity, high LDL cholesterol, and glucose intolerance. By 18 months the mice had increased weight gain, hypertension, insulin resistance, and endothelial dysfunction. At 22 months almost 40% of the low nitrate mice had died due to cardiovascular disease, where 100% of the normal nitrate mice survived. Adding sodium nitrate to the low nitrate group's diet prevented these outcomes.

Creatine Nitrate Supplementation

To our knowledge only four previous studies have examined supplementation with creatine nitrate. Joy et al. [43] investigated the safety of consuming either a 1 gram or 2 gram serving of creatine nitrate every day over a period of 28 days compared to a non-supplementation group. Whole blood cell counts and comprehensive metabolic profiles were analyzed before and after supplementation. They concluded creatine nitrate appears safe in both 1 and 2 gram per day doses for 28 days.

In a two part study, Galvan et al. [42] examined the effects of 1.5 gram and 3 gram daily servings of creatine nitrate on safety and performance, compared to 5 grams of creatine monohydrate and a dextrose placebo on resistance trained males. The first study examined seven day acute effects of creatine nitrate supplementation on heart rate, blood pressure, hepatorenal enzymes, muscle enzymes, and side effects over a 5 hour period among participants in a crossover fashion, with a seven day washout period between each treatment. Creatinine increased in the creatine nitrate and creatine monohydrate groups compared to placebo, but was not outside or normal clinical values. No other significant differences were observed among any of the other hepatorenal enzymes, muscle enzymes, heart rate, blood pressure, or reported side effects. In the second study participants, resistance trained males, engaged in a standardized resistance training program while supplementing for 28 days. The first week was a loading phase where participants consumed 4 doses of their prescribed supplement (1.5 grams creatine nitrate, 3 grams creatine nitrate, 5 grams creatine monohydrate, or placebo) each day. After the loading phase participants took one dose of their supplement each day for the remaining 21 days. Once again no significant differences in hepatorenal enzymes, muscle enzymes, heart rate, blood pressure, or reported side effects were found. The high dose creatine nitrate group experienced greater changes in bench press power and endurance, and in fat-free mass and lean mass compared to placebo. No other group differences were found.

Jung et al. conducted both an acute [44] and 8 week resistance training [45] study while administering a multi-ingredient supplement containing creatine nitrate (2 g), beta-

alanine (3 g), arginine alpha-ketoglutarate (2 g), N-acetyl-L-tryrosine (300 mg), caffeine (284 mg), 15% L-dopa (15 mg), ascorbic acid (500 mg), niacin (60 mg), folic acid (50 mg), and methylcobalamin (70 mg), with and without 30% p-synepherine (20 mg) compared to placebo on resistance trained male and female participants. During the acute crossover study [44] participants consumed one serving of the supplement during testing with a seven day washout period between each assigned supplement. No significant differences among treatments were found in heart rate, blood pressure, ECG, clinical blood measures, or in bench press, leg press, or Wingate performance. Significant increases were found in resting energy expenditure responses, participants reported increased optimism about performance and increased vigor and energy, and improved cognitive performance, assessed by Stroop test, were found after consuming the multi-ingredient supplement. During the 8 week study [45] participants consumed one serving of the same supplement each day for the duration of the study, while following a standardized resistance training program. No significant differences among groups were found in body composition, resting heart rate, blood pressure, readiness to perform questions, anaerobic sprint capacity, or clinical blood measures. Significant improvements in cognitive function and the change in bench press and leg press 1RM were found for the multi-ingredient supplement groups compared to placebo. No other group differences were found.

CHAPTER III

METHODS

The current report represents a study examining 7-day acute ingestion of a high dose (CNH) and low dose (CNL) creatine nitrate supplement. Participants ingested each respective supplement once a day for a 7 day period in a randomized, double blind, crossover manner. The study was performed at the Exercise & Sport Nutrition Laboratory (ESNL) at Texas A&M University after obtaining approval from Texas A&M University Institutional Review Board (IRB2015-0684F) and signed informed consent from each participant. The following describes our overall procedures for this study followed by a detailed methodology for each test used (see below, Testing Methodology).

Participants

Twenty-eight apparently healthy and recreationally active men and women (18 men, 10 women, age: 21.6 ± 3.7 y, height: 172.1 ± 8.2 cm, weight: 73.4 ± 10.9 kg) were recruited to participate in the study. Inclusion criteria required each participant have at least 6 months of resistance training immediately prior to entering the study inclusive of performing bench press and leg press or squats. Participants were excluded from participation if they had a history of treatment for metabolic disease (i.e., diabetes), hypertension, hypotension, thyroid disease, arrhythmias, and/or cardiovascular disease; they were currently using any prescription medication (birth control is allowed); they were a pregnant or lactating female or planned to become pregnant within the next

month; they had a history of smoking; they drank excessively (12 drinks per week); or they had a recent history of creatine or nitrate supplementation within 8 weeks of the start of supplementation.

Familiarization Session

Individuals who expressed interest in the study were interview by phone, email, or in person to determine if they met eligibility for this study. Qualified individuals were invited to attend a familiarization session (FAM). During the FAM participants received written and verbal explanations of the study design and protocols, testing procedures and equipment, and blood measurements that would occur during the study. Participants were able to view the facility where testing and exercise training were conducted. The participants read and signed informed consent statements, completed personal and medical histories, and were assessed for standard anthropological measurements including height, weight, blood pressure, and heart rate. Participants also completed a general health screening form which was reviewed by a registered nurse. A DXA (Dual-Energy X-ray Absorptiometer) and BIA (Bio-electrical Impedance Analysis) were performed to assess body composition and water. Then participants completed the 1-RM (1-Repetition Maximum) and 70% endurance protocols for bench press and leg press, and a 4 km cycle ergometer protocol that would be used for the duration of the study. After the exercise testing, participants scheduled their next 12 visits: 2 testing sessions followed by 3 days off followed by 2 testing sessions with a 7 day washout period between each cycle for 3 total cycles.

Supplementation Protocol

Participants were assigned in a randomized, double-blind, cross-over manner to one of the three supplement protocols each testing week. The supplements consisted of a (1) placebo (6.0 g dextrose, PLA), (2) creatine nitrate at 3.0 g (2 g creatine; 1 g.nitrate, CNL), or (3) creatine nitrate at 6.0 g (4 g creatine; 2 g nitrate, CNH). Nutrabolt International (Bryan, TX) provided all of the supplements for this study, prepared and packaged by Thermo-life International (Phoenix, Arizona). All supplements were provided in identical clear plastic bags with the only differentiating characteristic being the letter A, B, or C printed on the label for each bag. All supplements were indistinguishable from each other based on taste, texture, and appearance. All employees conducting testing sessions were blinded to the true identity of each supplement as were the participants. When consuming the supplements participants were instructed to mix the entire contents of the package with 16 ounces of water.

Testing Procedures

Participants arrived at the laboratory on days 0, 1, 5, and 6 for a total of four laboratory visits while on each supplement. Participants were requested to fast for 8 h and refrain from exercise, alcohol, and NSAIDs consumption for 48 h prior to each testing session. A fasting blood sample of approximately 20 ml was collected using standard venipuncture techniques of an antecubital vein in the arm after which participants completed a pre exercise side effects questionnaire.

On days 0 and 5 (Figure 3A), after the blood sample collection, participants were weighed and body composition and total body water was measured via bioelectrical

impedance analysis (BIA). After which, participants began the hemodynamic reactivity test, consisting of heart rate and blood pressure readings in supine and vertical positions, followed by the exercise tests, consisting of bench press 1-RM and leg press 1-RM and bench press and leg press 70% 1-RM endurance testing. Following their respective treatment assignment, participants were asked to ingest a dose of their respective supplement. Fifteen minutes post ingestion the hemodynamic and exercise tests were repeated, followed by the post exercise side effects questionnaire.

One days 1 and 6 (Figure 3B), participants ingested a dose of their respective supplement. Thirty minutes post supplement, the participant performed the 4 km time trial cycle ergometer test. During this test the participant was encouraged to complete a distance of 4 km as quickly as they could on the cycle ergometer. After this test the participant completed the post exercise side effects questionnaire.

Side effect questionnaires were completed each testing day, before and after exercise, for the duration of the study. The questionnaires were used both to determine how well participants tolerated each supplement based on any symptoms as a result of the supplementation and as a log to monitor the participants compliance with the supplementation protocol. After completing the 1st bout of exercise testing on day 0 participants were given their 1st dose of their respective supplement for that week. On day 1, post blood draw, the 2nd dose of supplement was consumed. After which, participants were given 3 more separately packaged doses with instructions to consume one package, mixed with water, each day for the next three days: 2, 3, & 4. On day 5, participants received their 6th dose of supplement immediately following the 1st round of

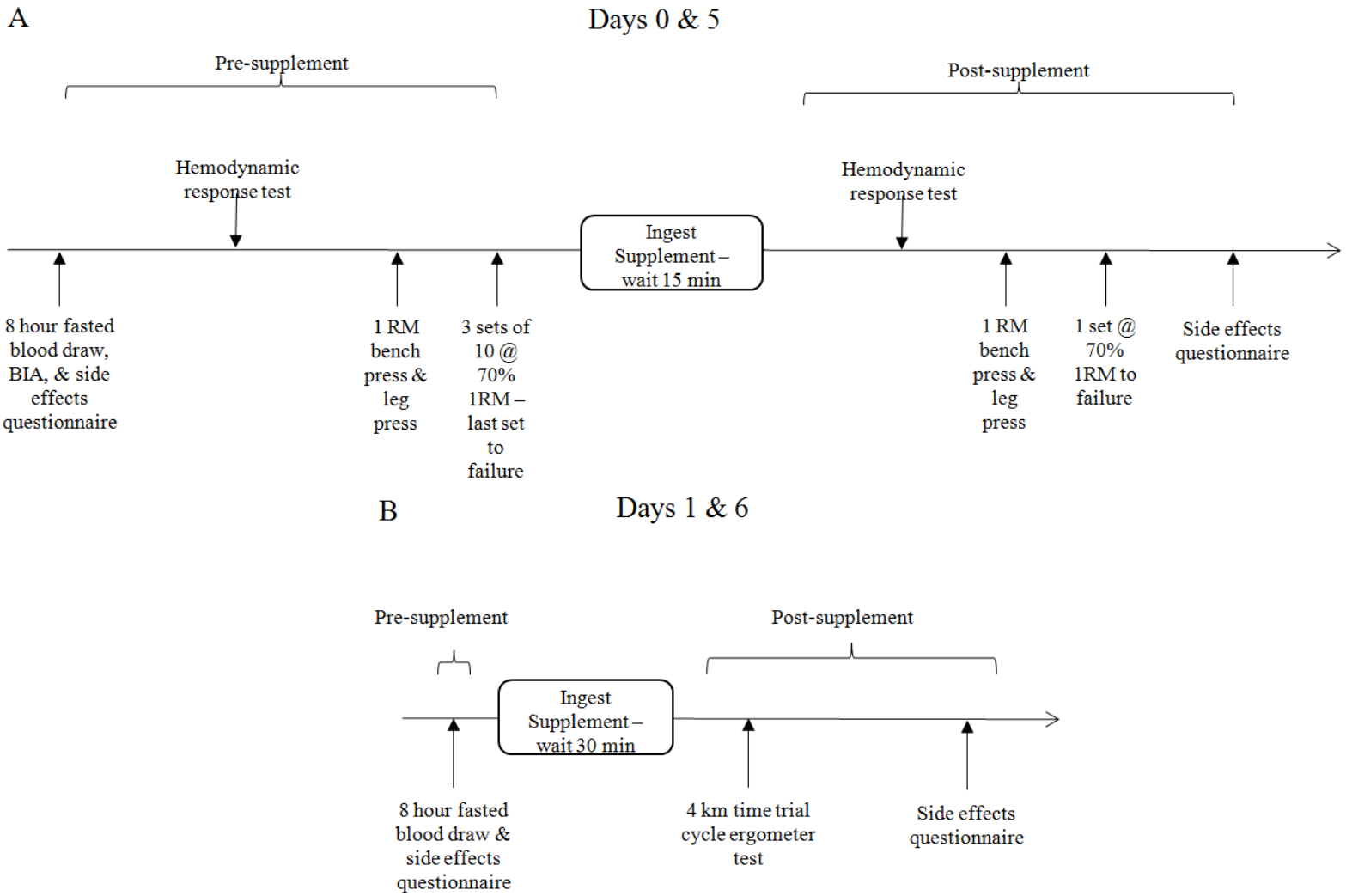

Figure 3. Testing Session Timelines. (A) Represents days 0 and 5. (B) Represents days 1 and 6.

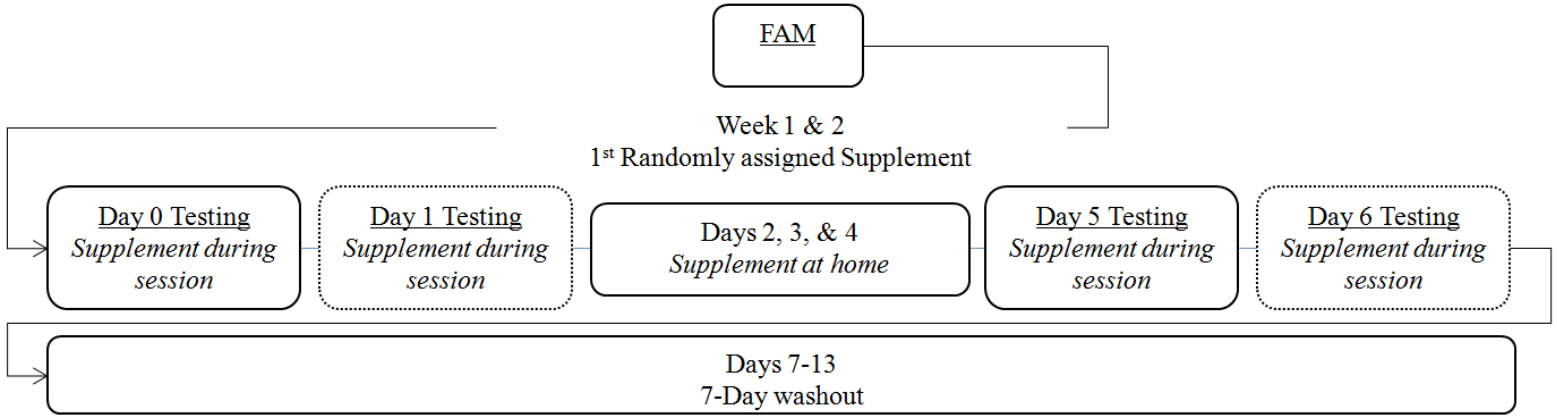

Figure 4. Study Timesline. Weeks 1 & 2 were repeated a total of three times for each participant over the duration of the study.

exercise testing on that day. On day 6, participants consumed their 7th and final dose of their respective supplement immediately post blood draw. Each 7-day period, participants consumed 7 total doses of their respective supplement followed by a 7-day washout period where no supplement was consumed nor testing performed. After which, the supplementation and testing schedule began again with the participants' next supplement. This cycle was repeated a total of 3 times (Figure 4), so each participant participated in the testing protocol while receiving all three possible formulas used in this study in randomized, double-blind, cross-over manner.

Testing Methodology

Hemodynamic Response and Reactivity Testing

Participants were placed on a modified inversion table (*IRONMAN Gravity 4000 Inversion Table; Paradigm Health & Wellness, Inc., City of Industry, CA, USA*) which allowed participants to lie supine at 0° or vertical at 180°. Participants began and remained in the supine position for each test for 15 minutes. After which, heart rate was taken at the radial artery and systolic and diastolic blood pressure was measured by listening for Korotkoff sounds from the brachial artery at the antecubital area of the elbow using standard stethoscopes and sphygmomanometers. Once the supine measurements were taken the participant was placed in the vertical position for 2 minutes, after which heart rate and blood pressure were repeated. Mean arterial pressure was calculated as 1/3 systolic blood pressure plus 2/3 diastolic blood pressure [134]. Pulse pressure was calculated as the difference in systolic and diastolic blood pressure [135]. Rate pressure product was calculated as heart rate multiplied by systolic blood

pressure [136]. Hemodynamic reactivity was determined as the difference between supine and standing measurements.

This test was repeated twice on days 0 and 5. Once before all exercise and supplementation and again 15 minutes post supplement. The test was completed a total of 4 times each 7-day testing period, once before any supplements and

three times after supplementation had begun. Participants underwent the hemodynamic reactivity test a total of 12 times over the duration of the study.

Blood Collection Procedures

Participants provided a (8 h) fasted blood sample via venipuncture from the antecubital vein in the forearm according to standard phlebotomy procedures. Approximately 10 mL of whole blood was collected at the beginning of each testing day, in one 7.5 mL BD Vacutainer® serum separation tube and in one 3.5 mL BD Vacutainer® K_2 EDTA tube (*Becton, Dickinson and Company, Franklin Lakes, New Jersey*). Both tubes were allowed to sit at room temperature for 15-min, then the 7.5 mL serum separation tube was centrifuged at 3500 rpm for 10-min using a 4° C refrigerated bench top Thermo Scientific Heraeus MegaFuge 40R Centrifuge (*Thermo Electron North America LLC, West Palm Beach, FL, USA*). Both tubes were stored at 4°C for 3-4 hours before analysis or storage. Serum was stored at -80°C in polypropylene microcentrifuge tubes for later analysis.

Blood Chemistry

Blood serum samples were analyzed for the following: alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT), creatinine, blood

urea nitrogen (BUN), creatine kinase (CK), lactate dehydrogenase (LDH), glucose, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), and triglycerides (TG) using a Cobas® c111 (*Roche Diagnostics, Basel, Switzerland*) automated clinical chemistry analyzer.

The Cobas® c111 automated clinical chemistry analyzer was calibrated according to manufacturer guidelines. This analyzer has been known to be valid and reliable in previously published reports [137]. The internal quality control for the Cobas® c111 was performed using two levels of control fluids purchased from the 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. Prior analysis in our lab yields a test-to-test reliability of a range of C_V from 0.4-2.4% for low control samples and 0.6-1.9% on high controls. Precision is between 0.8-2.4% on low controls and 0.5-1.7% on high controls.

A complete blood count with platelet differential (hemoglobin, hematocrit, red blood cell counts, MCV, MCH, MCHC, RDW, white blood cell counts, lymphocytes, granulocytes, and mid-range absolute count (MID)) was measured 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. Test-to-test reliability assessment of assays evaluated in the study yielded mean C_V's $< \pm 6.3\%$ with r values > 0.9.

Anthropometry and Body Composition

Standardized anthropological testing included assessments for body mass and height on a Healthometer Professional 500KL (*Pelstar LLC, Alsip, IL, USA*) self-calibrating digital scale with an accuracy of ±0.02 kg. Whole body bone density and body composition (excluding cranium) was measured using a Hologic Discovery W Dual-Energy X-ray Absorptiometer (DXA; *Hologic Inc, Waltham, MA, USA*) analyzed with APEX Software (*APEX Corporation Software, Pittsburg, PA, USA*) by a trained technician using procedures previously described [138]. Mean test-retest reliability studies performed on male athletes in our lab over repeated days revealed mean coefficients of variation (C_V) for total bone mineral content and total fat free / soft tissue mass of 0.31-0.45 % with a mean intraclass correlation of 0.985. On the day of each test the equipment was calibrated following manufacture's guidelines.

Total Body Water

Total body water was determined under standardized conditions using an ImpediMed DF50 bioelectrical impedance analyzer (BIA, *ImpediMed, San Diego, CA, USA*). Participants were laid in a supine position where two electrodes were placed on the dorsal side of the right hand and two other electrodes were placed on the dorsal side of the right foot. The participant's weight, height, age, and sex were entered into the BIA to determine total body, intracellular, and extracellular water.

Side Effects

The side effect questionnaire was completed both before and after each testing session to access side effects and monitor compliance with the supplementation protocol.

The questionnaire was completed a total of 24 times by each participant over the duration of the study; 2 times each testing day for 4 testing days per supplement for 3 different supplements. Participants were asked to rank the frequency and severity of – dizziness, headache, tachycardia, heart skipping or palpitations, shortness of breath, nervousness, blurred vision, and unusual or adverse effects. Participants were requested to rank their perceived 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).

Strength Testing

Bench press tests were performed using a standard isotonic Olympic bench press (Nebula Fitness, Versailles, OH) while leg press was determined using a hip/leg sled (Nebula Fitness, Versailles, OH) using standard procedures [139]. Participants warmed-up by performing 10 repetitions at 50% of their estimated 1RM, 5 repetitions using 70% of their estimated 1RM, and 3 repetition using 90% of their estimated 1RM. The participants 1 RM was determined within approximately 5, one-repetition sets following the warm-up. Participants rested 2 minutes between each warm-up set and each 1RM attempt. Hand, seat, and foot placement positions were recorded to standardize positions among testing sessions. Our bench press and leg press procedures show low day-to-day mean coefficients of variation and high reliability in our lab (1.1%, intra-class, r=0.99).

The endurance tests consisted of three total sets using a 70% of the 1RM measure at FAM load for both bench press and leg press for the duration of the study. Two sets of 10 repetitions followed by one set of repetitions to failure were performed. Participants had a 2-minute rest period between sets. During the 2nd round of strength

testing each day, participants only completed the final set to failure without the previous 2 sets of 10 repetitions. Day to day test reliability of performing this endurance test in our lab on resistance-trained participants has yielded a standard error of measurement (SEM) of 92 kg, a SEM as a percent of grand mean of 4.1%, a C_V of 0.34, and an intraclass correlation coefficient of 0.99 for 3 sets of bench press total lifting volume; and a SEM of 820 kg, a SEM as a percent of grand mean of 6.4%, a C_V of 0.32, and an intraclass correlation coefficient of 0.96 for 3 sets of leg press total lifting volume.

Strength testing was repeated on days 0 and 5 before and after supplementation immediately following the hemodynamic reactivity test. Participants completed the 1RM and endurance tests 4 times each 7-day testing period for a total of 12 times over the duration of the study.

4-K Time Trial Cycle Ergometer Test

On days 1 and 6 participants performed the 4-km time trial cycle ergometer test on a Lode Excalibur Sport 925900 cycle ergometer (*Lode BV, Groningen, The Netherlands*). The test began with a 3-min warm-up comprised of pedaling against a resistance of 25 W for the first minute, 50 W for the second minute, and 100 W for the third minute. Then participants completed 4 kilometers at a resistance of 4 J/kg/rev. They were instructed to complete the 4-km as quickly as they could and encouraged for the entire duration of the test. The seat height, seat position, handlebar height, and handlebar position were recorded for each participant to use for each testing session. A total of six 4-km time trials were conducted over the duration of the study: Two 4-km time trials while supplementing for each of three supplements. Mean test-retest

reliability studies performed in our lab over repeated days revealed mean C_V's for time to completion of 0.235 with a mean intraclass correlation of 0.850.

Data Analysis

All statistical analysis was completed utilizing SPSS 22.0 (*IBM Statistics, Chicago, IL*). Study data were analyzed using a repeated measured multivariate analysis of variance (MANOVA). Delta change values were calculated and used to determine changes from baseline, which were analyzed by repeated measures multivariate analysis of covariance (MANCOVA). Participant baseline demographic data were analyzed using one-way ANOVA. Overall MANOVA effects were examined as well as MANOVA univariate treatment effects for certain variables when significant interactions were seen. Greenhouse-Geisser univariate tests of within-subjects time, treatment x time, sex x time, and treatment x sex x time effects and between-subjects univariate treatment, sex, and treatment x sex effects were reported for each variable analyzed within the MANOVA model. When examining hematology relative to normal clinical limits, we examined the frequency of changes in hematology outside of normal clinical limits from baseline to follow-up using a Chi-square for each treatment as follows: (1) normal at baseline, normal at follow-up, (2) normal at baseline, high at follow-up, (3) high at baseline, normal at follow-up, (4) high at baseline, high at follow-up. Data were considered statistically significant when the probability of type I error was 0.05 or less and statistical trends were considered when the probability of error ranged between $p > 0.05$ to $p < 0.10$. When a significant treatment and/or interaction alpha level was observed, Fisher's least significant difference post-hoc analysis was performed to

determine where significance was obtained. When a non-significant treatment and/or interaction alpha level was observed, analyses of mean change from baseline with 95% CI with Sidak adjustment were performed. Data are presented as mean ± SD and mean change ± 95% confidence intervals as appropriate.

CHAPTER IV

RESULTS

Participant Characteristics

Initially, 38 participants were recruited for this study, signed consent forms, and completed the familiarization session. Of the original 38 participants, 28 participants (18 men, 10 women, 21.6±3.7 yr of age, 20.4±10.6% body fat, 24.7±2.9 kg/m^2 BMI) completed the study (Figure 5). Four participants decided to withdraw from the study after the familiarization session due to time constraints. Thirty four participants were randomized into the three treatments. Six participants withdrew after beginning supplementation, one due to illness unrelated to the study, one due to a family emergency, and four due to time constraints. Twenty eight participants completed all treatments and testing sessions. Table 1 represents participant demographics.

Primary Outcome Variables – Safety

Hemodynamic Response and Reactivity

Table 2 presents data from the the hemodynamic reactivity test. MANOVA analysis revealed overall Wilks' Lambda treatment (p=0.93), time (p=0.001), sex (p=0.03), treatment x time (p=0.38), treatment x sex (p=0.71), time x sex (p=0.005), and treatment x time x sex (p=0.45) for systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), pulse pressure (PP), heart rate (HR), and rate pulse product (RPP). Univariate analysis revealed significant time effects for DBP

(p=0.001), PP (p=0.002), HR (p=0.001), and RPP (p=0.001), sex effects for HR (p=0.01), and time x sex effects for SBP (p=0.02), MAP (p=0.001), and PP (p=0.01).

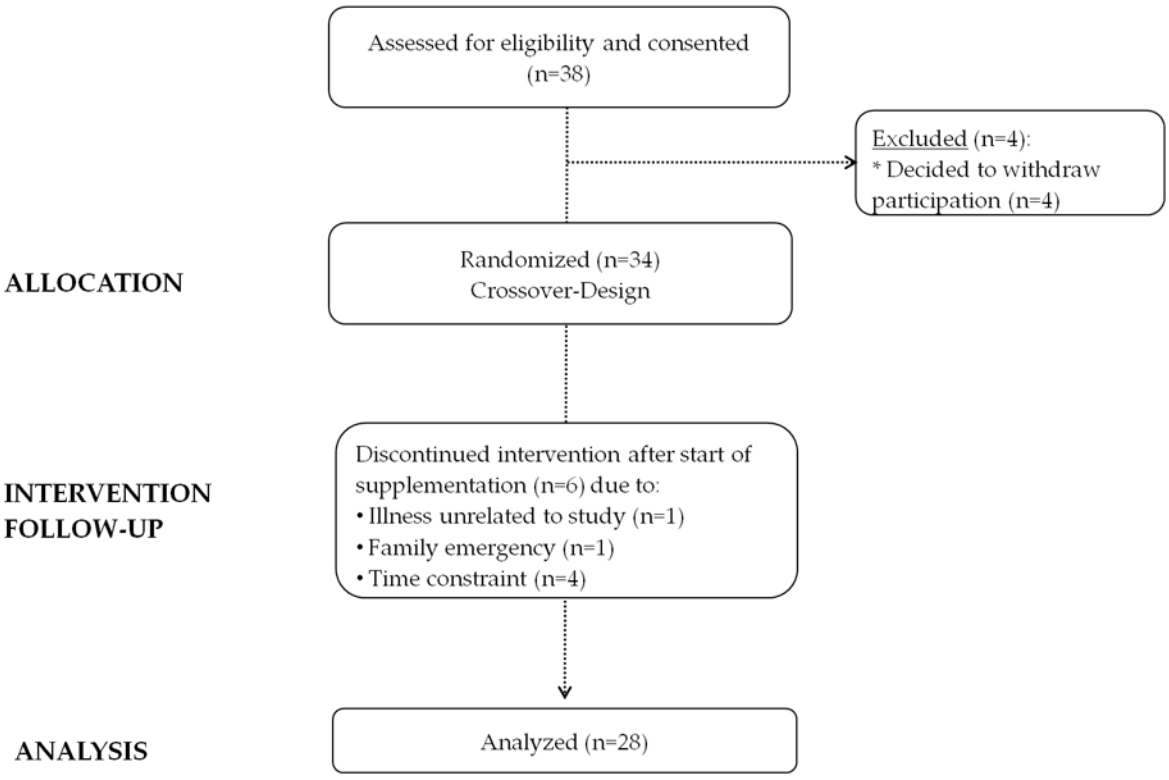

Figure 5. Consort Diagram.

Table 1: Participant Characteristics.

	Total	Male	Female	p-Level
N	28	18	10	
Age (y)	21.6±3.7	21.4±3.0	22.1±4.7	0.40
Height (m)	1.72±0.08	1.76±0.06	1.65±0.06†	0.001
Weight (kg)	67.3±10.3	70.6±8.5	61.4±10.7†	0.001
BMI (kg/m^2)	24.7±2.9	24.9±2.9	24.5±2.8	0.63
Fat Free Mass (kg)	53.5±10.3	59.9±6.1	41.9±4.2†	0.001
Fat Mass (kg)	13.8±8.0	10.7±6.2	19.6±7.7†	0.001
Body Fat (%)	20.4±10.5	14.6±7.0	30.9±7.1†	0.001

Data are mean ± SD. p<0.05 is considered significant. (†) denotes a significant difference from male.

Table 2: Hemodynamic response.

		Day										
	Treatment	0 Pre Supine	0 Pre Vertical	0 Post Supine	0 Post Vertical	5 Pre Supine	5 Pre Vertical	5 Post Supine	5 Post Vertical	Mean	Interaction	p-Level
Systolic	Overall	113.3±8.2	113.9±9.8	113.8±8.3	114.1±10.1	114.0±8.1	114.2±9.4	113.7±9.1	113.8±10.0	113.9±9.1	Time	0.92
Blood	PLA	113.9±10.2	114.4±11.2	114.2±8.9	115.0±10.0	113.6±8.5	114.9±10.3	112.2±10.3	112.7±9.0	114.4±10.1	Treatment	0.78
Pressure	CNL	114.9±7.1	114.4±10.0	113.1±7.2	113.6±9.6	115.5±7.9	115.7±8.9	115.1±8.3	115.2±10.6	114.7±8.7	Treatment x Time	0.93
(mm Hg)	CNH	111.2±6.7	112.9±8.2	114.1±8.9	113.8±11.0	113.0±7.8	111.9±8.8	113.9±8.8	113.4±10.4	113.0±8.8		
	Male	114.7±7.7	115.6±9.7	114.2±7.6	115.5±8.8	114.6±8.0	115.9±8.3	112.6±7.4	113.9±9.8	114.6±8.5	Sex	0.12
	Female	110.7±8.6†	110.7±9.2†	113.1±9.6	111.7±11.8	113.0±8.2	111.1±10.5†	115.9±11.5*	113.6±10.4	112.5±10.0	Time x Sex	0.02
	PLA M	116.7±8.9	117.0±10.6	114.9±8.7	116.6±10.0	115.1±8.7	117.2±9.5	109.4±7.0	110.9±8.0	114.7±9.2	Treatment x Sex	0.56
	PLA F	108.8±11.0	109.6±11.1	112.8±9.6	112.2±9.8	110.8±7.7	110.6±10.8	117.2±13.4	116.0±10.1	112.3±10.5	Treatment x Time x Sex	0.23
	CNL M	116.7±6.9	116.6±10.3	114.1±7.4	114.9±8.7	116.9±7.6	117.6±7.4	114.6±5.4	117.0±10.1	116.0±8.0		
	CNL F	111.6±6.5	110.4±8.4	111.4±6.8	111.4±11.2	113.0±8.2	112.4±10.6	116.2±12.3	112.0±11.4	112.3±9.3		
	CNH M	110.9±6.0	113.2±8.2	113.4±6.8	115.1±8.0	111.8±7.2	112.8±7.4	113.7±8.6	113.7±10.8	113.1±7.9		
	CNH F	111.8±8.2	112.2±8.6	115.2±12.2	111.4±15.1	115.2±8.9	110.4±11.2	114.2±9.5	112.8±10.3	112.9±10.4		
Diastolic	Overall	72.7±8.5	75.5±8.9*	73.5±7.9	76.7±7.8*	73.5±8.7	76.6±10.1*	73.7±8.5	76.2±7.7*	74.8±8.6	Time	0.001
Blood	PLA	72.6±9.1	76.8±8.8	74.5±7.6	77.9±7.6	74.5±9.2	76.2±9.7	74.1±8.8	76.1±6.4	75.4±8.5	Treatment	0.78
Pressure	CNL	73.9±7.1	74.8±8.0	72.8±7.0	76.1±7.0	73.9±9.0	77.4±11.3	72.3±7.9	76.4±8.5	74.7±8.4	Treatment x Time	0.88
(mm Hg)	CNH	71.6±9.2	74.9±10.0	73.1±9.2	76.1±8.9	72.1±8.0	76.0±9.5	74.6±8.8	76.0±8.4	74.3±9.0		
	Male	74.0±7.5	77.2±6.8	73.8±6.5	76.5±5.9	74.6±7.4	77.6±7.3	73.2±6.1	76.0±6.4	75.4±6.9	Sex	0.25
	Female	70.3±9.7	72.5±11.3	72.9±10.1	77.1±10.5	71.5±10.5	74.7±13.8	74.5±11.7	76.5±9.8	73.7±11.1	Time x Sex	0.051
	PLA M	74.1±9.5	78.9±8.1	74.7±7.0	76.9±6.6	76.7±6.5	76.8±7.1	72.9±6.0	74.9±4.4	75.7±7.1	Treatment x Sex	0.95
	PLA F	70.0±8.1	73.0±9.0	74.2±9.0	79.8±9.2	70.6±12.3	75.2±13.7	76.4±12.5	78.4±8.7	74.7±10.5	Treatment x Time x Sex	0.69
	CNL M	74.7±6.4	75.9±6.3	73.7±5.9	76.6±6.0	74.9±8.8	78.7±7.4	71.6±5.6	76.9±7.0	75.4±6.9		
	CNL F	72.4±8.4	72.8±10.5	71.2±8.9	75.4±8.7	72.2±9.7	75.2±16.5	73.6±11.3	75.6±11.0	73.6±10.5		
	CNH M	73.3±6.5	76.8±5.8	73.0±6.8	76.1±5.3	72.3±6.6	77.3±7.6	75.2±6.4	76.2±7.5	75.0±6.7		
	CNH F	68.6±12.6	71.6±14.7	73.2±12.8	76.0±13.6	71.8±10.6	73.6±12.3	73.4±12.4	75.6±10.3	73.0±12.1		

Table 2: Continued.

		Day										
	Treatment	0 Pre Supine	0 Pre Vertical	0 Post Supine	0 Post Vertical	5 Pre Supine	5 Pre Vertical	5 Post Supine	5 Post Vertical	Mean	Interaction	p-Level
Mean	Overall	99.8±7.4	101.1±8.6	100.3±7.5	101.7±8.4	100.5±7.3	101.6±8.9	100.4±8.0	101.2±8.2	100.8±8.0	Time	0.41
Arterial	PLA	100.1±8.9	101.8±9.7	101.0±7.8	102.6±7.9	100.5±7.6	102.0±9.3	99.5±9.0	100.5±7.2	101.0±8.4	Treatment	0.82
Pressure	CNL	101.2±6.5	101.2±8.7	99.7±6.4	101.1±7.8	101.6±7.1	103.0±9.0	100.9±7.6	102.3±8.4	101.4±7.7	Treatment x Time	0.91
(mm Hg)	CNH	98.0±6.4	100.2±7.5	100.4±8.4	101.2±9.7	99.4±7.2	100.0±8.4	100.8±7.7	100.9±8.9	100.1±8.0		
	Male	101.2±6.7	102.8±8.0*	100.7±6.5	102.5±6.7	101.3±6.4	103.1±7.0	99.4±6.0	101.2±7.5	101.5±6.9	Sex	0.13
	Female	97.3±8.0†	98.0±8.9†	99.7±9.2*	100.1±10.9	99.2±8.5	99.0±11.2†	102.1±10.7*	101.2±9.4*	99.6±9.6	Time x Sex	0.01
	PLA M	102.5±8.0	104.3±8.9	101.5±7.3	103.3±7.5	102.3±6.5	103.7±7.6	97.3±6.0	98.9±5.7	101.7±7.4	Treatment x Sex	0.77
	PLA F	95.9±9.2	97.4±9.8	99.9±9.0	101.4±8.8	97.4±8.8	98.8±11.4	103.6±12.0	103.5±9.0	99.7±9.8	Treatment x Time x Sex	0.26
	CNL M	102.7±6.4	103.0±8.5	100.6±6.3	102.1±6.5	102.9±6.2	104.6±6.6	100.2±4.5	103.6±7.1	102.5±6.6		
	CNL F	98.5±6.3	97.9±8.4	98.0±6.6	99.4±9.9	99.4±8.3	100.0±12.1	102.0±11.6	99.9±10.3	99.4±9.1		
	CNH M	98.4±4.8	101.1±6.3	100.0±6.0	102.1±6.3	98.6±6.1	101.0±6.7	100.9±7.0	101.2±8.9	100.4±6.6		
	CNH F	97.4±8.9	98.7±9.5	101.2±11.8	99.6±14.2	100.7±9.0	98.1±11.1	100.6±9.3	100.4±9.5	99.6±10.2		
Pulse	Overall	40.6±7.9	38.4±8.5*	40.3±6.9	37.4±8.7*	40.5±8.4	37.6±7.8*	40.1±8.2	37.6±9.3*	39.1±8.3	Time	0.002
Pressure	PLA	41.2±9.0	37.6±8.3	39.7±7.2	37.1±10.2	39.1±9.0	38.6±8.7	38.1±8.4	36.6±8.0	38.5±8.6	Treatment	0.52
(mm Hg)	CNL	41.0±5.8	39.6±7.3	40.4±6.7	37.5±8.5	41.6±9.2	38.3±7.9	42.9±6.5	38.8±11.3	40.0±8.1	Treatment x Time	0.96
	CNH	39.6±8.7	37.9±9.9	41.0±7.0	37.7±7.5	40.9±7.0	35.9±6.8	39.3±8.9	37.4±8.5	38.7±8.2		
	Male	40.7±7.8	38.4±8.2	40.4±6.9	39.0±9.1	40.0±9.3	38.3±8.0	39.3±7.4	37.9±10.0	39.2±8.4	Sex	0.63
	Female	40.4±8.4	38.3±9.1	40.3±7.2	34.6±7.3	41.5±6.5	36.5±7.5	41.4±9.4	37.1±8.2	38.7±8.2	Time x Sex	0.23
	PLA M	42.6±9.1	38.1±8.7	40.3±7.9	39.7±10.4	38.4±9.9	40.4±9.3	36.6±6.2	36.0±8.6	39.0±8.9	Treatment x Sex	0.25
	PLA F	38.8±8.8	36.6±7.8	38.6±5.8	32.4±8.3	40.2±7.6	35.4±6.8	40.8±11.3	37.6±7.3	37.6±8.2	Treatment x Time x Sex	0.69
	CNL M	42.0±4.6	40.7±7.2	40.4±6.2	38.3±9.4	42.0±10.8	38.9±7.3	43.0±6.8	40.1±12.3	40.7±8.3		
	CNL F	39.2±7.6	37.6±7.5	40.2±7.9	36.0±6.7	40.8±5.9	37.2±9.2	42.6±6.3	36.4±9.4	38.8±7.6		
	CNH M	37.6±8.2	36.4±8.6	40.4±6.7	39.0±7.6	39.4±7.2	35.4±6.9	38.4±8.0	37.4±8.8	38.0±7.7		
	CNH F	43.2±8.9	40.6±11.8	42.0±7.9	35.4±7.0	43.4±6.0	36.8±7.0	40.8±10.7	37.2±8.5	39.9±8.8		

Table 2: Continued.

		Day										
	Treatment	0 Pre Supine	0 Pre Vertical	0 Post Supine	0 Post Vertical	5 Pre Supine	5 Pre Vertical	5 Post Supine	5 Post Vertical	Mean	Interaction	p-Level
Heart Rate	Overall	61.0±9.7	72.7±11.9*	75.5±9.3*	85.4±11.7*	59.5±9.0	73.4±11.3*	76.1±10.1*	85.7±12.6*	73.7±14.0	Time	0.001
(BPM)	PLA	61.6±10.9	72.4±14.1	75.1±9.2	85.2±11.1	57.8±8.6	72.4±11.6	77.4±10.5	85.3±13.0	73.4±14.4	Treatment	0.94
	CNL	61.3±10.4	72.7±10.7	77.0±9.1	87.9±12.6	59.8±7.3	74.0±10.5	73.8±9.4	83.9±13.3	73.8±13.8	Treatment x Time	0.18
	CNH	59.9±7.8	73.1±10.9	74.3±9.7	83.0±11.4	61.0±10.9	73.7±12.1	77.2±10.2	87.9±11.7	73.8±13.9		
	Male	57.8±7.8	69.1±8.5*	75.0±9.0*	84.0±11.4*	57.8±8.9	70.6±9.6*	76.6±9.9*	85.9±11.9*	72.1±13.8	Sex	0.01
	Female	66.7±10.4†	79.3±14.3†*	76.2±10.0*	87.7±12.1*	62.7±8.4†*	78.4±12.4†*	75.3±10.5*	85.3±14.1*	76.4±14.0†	Time x Sex	0.001
	PLA M	58.3±7.2	67.4±8.6	76.2±8.0	85.1±12.2	56.6±8.5	69.7±10.8	78.3±9.5	85.2±12.2	72.1±14.1	Treatment x Sex	0.84
	PLA F	67.6±14.0	81.2±17.9	73.0±11.1	85.3±9.4	60.0±8.6	77.2±11.8	75.6±12.4	85.4±15.0	75.7±14.8	Treatment x Time x Sex	0.36
	CNL M	57.8±10.4	69.4±9.0	75.3±8.9	83.7±11.3	58.1±6.4	71.8±8.5	74.6±10.2	83.4±12.7	71.8±13.4		
	CNL F	67.6±7.2	78.6±11.5	80.0±9.2	95.4±11.7	62.8±8.1	78.0±12.9	72.4±8.2	84.8±14.9	77.5±14.0		
	CNH M	57.2±5.1	70.3±7.9	73.6±10.2	83.3±11.4	58.7±11.5	70.2±9.8	76.9±10.1	89.2±10.5	72.4±14.1		
	CNH F	64.8±9.5	78.0±14.0	75.6±9.3	82.5±12.0	65.2±8.7	80.0±13.6	77.8±10.9	85.6±13.9	76.2±13.2		
Rate	Overall	6899±1173	8261±1436*	8580±1167*	9727±1493*	6789±1137	8363±1384*	8641±1215*	9733±1590*	8374±1681	Time	0.001
Pressure	PLA	7001±1332	8248±1690	8581±1286	9793±1496	6542±941	8296±1413	8673±1376	9600±1586	8342±1742	Treatment	0.87
Product	CNL	7040±1259	8283±1227	8696±1022	9945±1432	6900±919	8553±1322	8493±1218	9644±1686	8445±1616	Treatment x Time	0.50
(mm Hg x	CNH	6658±883	8252±1405	8463±1210	9442±1556	6924±1465	8239±1445	8756±1060	9954±1528	8336±1688		
BPM)	Male	6629±983	7970±1081	8551±1030	9691±1382	6621±1097	8176±1261	8604±1105	9773±1539	8252±1632	Sex	0.10
	Female	7386±1339	8785±1822	8632±1400	9791±1697	7091±1164	8698±1548	8707±1411	9660±1701	8594±1747	Time x Sex	0.07
	PLA M	6790±842	7890±1225	8750±1024	9926±1674	6491±946	8192±1554	8572±1171	9446±1492	8258±1672	Treatment x Sex	0.94
	PLA F	7380±1934	8892±2239	8278±1680	9554±1149	6634±977	8483±1171	8855±1742	9877±1790	8494±1862	Treatment x Time x Sex	0.23
	CNL M	6757±1347	8061±1058	8579±997	9556±1042	6791±840	8431±1074	8533±1135	9730±1575	8305±1528		
	CNL F	7549±935	8685±1458	8906±1086	10644±1805	7098±1065	8774±1727	8422±1418	9491±1950	8696±1745		
	CNH M	6340±595	7959±1004	8324±1080	9591±1405	6581±1449	7906±1115	8706±1062	10144±1555	8194±1701		
	CNH F	7230±1052	8779±1880	8714±1441	9175±1847	7541±1348	8838±1815	8846±1109	9612±1495	8592±1644		

Data are means ± SD. MANOVA analysis revealed overall Wilks' Lambda treatment (p=0.93), time (p=0.001), sex (p=0.03), treatment x time (p=0.38), treatment x sex (p=0.71), time x sex (p=0.005), and treatment x time x sex (p=0.45).Univariate ANOVA p-levels from MANOVA analysis are presented for each variable. p<0.05 is considered significant. Statistical notations. (*) denotes a significant difference from baseline. (†) denotes a significant difference from male.

However, there were no significant effects found in treatment, treatment x time, treatment x sex, or treatment x time x sex among any of the hemodynamic variables.

Figures 6 through 11 presents the mean change from baseline with 95% CI;s for systolic blood pressure, diastolic blood pressure, mean arterial pressure, pulse pressure, heart rate, and rate pressure product, respectively. Analysis of mean change from baseline with 95% CI revealed the change in diastolic blood pressure was significantly higher than baseline only for PLA at day 0 post supplement vertical ($p<0.05$). The change in heart rate ($p<0.05$) and rate pressure product ($p<0.05$) was significantly higher for all groups at every time point except day 5 pre supplement, with no differences among treatments. No other significant effects were observed among the changes from baseline.

Figures 12 through 17 present mean reactivity (difference from supine to standing) with 95% CI;s for systolic blood pressure, diastolic blood pressure, mean arterial pressure, pulse pressure, heart rate, and rate pressure product, respectively. Analysis of mean reactivity with 95% CI revealed the change in diastolic blood pressure was significantly higher only for PLA at baseline pre-ingestion ($p<0.05$) and only for CNL at follow-up post-ingestion ($p<0.05$). Mean reactivity for pulse pressure was significantly lower only for CNH at follow-up pre-ingestion ($p<0.05$). Mean reactivity was significantly higher for all groups at all time points for heart rate ($p<0.05$) and rate pressure product ($p<0.05$). No differences among treatments were observed for any of the measures of reactivity. These data fail to reject $H_0$1: There will be no significant

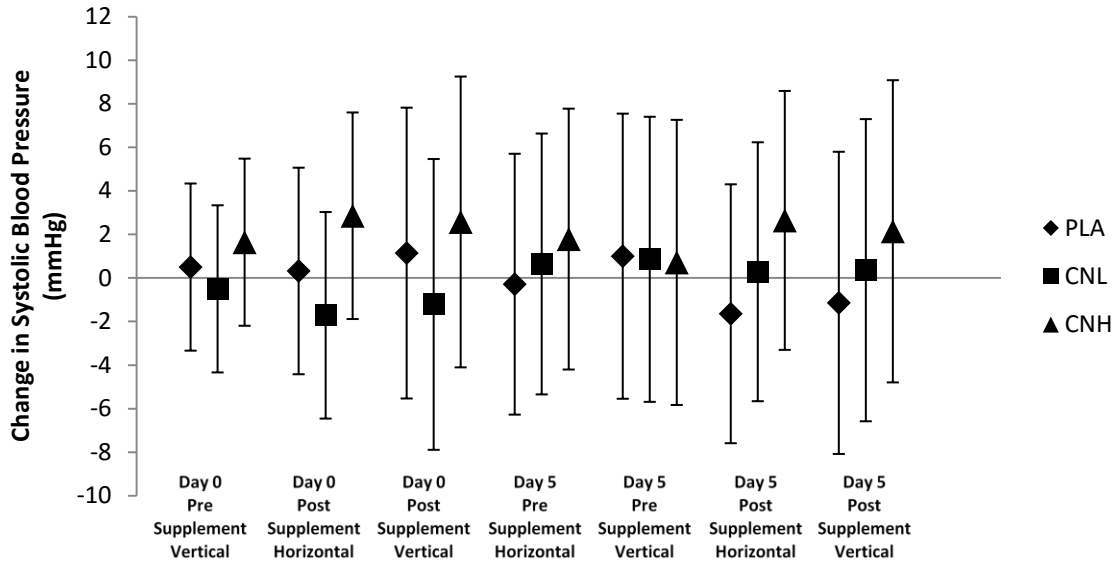

Figure 6. Changes in systolic blood pressure. Data are means ± CI. p<0.05 is considered significant..

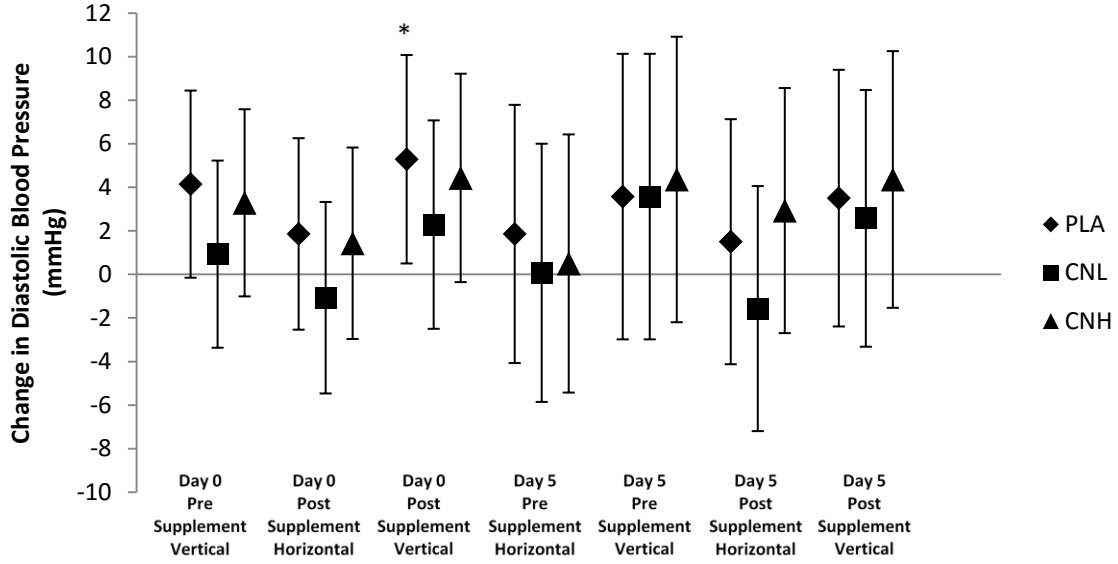

Figure 7. Changes in diastolic blood pressure. Data are means ± CI. p<0.05 is considered significant. Statistical notations. (*) denotes a significant difference from baseline.

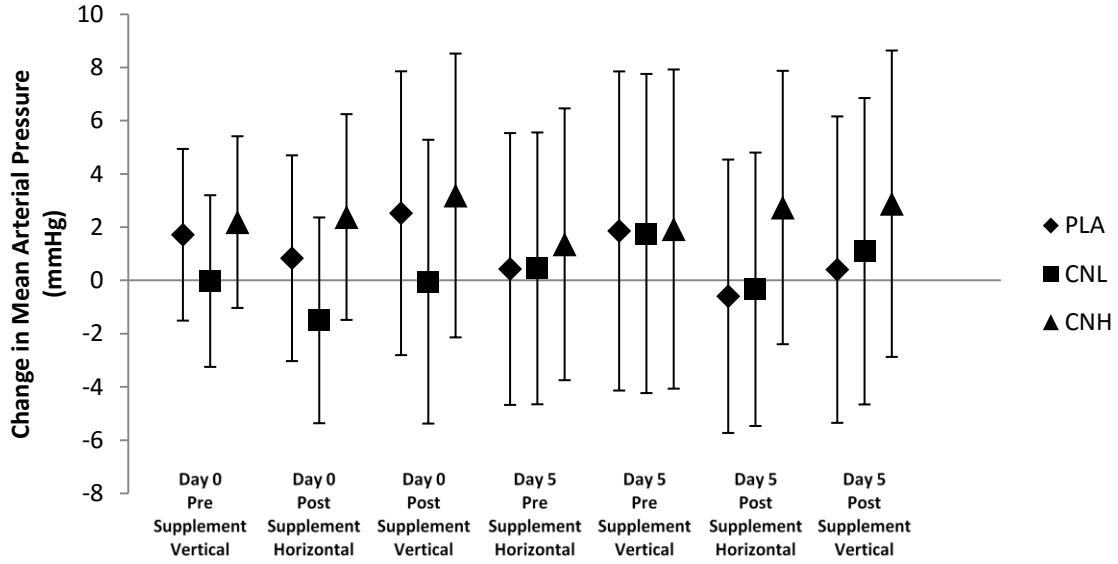

Figure 8. Changes in mean arterial pressure. Data are means ± CI. p<0.05 is considered significant.

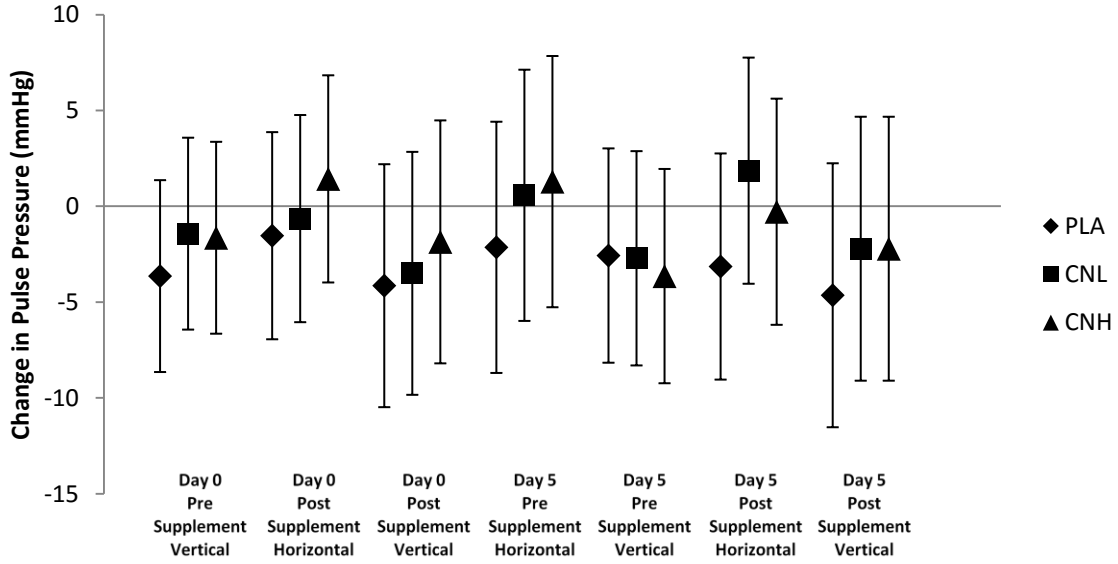

Figure 9. Changes in pulse pressure. Data are means ± CI. p<0.05 is considered significant.

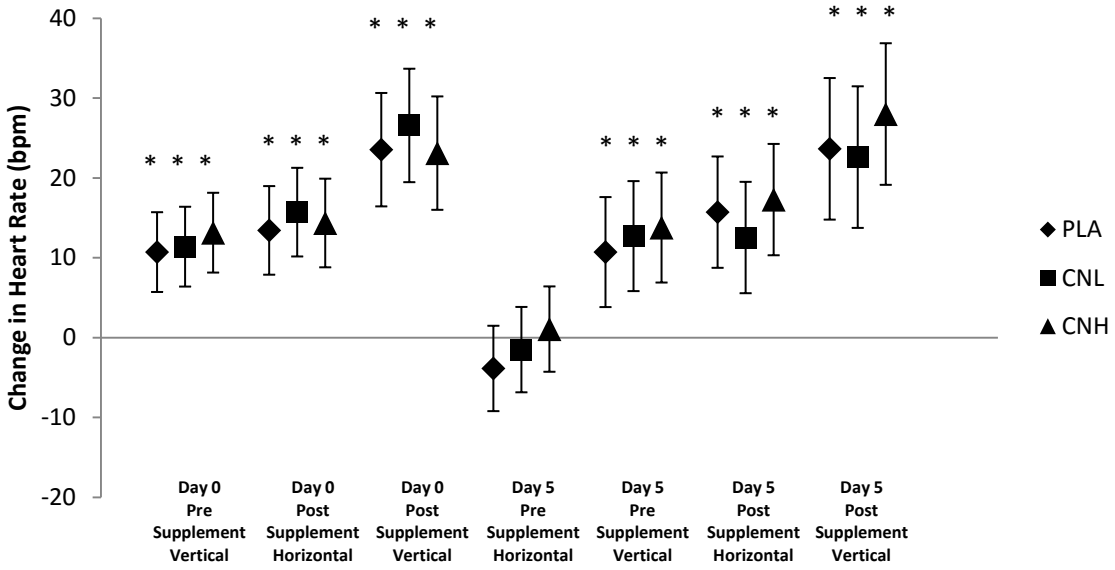

Figure 10. Changes in heart rate. Data are means ± CI. p<0.05 is considered significant. Statistical notations. (*) denotes a significant difference from baseline.

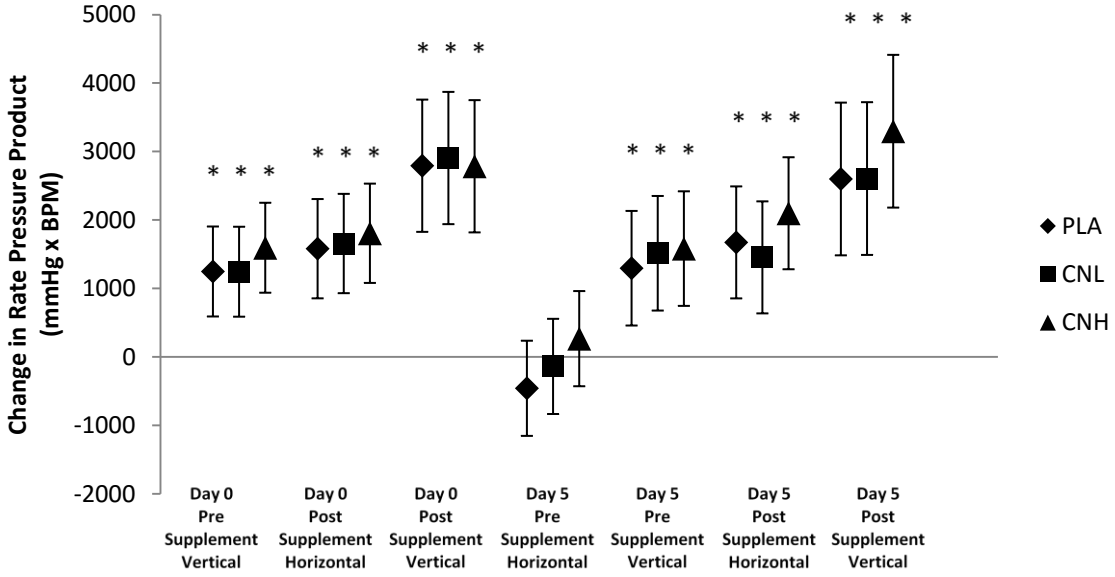

Figure 11. Changes in rate pressure product. Data are means ± CI. p<0.05 is considered significant. Statistical notations. (*) denotes a significant difference from baseline.

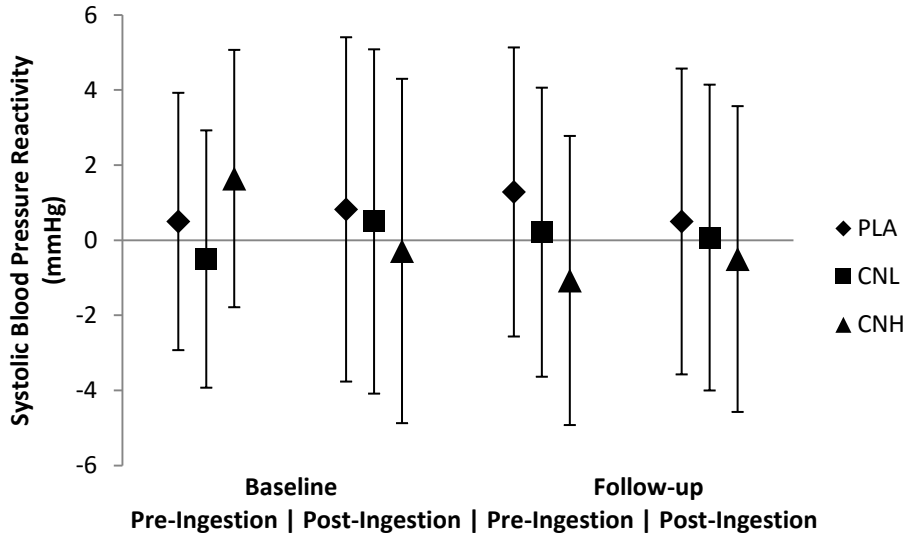

Figure 12. Systolic blood pressure reactivity. Data are means ± CI. p<0.05 is considered significant.

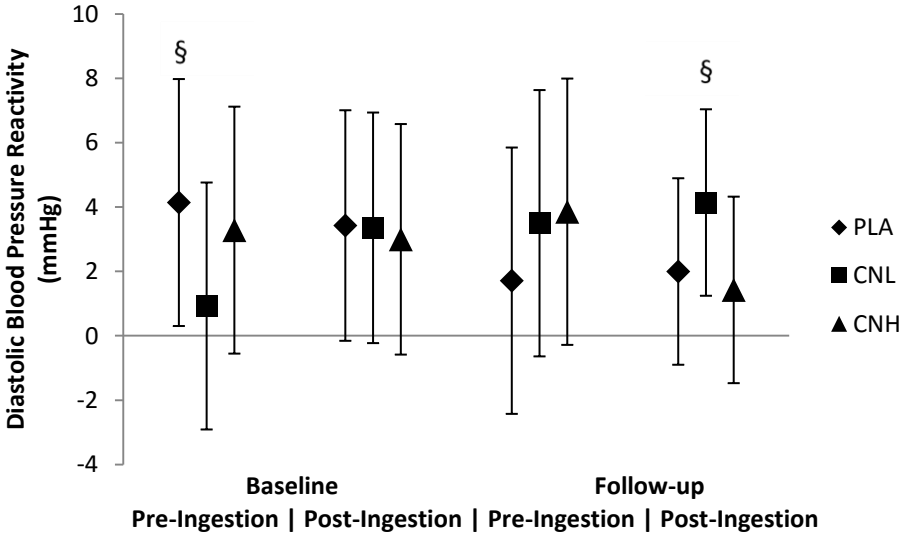

Figure 13. Diastolic blood pressure reactivity. Data are means ± CI. p<0.05 is considered significant. Statistical notations. (§) denotes a significant difference from supine to standing.

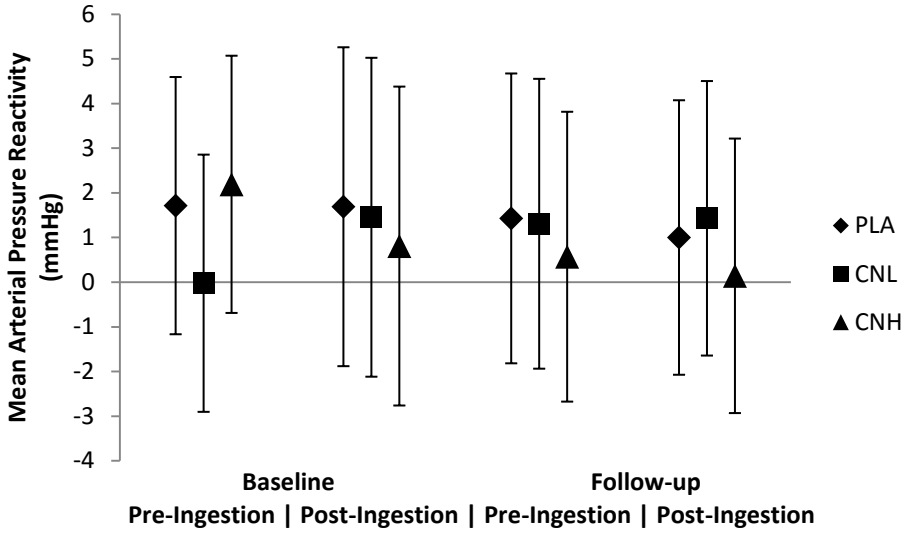

Figure 14. Mean arterial pressure reactivity. Data are means ± CI. p<0.05 is considered significant.

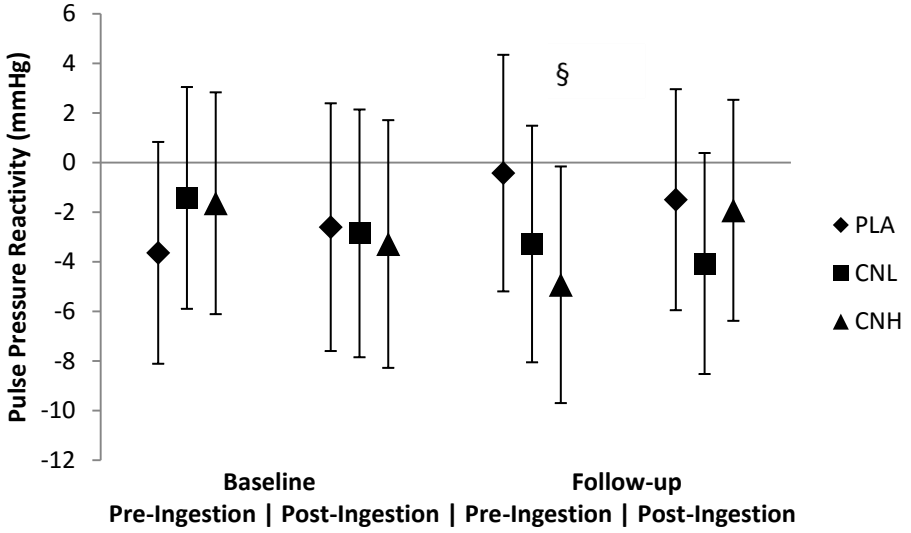

Figure 15. Pulse pressure reactivity. Data are means ± CI. p<0.05 is considered significant. Statistical notations. (§) denotes a significant difference from supine to standing.

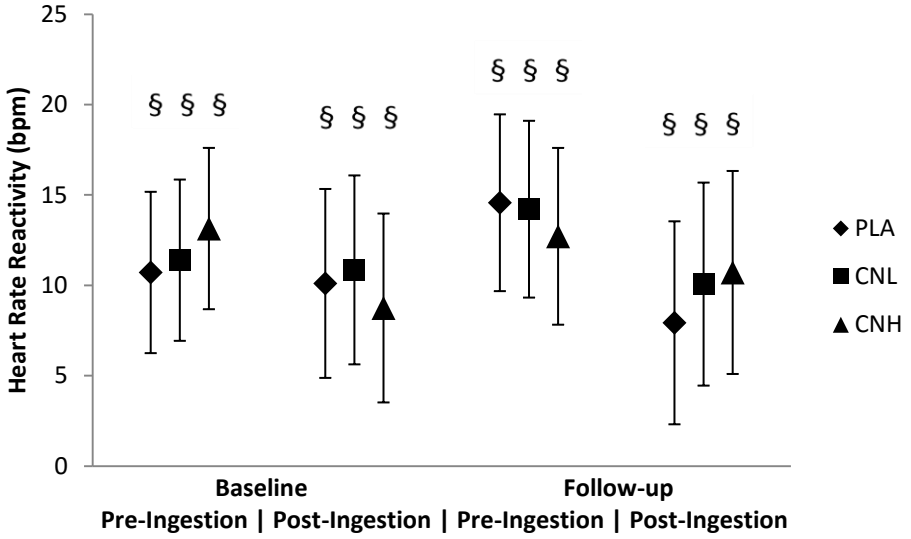

Figure 16. Heart rate reactivity. Data are means ± CI. p<0.05 is considered significant. Statistical notations. (§) denotes a significant difference from supine to standing.

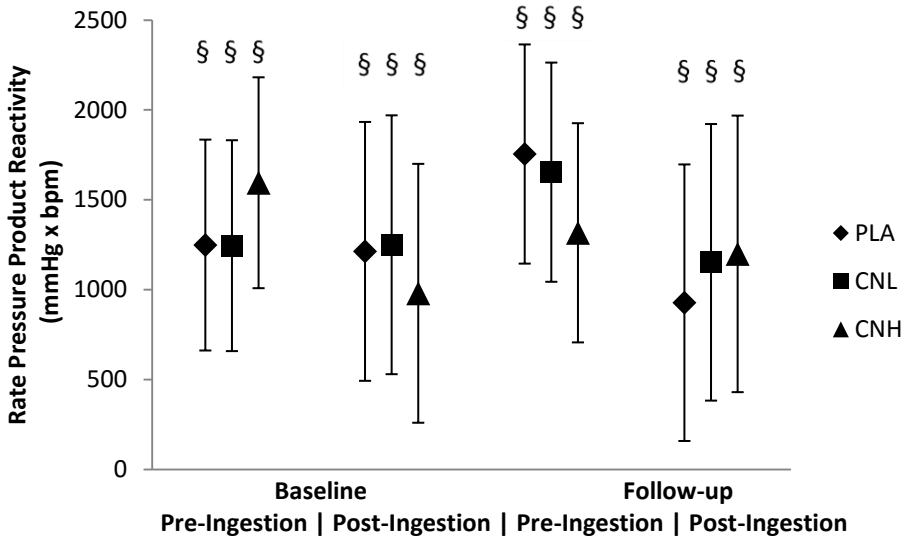

Figure 17. Rate pressure product reactivity. Data are means ± CI. p<0.05 is considered significant. Statistical notations. (§) denotes a significant difference from supine to standing.

differences among treatments in systolic or diastolic blood pressure, mean arterial pressure, pulse pressure, heart rate, or rate pulse product.

Clinical Blood Markers

Tables 3 through 9 present results of whole blood and serum markers monitored in this study. No significant overall MANOVA or univariate ANOVA interactions were observed among treatments in any of the analyses performed. Table 10 shows Chi square analysis of changes from baseline values observed. This analysis also showed no significant changes were observed among treatments. These data fail to reject $H_0$2: There will be no significant differences among treatments in any of the clinical markers of health.

Body Composition

Table 13 presents body water. MANOVA analysis revealed overall Wilks' Lambda treatment (p=0.98), time (p=0.04), sex (p=0.001), treatment x time (p=0.007), treatment x sex (p=0.98), time x sex (p=0.15), and treatment x time x sex (p=0.005). Univariate analysis revealed significant time effects for extracellular water (L) (p=0.04), sex effects for total body water (L) (p=0.001), intracellular water (L) (p=0.001), extracellular water (L) (p=0.001), total body water (%) (p=0.001), intracellular water (%) (p=0.001), extracellular water (%) (p=0.001), and treatment x time x sex effects for intracellular water (L) (p=0.03),) intracellular water (%) (p=0.006), extracellular water (%) (p=0.006). These data fail to reject $H_0$3: There will be no significant difference among treatments in hydration status.

Table 3: White Blood Cell Counts.

		Day						
	Treatment	0	1	5	6	Mean	Interaction	p-Level
White	Overall	6.29±1.18	6.33±1.03	6.48±1.18	6.22±1.09	6.35±1.09	Time	0.34
Blood	PLA	6.25±1.21	6.26±1.13	6.51±1.16	6.37±1.05	6.30±1.12	Treatment	0.97
Cell	CNL	6.27±1.19	6.51±1.06	6.35±1.25	6.31±1.00	6.39±1.03	Treatment x Time	0.64
(K/µL)	CNH	6.35±1.18	6.25±0.91	6.56±1.18	6.00±1.22	6.36±1.13		
	Male	6.33±1.18	6.49±1.00	6.51±1.22	6.32±0.99	6.42±1.07	Sex	0.31
	Female	6.23±1.19	6.08±1.06	6.43±1.13	6.06±1.25	6.22±1.11	Time x Sex	0.62
	PLA M	6.43±1.09	6.34±1.18	6.69±1.21	6.52±1.11	6.46±1.12	Treatment x Sex	0.62
	PLA F	5.89±1.43	6.09±1.10	6.15±1.05	6.06±0.93	5.99±1.07	Treatment x Time x Sex	0.63
	CNL M	6.20±1.34	6.85±0.64	6.41±1.44	6.41±0.94	6.43±1.07		
	CNL F	6.38±1.01	6.01±1.37	6.28±0.97	6.17±1.13	6.33±0.95		
	CNH M	6.33±1.20	6.32±1.01	6.40±1.10	6.03±0.90	6.38±1.04		
	CNH F	6.39±1.20	6.13±0.75	6.83±1.33	5.94±1.69	6.33±1.28		
Lymphocyte	Overall	2.29±0.60	2.34±0.63	2.43±0.60*	2.23±0.59	2.31±0.60	Time	0.006
(K/µL)	PLA	2.31±0.62	2.33±0.67	2.42±0.65	2.30±0.65	2.32±0.63	Treatment	0.84
	CNL	2.28±0.60	2.35±0.66	2.45±0.53	2.35±0.47	2.32±0.59	Treatment x Time	0.39
	CNH	2.28±0.62	2.36±0.59	2.41±0.61	2.05±0.60	2.29±0.60		
	Male	2.32±0.51	2.44±0.57	2.44±0.55	2.39±0.54	2.38±0.53	Sex	0.12
	Female	2.24±0.75	2.19±0.70	2.41±0.68	1.96±0.58†*	2.17±0.70	Time x Sex	0.01
	PLA M	2.38±0.51	2.46±0.65	2.46±0.65	2.44±0.66	2.42±0.60	Treatment x Sex	0.89
	PLA F	2.19±0.82	2.06±0.68	2.34±0.70	2.01±0.56	2.12±0.65	Treatment x Time x Sex	0.66
	CNL M	2.21±0.45	2.46±0.49	2.45±0.48	2.54±0.37	2.37±0.48		
	CNL F	2.38±0.79	2.19±0.85	2.46±0.63	2.08±0.48	2.21±0.74		
	CNH M	2.35±0.57	2.39±0.60	2.40±0.51	2.21±0.49	2.35±0.51		
	CNH F	2.14±0.71	2.30±0.61	2.43±0.78	1.79±0.70	2.17±0.72		

Table 3: Continued.

		Day						
	Treatment	0	1	5	6	Mean	Interaction	p-Level
Mid-Range	Overall	0.64±0.26	0.69±0.34	0.67±0.23	0.61±0.23	0.65±0.26	Time	0.13
(K/µL)	PLA	0.61±0.20	0.67±0.25	0.67±0.27	0.62±0.22	0.64±0.23	Treatment	0.58
	CNL	0.65±0.31	0.73±0.46	0.67±0.22	0.67±0.23	0.67±0.30	Treatment x Time	0.66
	CNH	0.67±0.26	0.69±0.30	0.67±0.19	0.53±0.23	0.65±0.26		
	Male	0.59±0.19	0.66±0.20	0.67±0.25	0.62±0.24	0.63±0.22	Sex	0.28
	Female	0.72±0.34	0.76±0.49	0.67±0.17	0.58±0.21	0.69±0.33	Time x Sex	0.16
	PLA M	0.62±0.21	0.68±0.26	0.68±0.32	0.63±0.22	0.65±0.25	Treatment x Sex	0.39
	PLA F	0.59±0.20	0.65±0.24	0.64±0.18	0.60±0.21	0.62±0.21	Treatment x Time x Sex	0.72
	CNL M	0.54±0.10	0.66±0.20	0.65±0.25	0.70±0.21	0.62±0.19		
	CNL F	0.81±0.44	0.82±0.69	0.70±0.18	0.62±0.25	0.75±0.43		
	CNH M	0.61±0.22	0.63±0.13	0.67±0.20	0.55±0.26	0.63±0.22		
	CNH F	0.76±0.31	0.79±0.46	0.68±0.18	0.51±0.17	0.68±0.31		
Granulocyte	Overall	3.37±0.95	3.30±0.89	3.38±0.94	3.39±1.03	3.39±0.91	Time	0.78
(K/µL)	PLA	3.33±0.78	3.27±0.83	3.41±0.99	3.44±0.94	3.34±0.86	Treatment	0.90
	CNL	3.35±1.26	3.42±1.07	3.24±0.89	3.30±0.75	3.41±0.89	Treatment x Time	0.83
	CNH	3.43±0.81	3.22±0.77	3.49±0.95	3.41±1.32	3.43±0.98		
	Male	3.43±0.96	3.39±0.75	3.40±0.96	3.30±0.94	3.41±0.88	Sex	0.71
	Female	3.28±0.94	3.13±1.08	3.35±0.92	3.52±1.16	3.37±0.97	Time x Sex	0.30
	PLA M	3.45±0.76	3.21±0.83	3.54±1.10	3.44±1.09	3.40±0.93	Treatment x Sex	0.66
	PLA F	3.10±0.81	3.39±0.87	3.16±0.74	3.44±0.59	3.24±0.72	Treatment x Time x Sex	0.44
	CNL M	3.46±1.30	3.72±0.62	3.32±0.95	3.17±0.79	3.43±0.88		
	CNL F	3.20±1.25	2.99±1.45	3.13±0.84	3.49±0.69	3.38±0.93		
	CNH M	3.38±0.87	3.31±0.71	3.34±0.85	3.27±0.94	3.40±0.84		
	CNH F	3.51±0.73	3.06±0.88	3.73±1.10	3.63±1.85	3.48±1.19		

Data are means ± SD. MANOVA analysis revealed overall Wilks' Lambda treatment (p=0.94), time (p=0.02), sex (p=0.10), treatment x time (p=0.73), treatment x sex (p=0.86), time x sex (p=0.36), and treatment x time x sex (p=0.96). Univariate ANOVA p-levels from MANOVA analysis are presented for each variable. p<0.05 is considered significant. Statistical notations. (*) denotes a significant difference from baseline. (†) denotes a significant difference from male.

Table 4: Red Blood Cell Counts.

		Day						
	Treatment	0	1	5	6	Mean	Interaction	p-Level
Red	Overall	4.94±0.55	4.94±0.52	4.96±0.55	4.88±0.54	4.94±0.53	Time	0.57
Blood	PLA	4.98±0.47	4.95±0.39	4.95±0.49	4.90±0.52	4.94±0.47	Treatment	0.97
Cell	CNL	4.97±0.64	4.94±0.52	4.96±0.54	4.85±0.59	4.94±0.57	Treatment x Time	0.93
(M/µL)	CNH	4.87±0.54	4.92±0.66	4.97±0.63	4.89±0.52	4.94±0.57		
	Male	5.21±0.44	5.14±0.46	5.25±0.43	5.12±0.46	5.19±0.43	Sex	0.001
	Female	4.48±0.38	4.59±0.43	4.45±0.31	4.46±0.39	4.48±0.38†	Time x Sex	0.19
	PLA M	5.19±0.39	5.06±0.37	5.20±0.39	5.12±0.45	5.15±0.39	Treatment x Sex	0.72
	PLA F	4.55±0.29	4.74±0.36	4.44±0.19	4.46±0.34	4.52±0.31	Treatment x Time x Sex	0.60
	CNL M	5.27±0.55	5.14±0.33	5.27±0.36	5.12±0.54	5.21±0.44		
	CNL F	4.45±0.42	4.59±0.62	4.42±0.34	4.36±0.29	4.44±0.42		
	CNH M	5.17±0.38	5.25±0.67	5.30±0.56	5.12±0.40	5.20±0.48		
	CNH F	4.44±0.44	4.46±0.25	4.49±0.40	4.56±0.52	4.48±0.40		
Hemoglobin	Overall	14.8±1.8	14.8±1.7	14.9±1.8	14.6±1.7	14.8±1.7	Time	0.62
(g/dl)	PLA	14.9±1.8	14.8±1.4	14.9±1.8	14.6±1.7	14.8±1.6	Treatment	0.94
	CNL	14.9±2.0	14.7±1.7	15.0±1.6	14.7±2.0	14.8±1.8	Treatment x Time	0.96
	CNH	14.6±1.8	14.8±2.2	15.0±2.0	14.6±1.5	14.8±1.8		
	Male	15.8±1.4	15.6±1.4	16.0±1.3	15.5±1.3	15.7±1.3	Sex	0.001
	Female	13.2±1.3	13.4±1.5	13.2±1.0	13.1±1.3	13.2±1.2†	Time x Sex	0.48
	PLA M	15.7±1.4	15.3±1.1	15.8±1.2	15.4±1.2	15.6±1.2	Treatment x Sex	0.79
	PLA F	13.4±1.3	13.7±1.4	13.0±0.9	13.1±1.4	13.3±1.2	Treatment x Time x Sex	0.60
	CNL M	16.0±1.7	15.5±0.8	16.0±0.9	15.7±1.7	15.8±1.3		
	CNL F	13.2±1.2	13.5±2.0	13.2±0.7	12.9±1.3	13.1±1.3		
	CNH M	15.7±1.0	16.0±2.0	16.2±1.6	15.4±1.0	15.8±1.4		
	CNH F	13.0±1.5	13.0±0.9	13.4±1.4	13.4±1.2	13.2±1.2		

Table 4: Continued.

		Day						
	Treatment	0	1	5	6	Mean	Interaction	p-Level
Hematocrit	Overall	46.1±4.8	46.0±4.6	46.1±5.1	45.2±4.8	45.9±4.8	Time	0.36
(%)	PLA	46.2±4.4	46.0±3.8	45.8±4.8	45.2±4.6	45.8±4.4	Treatment	0.96
	CNL	46.5±5.4	46.1±4.7	46.1±4.8	45.0±5.6	45.9±5.1	Treatment x Time	0.86
	CNH	45.4±4.7	45.7±5.5	46.3±5.8	45.4±4.2	46.0±4.9		
	Male	48.5±3.5	47.7±3.8	48.8±3.9	47.5±3.9	48.1±3.7	Sex	0.001
	Female	41.9±3.7	43.0±4.6	41.4±3.1	41.3±3.5	41.8±3.7†	Time x Sex	0.12
	PLA M	48.2±3.4	46.9±3.3	48.3±3.6	47.3±3.4	47.8±3.4	Treatment x Sex	0.75
	PLA F	42.3±3.6	44.2±4.3	41.0±2.5	41.0±3.8	42.1±3.5	Treatment x Time x Sex	0.65
	CNL M	49.2±4.3	47.7±2.6	49.0±3.3	47.6±5.0	48.4±3.7		
	CNL F	41.8±3.5	43.4±6.3	41.2±2.0	40.5±3.5	41.4±4.1		
	CNH M	48.1±2.6	48.5±5.3	49.3±4.8	47.6±3.4	48.3±3.9		
	CNH F	41.5±4.4	41.7±2.8	41.9±4.3	42.4±3.3	41.9±3.6		
MCV	Overall	93.3±4.0	93.2±4.4	93.0±3.9	92.8±4.0	93.0±4.0	Time	0.07
(fL)	PLA	92.9±4.2	93.0±4.2	92.6±3.9	92.3±4.0	92.9±4.0	Treatment	0.81
	CNL	93.7±3.9	93.6±5.1	93.2±3.9	92.9±4.1	93.1±4.2	Treatment x Time	0.55
	CNH	93.4±4.0	93.0±4.0	93.3±4.0	93.2±4.0	93.1±4.0		
	Male	93.2±3.8	92.9±3.6	93.0±3.7	92.8±3.7	92.9±3.6	Sex	0.79
	Female	93.6±4.4	93.7±5.5	93.0±4.3	92.7±4.5	93.3±4.7	Time x Sex	0.14
	PLA M	92.9±4.1	92.9±3.8	92.8±3.9	92.5±3.9	92.8±3.7	Treatment x Sex	0.95
	PLA F	93.0±4.6	93.2±5.3	92.3±4.3	91.8±4.3	93.1±4.6	Treatment x Time x Sex	0.95
	CNL M	93.6±3.7	93.1±3.9	93.1±3.8	93.0±3.7	93.0±3.6		
	CNL F	94.1±4.6	94.5±7.0	93.5±4.4	92.9±4.9	93.4±5.0		
	CNH M	93.2±3.7	92.6±3.3	93.2±3.7	93.0±3.7	93.0±3.6		
	CNH F	93.7±4.6	93.5±4.9	93.3±4.7	93.3±4.7	93.5±4.5		

Table 4: Continued.

		Day						
	Treatment	0	1	5	6	Mean	Interaction	p-Level
MCH	Overall	30.0±1.3	29.9±1.5	30.2±1.4	30.0±1.2	30.0±1.3	Time	0.02
(pg/cell)	PLA	29.9±1.5	29.8±1.5	30.0±1.3	29.8±1.2	29.9±1.4	Treatment	0.72
	CNL	30.1±1.2	29.8±1.3	30.2±1.4	30.2±1.4	30.0±1.3	Treatment x Time	0.66
	CNH	30.0±1.3	30.0±1.5	30.3±1.3	29.8±1.2	30.0±1.3		
	Male	30.3±1.1	30.3±1.2	30.4±1.0	30.3±1.1	30.3±1.1	Sex	0.00
	Female	29.5±1.5	29.1±1.5	29.7±1.8	29.4±1.4	29.5±1.6†	Time x Sex	0.20
	PLA M	30.2±1.4	30.2±1.4	30.3±1.1	30.2±1.1	30.2±1.2	Treatment x Sex	0.91
	PLA F	29.3±1.5	28.9±1.5	29.3±1.5	29.2±1.3	29.4±1.5	Treatment x Time x Sex	0.68
	CNL M	30.3±0.8	30.2±1.1	30.4±1.0	30.6±1.0	30.2±1.0		
	CNL F	29.7±1.7	29.3±1.6	29.9±2.1	29.6±1.8	29.5±1.7		
	CNH M	30.4±1.0	30.5±1.3	30.5±0.8	30.1±1.1	30.3±1.1		
	CNH F	29.4±1.5	29.2±1.6	29.9±1.8	29.5±1.2	29.5±1.5		
MCHC	Overall	32.1±1.0	32.0±1.3	32.4±0.9*	32.3±0.9	32.2±1.0	Time	0.007
(g/dl)	PLA	32.2±1.2	32.1±1.4	32.4±0.7	32.3±0.9	32.3±1.0	Treatment	0.98
	CNL	32.1±1.0	31.9±1.3	32.5±0.9	32.5±0.8	32.2±1.1	Treatment x Time	0.38
	CNH	32.1±1.0	32.2±1.3	32.4±1.0	32.0±0.9	32.2±1.0		
	Male	32.5±0.9	32.6±1.0	32.7±0.6	32.6±0.7	32.6±0.8	Sex	0.001
	Female	31.5±0.9	31.1±1.2	31.9±1.0	31.8±0.9	31.6±1.1†	Time x Sex	0.06
	PLA M	32.5±1.1	32.6±1.1	32.7±0.5	32.6±0.8	32.6±0.9	Treatment x Sex	0.94
	PLA F	31.6±1.0	31.1±1.3	31.7±0.5	31.8±0.8	31.6±1.0	Treatment x Time x Sex	0.92
	CNL M	32.4±0.8	32.3±1.0	32.7±0.7	32.9±0.5	32.5±0.9		
	CNL F	31.6±1.0	31.0±1.3	32.0±1.0	31.9±0.9	31.6±1.1		
	CNH M	32.6±0.8	32.9±0.9	32.7±0.6	32.3±0.6	32.6±0.7		
	CNH F	31.4±0.9	31.2±1.2	32.0±1.3	31.6±1.1	31.5±1.1		

Table 4: Continued.

		Day						
	Treatment	0	1	5	6	Mean	Interaction	p-Level
RBCDW	Overall	13.4±1.1	13.4±1.2	13.4±1.1	13.4±1.1	13.3±1.1	Time	0.75
(%)	PLA	13.4±0.9	13.3±1.2	13.3±1.0	13.4±1.1	13.3±1.0	Treatment	0.99
	CNL	13.4±1.1	13.5±1.2	13.3±1.1	13.3±1.0	13.4±1.0	Treatment x Time	0.54
	CNH	13.4±1.3	13.4±1.3	13.5±1.2	13.6±1.2	13.4±1.2		
	Male	13.1±0.7	13.0±0.6	13.1±0.7	13.2±0.7	13.1±0.7	Sex	0.002
	Female	13.8±1.4	14.1±1.6	13.8±1.5	13.8±1.5	13.8±1.5†	Time x Sex	0.06
	PLA M	13.1±0.5	13.0±0.8	13.1±0.8	13.1±0.6	13.1±0.7	Treatment x Sex	0.95
	PLA F	13.9±1.2	14.1±1.5	13.7±1.4	14.0±1.6	13.7±1.4	Treatment x Time x Sex	0.75
	CNL M	13.1±0.7	13.1±0.6	13.0±0.7	13.2±0.8	13.1±0.7		
	CNL F	13.8±1.5	14.1±1.7	13.8±1.5	13.6±1.4	13.8±1.4		
	CNH M	13.0±0.8	12.9±0.4	13.1±0.7	13.2±0.7	13.1±0.6		
	CNH F	13.8±1.7	14.0±1.8	14.0±1.7	14.0±1.6	13.9±1.6		
Platelet	Overall	213±55	219±56	213±55	217±56	211±57	Time	0.32
Count	PLA	216±59	223±65	206±63	216±60	212±61	Treatment	0.84
(x103/µL)	CNL	202±52	214±52	216±53	214±60	208±55	Treatment x Time	0.20
	CNH	220±56	221±52	219±48	220±50	213±53		
	Male	194±45	197±42	192±38	196±43	190±43	Sex	0.001
	Female	245±58	257±58	250±59	252±59	250±58†	Time x Sex	0.68
	PLA M	190±46	196±42	186±39	193±35	189±41	Treatment x Sex	0.56
	PLA F	268±46	276±72	246±83	261±76	256±69	Treatment x Time x Sex	0.37
	CNL M	187±47	196±41	194±39	190±52	185±43		
	CNL F	227±55	246±55	255±54	256±51	249±52		
	CNH M	207±43	201±46	197±39	206±43	196±46		
	CNH F	239±68	251±47	249±44	241±54	245±52		

Table 4: Continued.

		Day						
	Treatment	0	1	5	6	Mean	Interaction	p-Level
MPV	Overall	10.0±1.1	9.9±1.2	10.0±1.3	9.8±1.2	10.1±1.5	Time	0.33
(fL)	PLA	9.9±1.0	9.8±1.0	10.2±1.6	9.9±1.2	10.0±1.3	Treatment	0.95
	CNL	10.0±1.1	10.0±1.4	9.8±0.9	10.0±1.5	10.1±1.4	Treatment x Time	0.21
	CNH	10.0±1.2	9.9±1.3	9.9±1.2	9.6±1.0	10.2±1.6		
	Male	10.2±1.0	10.1±1.0	10.3±1.2	10.2±1.1	10.5±1.4	Sex	0.004
	Female	9.6±1.1	9.5±1.4	9.4±1.2	9.1±1.1	9.4±1.2†	Time x Sex	0.11
	PLA M	10.0±1.0	10.1±0.9	10.6±1.5	10.3±1.0	10.4±1.2	Treatment x Sex	0.79
	PLA F	9.6±1.1	9.1±0.9	9.3±1.4	9.0±1.2	9.3±1.1	Treatment x Time x Sex	0.34
	CNL M	10.3±1.1	10.2±1.0	9.9±0.7	10.4±1.4	10.4±1.3		
	CNL F	9.5±1.0	9.6±1.9	9.5±1.1	9.4±1.5	9.4±1.3		
	CNH M	10.3±1.0	10.1±1.2	10.2±1.2	10.0±0.9	10.5±1.7		
	CNH F	9.7±1.4	9.7±1.5	9.5±1.2	9.1±0.9	9.5±1.2		

Data are means ± SD. MANOVA analysis revealed overall Wilks' Lambda treatment (p=0.99), time (p=0.008), sex (p=0.001), treatment x time (p=0.90), treatment x sex (p=0.99), time x sex (p=0.15), and treatment x time x sex (p=0.56). Univariate ANOVA p-levels from MANOVA analysis are presented for each variable. p<0.05 is considered significant. Statistical notations. (*) denotes a significant difference from baseline. (†) denotes a significant difference from male.

Table 5: Kidney Function Markers.

		Day						
	Treatment	0	1	5	6	Mean	Interaction	p-Level
BUN	Overall	14.2±3.6	14.0±4.1	14.8±4.0	14.7±4.2	14.4±4.0	Time	0.15
(mg/dl)	PLA	14.7±4.1	13.7±4.0	14.9±3.9	14.6±4.8	14.5±4.2	Treatment	0.91
	CNL	13.8±3.1	13.4±3.8	14.8±4.4	15.0±4.2	14.3±3.9	Treatment x Time	0.24
	CNH	14.1±3.7	14.9±4.6	14.8±3.7	14.5±3.7	14.6±3.9		
	Male	15.4±3.4	15.6±3.9	16.7±3.3*	16.7±3.5*	16.1±3.6	Sex	0.001
	Female	12.1±2.9†	11.1±2.7†	11.5±2.8†	11.2±2.7†	11.5±2.8†	Time x Sex	0.01
	PLA M	16.3±3.6	15.2±3.7	16.8±3.2	16.9±4.2	16.3±3.7	Treatment x Sex	0.89
	PLA F	11.8±3.4	11.1±3.0	11.5±2.8	10.5±2.3	11.2±2.8	Treatment x Time x Sex	0.25
	CNL M	14.5±3.0	15.2±3.1	16.9±3.9	17.0±3.3	15.9±3.4		
	CNL F	12.6±2.8	10.1±2.4	11.0±2.2	11.6±3.4	11.3±2.8		
	CNH M	15.4±3.6	16.5±4.7	16.3±2.9	16.2±3.2	16.1±3.6		
	CNH F	11.8±2.6	12.0±2.7	12.0±3.4	11.5±2.4	11.8±2.7		
Creatinine	Overall	0.97±0.19	0.98±0.18	0.98±0.19	0.96±0.17	0.97±0.18	Time	0.24
(mg/dl)	PLA	0.96±0.18	0.98±0.18	0.95±0.20	0.95±0.17	0.96±0.18	Treatment	0.70
	CNL	1.01±0.20	0.99±0.18	0.97±0.16	0.94±0.15*	0.98±0.17	Treatment x Time	0.007
	CNH	0.93±0.17	0.98±0.18	1.02±0.20*	0.99±0.18	0.98±0.19		
	Male	1.04±0.16	1.07±0.15	1.05±0.15	1.04±0.14	1.05±0.15	Sex	0.001
	Female	0.83±0.17	0.83±0.11	0.85±0.19	0.82±0.11	0.83±0.15†	Time x Sex	0.46
	PLA M	1.04±0.16	1.06±0.16	1.04±0.18	1.03±0.14	1.04±0.15	Treatment x Sex	0.82
	PLA F	0.81±0.14	0.83±0.12	0.79±0.10	0.79±0.12	0.80±0.12	Treatment x Time x Sex	0.43
	CNL M	1.08±0.15	1.08±0.16	1.04±0.14	1.01±0.12	1.05±0.14		
	CNL F	0.87±0.21	0.82±0.08	0.83±0.09	0.82±0.11	0.84±0.13		
	CNH M	1.00±0.16	1.07±0.15	1.07±0.13	1.07±0.15	1.05±0.15		
	CNH F	0.82±0.15	0.83±0.14	0.93±0.29	0.84±0.12	0.86±0.18		

Table 5: Continued.

		Day						
	Treatment	0	1	5	6	Mean	Interaction	p-Level
BUN/	Overall	14.9±3.5	14.4±3.8	15.2±3.2	15.4±3.7	15.0±3.6	Time	0.11
Creatinine	PLA	15.5±4.0	14.3±3.8	15.7±3.1	15.4±4.1	15.2±3.8	Treatment	0.80
Ratio	CNL	14.0±2.8	13.7±3.6	15.2±3.4	15.9±3.4*	14.7±3.4	Treatment x Time	0.03
	CNH	15.3±3.7	15.3±4.0	14.6±3.2	14.9±3.6	15.0±3.6		
	Male	15.0±3.7	14.8±3.8	16.0±3.1*	16.3±3.6*	15.5±3.6	Sex	0.02
	Female	14.7±3.4	13.6±3.8	13.7±3.0†	13.9±3.3†	14.0±3.4†	Time x Sex	0.01
	PLA M	15.9±3.7	14.5±3.5	16.2±2.8	16.5±4.1	15.8±3.6	Treatment x Sex	1.00
	PLA F	14.9±4.6	13.9±4.6	14.7±3.5	13.5±3.3	14.2±3.9	Treatment x Time x Sex	0.17
	CNL M	13.5±2.9	14.4±3.7	16.2±3.3	16.9±3.0	15.3±3.4		
	CNL F	14.8±2.5	12.4±3.0	13.3±2.8	14.2±3.5	13.7±3.0		
	CNH M	15.7±4.1	15.6±4.2	15.5±3.3	15.5±3.6	15.6±3.7		
	CNH F	14.5±3.0	14.6±3.7	13.1±2.7	13.9±3.4	14.0±3.2		

Data are means ± SD. MANOVA analysis revealed overall Wilks' Lambda treatment (p=0.92), time (p=0.08), sex (p=0.001), treatment x time (p=0.23), treatment x sex (p=0.94), time x sex (p=0.15), and treatment x time x sex (p=0.19). Univariate ANOVA p-levels from MANOVA anlysis are presented for each variable. p<0.05 is considered significant. Statistical notations. (*) denotes a significant difference from baseline. (†) denotes a significant difference from male.

Table 6: Liver Enzymes.

		Day						
	Treatment	0	1	5	6	Mean	Interaction	p-Level
ALP	Overall	80.4±22.2	77.9±20.1*	81.1±21.4	79.6±20.8	80.0±21.0	Time	0.04
(U/L)	PLA	80.1±21.5	78.4±20.5	80.1±19.5	78.6±20.0	80.2±20.2	Treatment	0.99
	CNL	81.5±23.7	77.5±21.4	81.8±22.9	79.9±20.6	80.4±21.8	Treatment x Time	0.73
	CNH	79.7±22.1	77.7±19.2	81.5±22.2	80.4±22.3	79.3±21.3		
	Male	84.4±22.4	81.6±19.5	84.5±19.7	83.4±20.0	82.6±20.5	Sex	0.03
	Female	73.4±20.3	71.2±19.8	75.0±23.1	72.7±20.7	75.1±21.2†	Time x Sex	0.88
	PLA M	83.8±20.6	80.7±19.6	84.0±17.6	82.3±18.6	82.4±19.4	Treatment x Sex	0.80
	PLA F	73.3±22.5	74.3±22.7	72.9±21.7	71.9±21.7	75.9±21.3	Treatment x Time x Sex	0.68
	CNL M	86.7±24.0	83.7±21.7	86.2±21.4	84.7±20.8	84.6±21.7		
	CNL F	72.1±21.0	66.4±16.4	73.9±24.6	71.3±18.3	72.8±20.3		
	CNH M	82.5±23.6	80.4±18.2	83.2±21.0	83.3±21.7	80.8±20.7		
	CNH F	74.7±19.5	72.9±20.9	78.4±25.0	75.1±23.7	76.6±22.4		
ALT	Overall	20.7±8.7	20.6±9.2	20.3±9.0	21.1±11.3	21.1±9.8	Time	0.67
(U/L)	PLA	21.5±9.5	21.1±9.1	20.5±9.1	21.0±8.4	21.5±9.1	Treatment	0.78
	CNL	20.9±7.1	20.7±7.3	21.5±10.6	23.1±15.7	21.7±11.1	Treatment x Time	0.64
	CNH	19.9±9.4	19.9±11.2	19.1±7.2	19.4±8.0	20.1±9.3		
	Male	22.8±8.7	22.6±9.7	22.7±9.9	24.0±12.8	23.3±10.7	Sex	0.001
	Female	17.0±7.4	16.9±7.0	16.0±5.2	16.0±4.7	17.1±6.2†	Time x Sex	0.37
	PLA M	23.1±8.5	22.3±8.1	22.6±9.6	23.4±8.3	23.1±8.7	Treatment x Sex	0.55
	PLA F	18.6±11.0	18.9±10.7	16.6±7.1	16.6±6.9	18.5±9.2	Treatment x Time x Sex	0.68
	CNL M	23.6±6.6	23.8±7.1	25.1±11.7	27.3±18.3	25.0±12.4		
	CNL F	16.0±5.3	15.3±3.7	15.0±3.1	15.6±2.6	15.8±3.7		
	CNH M	21.9±10.9	21.8±13.2	20.5±8.0	21.3±9.1	21.8±10.8		
	CNH F	16.3±4.8	16.5±5.0	16.6±5.0	15.9±4.0	17.0±4.3		

Table 6: Continued.

		Day						
	Treatment	0	1	5	6	Mean	Interaction	p-Level
AST	Overall	25.2±9.3	26.3±9.2	25.3±14.4	26.4±13.2	26.0±12.1	Time	0.65
(U/L)	PLA	25.2±9.4	27.1±8.9	26.5±17.4	27.3±11.2	26.8±12.4	Treatment	0.37
	CNL	28.4±10.9	28.0±9.6	25.5±16.6	27.2±17.5	27.4±14.4	Treatment x Time	0.72
	CNH	22.0±5.9	23.9±9.1	23.8±7.5	24.6±9.7	23.8±8.5		
	Male	27.4±9.9	28.9±9.6	28.0±16.9	29.5±15.2	28.4±13.8	Sex	0.001
	Female	21.1±6.3	21.7±6.5	20.4±5.9	20.7±4.9	21.5±6.0†	Time x Sex	0.69
	PLA M	26.8±10.0	28.4±8.9	29.0±21.0	29.9±12.5	28.4±14.1	Treatment x Sex	0.22
	PLA F	22.2±7.6	24.8±8.8	21.9±6.6	22.6±6.9	23.6±7.5	Treatment x Time x Sex	0.86
	CNL M	32.4±11.3	32.3±9.3	30.2±19.2	31.4±20.7	31.4±16.3		
	CNL F	21.2±5.3	20.4±3.0	17.2±3.2	19.5±3.0	20.2±3.8		
	CNH M	23.1±5.7	26.0±10.0	24.7±8.2	27.2±11.0	25.4±9.4		
	CNH F	19.9±6.0	20.0±5.8	22.2±6.3	19.9±3.9	20.9±5.5		

Data are means ± SD. MANOVA analysis revealed overall Wilks' Lambda treatment (p=0.90), time (p=0.001), sex (p=0.001), treatment x time (p=0.66), treatment x sex (p=0.78), time x sex (p=0.98), and treatment x time x sex (p=0.87). Univariate ANOVA p-levels from MANOVA analysis are presented for each variable. p<0.05 is considered significant. Statistical notations. (*) denotes a significant difference from baseline. (†) denotes a significant difference from male.

Table 7: Muscle Catabolism Markers.

		Day						
	Treatment	0	1	5	6	Mean	Interaction	p-Level
CK	Overall	227±268	334±306	404±1329	498±1204	371±967	Time	0.26
(U/L)	PLA	226±177	327±242	345±609	432±441	322±407	Treatment	0.60
	CNL	263±418	401±429	624±2196	714±1988	521±1592	Treatment x Time	0.82
	CNH	193±113	275±191	244±398	350±482	272±349		
	Male	287±315	436±337	552±1638	675±1469	500±1181	Sex	0.03
	Female	120±80	152±83	138±188	181±209	133±80†	Time x Sex	0.39
	PLA M	280±194	420±254	423±719	541±461	422±472	Treatment x Sex	0.62
	PLA F	129±83	158±72	204±317	235±341	132±65	Treatment x Time x Sex	0.78
	CNL M	346±505	530±488	914±2721	1004±2454	726±1954		
	CNL F	114±63	169±100	103±50	191±116	146±96		
	CNH M	236±98	357±186	320±482	479±564	356±409		
	CNH F	116±97	129±78	107±61	118±64	122±76		
LDH	Overall	158±21	160±25	164±46	165±37	161±34	Time	0.38
(U/L)	PLA	158±17	157±19	163±27	162±21	159±21	Treatment	0.93
	CNL	158±24	159±29	167±68	173±55	163±49	Treatment x Time	0.66
	CNH	158±23	163±25	163±34	160±25	160±27		
	Male	159±19	162±26	170±52	171±42	164±38	Sex	0.09
	Female	156±25	155±22	154±31	154±20	156±25	Time x Sex	0.18
	PLA M	160±16	158±20	168±28	166±21	162±22	Treatment x Sex	0.80
	PLA F	154±18	156±19	155±22	154±21	154±20	Treatment x Time x Sex	0.39
	CNL M	159±21	161±31	179±83	181±66	168±58		
	CNL F	157±29	154±25	145±14	157±19	156±23		
	CNH M	159±20	167±26	163±25	165±24	162±24		
	CNH F	156±27	155±24	162±48	151±23	158±32		

Data are means ± SD. MANOVA analysis revealed overall Wilks' Lambda treatment (p=0.81), time (p=0.001), sex (p=0.10), treatment x time (p=0.55), treatment x sex (p=0.91), time x sex (p=0.03), and treatment x time x sex (p=0.87). Univariate ANOVA p-levels from MANOVA analysis are presented for each variable. p<0.05 is considered significant. Statistical notations. (†) denotes a significant difference from male.

Table 8: Lipid Profile.

		Day						
	Treatment	0	1	5	6	Mean	Interaction	p-Level
Total-C	Overall	3.92±1.03	3.84±0.92	3.82±0.92*	3.82±0.90*	3.83±0.97	Time	0.005
(mmol/l)	PLA	3.88±0.88	3.84±0.87	3.83±0.91	3.80±0.88	3.82±0.90	Treatment	0.99
	CNL	4.03±1.13	3.81±0.81	3.79±0.95	3.83±0.87	3.83±0.97	Treatment x Time	0.21
	CNH	3.86±1.08	3.85±1.08	3.84±0.93	3.82±0.98	3.82±1.04		
	Male	3.84±1.10	3.76±1.04	3.85±1.10	3.86±1.06	3.80±1.09	Sex	0.77
	Female	4.07±0.89	3.97±0.63	3.77±0.45*	3.75±0.53*	3.88±0.69	Time x Sex	0.001
	PLA M	3.83±1.02	3.71±0.99	3.91±1.11	3.86±1.04	3.79±1.05	Treatment x Sex	0.99
	PLA F	3.98±0.58	4.08±0.56	3.69±0.36	3.69±0.50	3.87±0.54	Treatment x Time x Sex	0.22
	CNL M	3.92±1.13	3.81±0.97	3.82±1.15	3.85±1.02	3.80±1.07		
	CNL F	4.23±1.17	3.83±0.44	3.75±0.41	3.79±0.54	3.90±0.76		
	CNH M	3.78±1.20	3.76±1.20	3.82±1.08	3.86±1.17	3.79±1.17		
	CNH F	4.00±0.89	4.00±0.86	3.87±0.59	3.76±0.58	3.88±0.75		
HDL-C	Overall	1.44±0.39	1.41±0.35	1.45±0.35	1.44±0.33	1.44±0.37	Time	0.48
(mmol/l)	PLA	1.43±0.36	1.43±0.37	1.43±0.32	1.45±0.34	1.44±0.36	Treatment	0.99
	CNL	1.49±0.46	1.38±0.30*	1.43±0.32	1.43±0.32	1.43±0.37	Treatment x Time	0.02
	CNH	1.40±0.36	1.41±0.38	1.49±0.42*	1.44±0.35	1.45±0.39		
	Male	1.32±0.29	1.27±0.29	1.34±0.28	1.33±0.29	1.31±0.29	Sex	0.001
	Female	1.65±0.47	1.65±0.33	1.64±0.38	1.63±0.33	1.69±0.37†	Time x Sex	0.28
	PLA M	1.33±0.33	1.29±0.32	1.36±0.31	1.37±0.32	1.34±0.33	Treatment x Sex	0.88
	PLA F	1.60±0.35	1.69±0.33	1.57±0.29	1.60±0.35	1.65±0.32	Treatment x Time x Sex	0.39
	CNL M	1.35±0.24	1.26±0.26	1.31±0.26	1.30±0.27	1.29±0.25		
	CNL F	1.75±0.65	1.59±0.27	1.64±0.30	1.65±0.31	1.70±0.40		
	CNH M	1.29±0.30	1.26±0.29	1.36±0.29	1.33±0.27	1.30±0.29		
	CNH F	1.60±0.40	1.67±0.39	1.73±0.53	1.65±0.38	1.72±0.41		

Table 8: Continued.

		Day						
	Treatment	0	1	5	6	Mean	Interaction	p-Level
Total-C/	Overall	2.61±1.32	2.53±1.17	2.29±0.96*	2.35±0.96*	2.77±0.77	Time	0.05
HDL-C	PLA	2.53±1.19	2.57±1.16	2.38±0.97	2.43±0.97	2.75±0.67	Treatment	0.84
Ratio	CNL	3.05±1.60	2.64±1.32	2.23±0.95	2.32±0.94	2.79±0.78	Treatment x Time	0.16
	CNH	2.26±1.02	2.38±1.04	2.27±0.98	2.31±0.99	2.77±0.85		
	Male	2.74±1.53	2.63±1.33	2.40±1.08	2.47±1.06	2.98±0.80	Sex	0.15
	Female	2.38±0.76	2.35±0.80	2.11±0.65	2.15±0.72	2.37±0.50	Time x Sex	0.91
	PLA M	2.62±1.43	2.64±1.39	2.51±1.14	2.54±1.13	2.93±0.69	Treatment x Sex	0.34
	PLA F	2.37±0.59	2.44±0.61	2.14±0.49	2.23±0.59	2.41±0.46	Treatment x Time x Sex	0.79
	CNL M	3.41±1.81	2.87±1.50	2.42±1.12	2.56±1.06	3.02±0.81		
	CNL F	2.40±0.86	2.22±0.82	1.89±0.37	1.90±0.46	2.37±0.52		
	CNH M	2.19±1.10	2.38±1.10	2.26±1.03	2.31±1.02	3.01±0.90		
	CNH F	2.37±0.88	2.38±0.99	2.30±0.94	2.32±0.99	2.34±0.54		
LDL-C	Overall	0.88±0.39	0.81±0.31	0.91±0.42*	0.83±0.33	2.42±1.15	Time	0.03
(mmol/l)	PLA	0.86±0.30	0.79±0.26	0.89±0.42	0.86±0.38	2.47±1.11	Treatment	0.95
	CNL	0.88±0.44	0.80±0.30	0.91±0.39	0.82±0.26	2.52±1.29	Treatment x Time	0.87
	CNH	0.91±0.44	0.83±0.36	0.93±0.45	0.81±0.35	2.28±1.03		
	Male	0.85±0.42	0.81±0.30	0.95±0.41*	0.87±0.34	2.54±1.30	Sex	0.65
	Female	0.94±0.35	0.81±0.32	0.85±0.42	0.76±0.30	2.21±0.76	Time x Sex	0.04
	PLA M	0.85±0.34	0.77±0.27	0.98±0.45	0.90±0.40	2.58±1.30	Treatment x Sex	0.92
	PLA F	0.89±0.22	0.82±0.26	0.73±0.33	0.78±0.35	2.25±0.58	Treatment x Time x Sex	0.35
	CNL M	0.82±0.42	0.80±0.31	0.96±0.38	0.84±0.22	2.75±1.46		
	CNL F	0.97±0.50	0.80±0.30	0.83±0.40	0.80±0.32	2.09±0.71		
	CNH M	0.88±0.51	0.85±0.34	0.91±0.42	0.88±0.38	2.28±1.08		
	CNH F	0.95±0.29	0.80±0.41	0.98±0.52	0.69±0.26	2.29±0.95		

Table 8: Continued.

		Day						
	Treatment	0	1	5	6	Mean	Interaction	p-Level
Triglyceride	Overall	2.84±0.79	2.84±0.74*	2.75±0.77	2.76±0.76*	0.86±0.37	Time	0.001
(mmol/l)	PLA	2.83±0.69	2.80±0.67	2.77±0.66	2.70±0.66	0.86±0.34	Treatment	0.99
	CNL	2.81±0.75	2.89±0.81	2.75±0.77	2.80±0.79	0.84±0.36	Treatment x Time	0.64
	CNH	2.89±0.93	2.84±0.77	2.73±0.88	2.76±0.84	0.87±0.41		
	Male	2.99±0.83	3.04±0.77	2.95±0.80	2.97±0.80	0.86±0.37	Sex	0.002
	Female	2.57±0.63	2.48±0.55*	2.39±0.54	2.37±0.50*	0.85±0.36†	Time x Sex	0.045
	PLA M	2.97±0.73	2.97±0.69	2.96±0.69	2.89±0.68	0.87±0.37	Treatment x Sex	0.97
	PLA F	2.56±0.57	2.49±0.52	2.42±0.44	2.38±0.48	0.83±0.29	Treatment x Time x Sex	0.80
	CNL M	2.95±0.75	3.11±0.84	2.98±0.84	3.04±0.84	0.84±0.34		
	CNL F	2.56±0.72	2.49±0.60	2.35±0.41	2.36±0.48	0.85±0.39		
	CNH M	3.06±1.03	3.05±0.80	2.91±0.91	2.98±0.90	0.87±0.41		
	CNH F	2.59±0.66	2.46±0.57	2.41±0.75	2.37±0.58	0.86±0.40		

Data are means ± SD. MANOVA analysis revealed overall Wilks' Lambda treatment (p=0.99), time (p=0.001), sex (p=0.001), treatment x time (p=0.62), treatment x sex (p=0.82), time x sex (p=0.004), and treatment x time x sex (p=0.28). Univariate ANOVA p-levels from MANOVA analysis are presented for each variable. p<0.05 is considered significant. Statistical notations. (*) denotes a significant difference from baseline. (†) denotes a significant difference from male.

Table 9: Blood Glucose.

		Day						
	Treatment	0	1	5	6	Mean	Interaction	p-Level
Glucose	Overall	5.10±0.52	4.89±0.40*	4.96±0.54	5.03±0.44	5.00±0.50	Time	0.04
(mmol/l)	PLA	5.09±0.34	4.85±0.39	4.84±0.48	5.05±0.38	4.97±0.42	Treatment	0.34
	CNL	5.16±0.69	4.84±0.37	4.85±0.32	4.97±0.47	4.97±0.52	Treatment x Time	0.06
	CNH	5.05±0.48	4.98±0.42	5.20±0.68	5.06±0.46	5.07±0.54		
	Male	5.16±0.41	4.90±0.47	5.00±0.42	5.16±0.41	5.07±0.44	Sex	0.09
	Female	4.98±0.68	4.86±0.23	4.89±0.70	4.79±0.39	4.89±0.56	Time x Sex	0.09
	PLA M	5.19±0.33	4.81±0.46	4.90±0.56	5.18±0.34	5.03±0.46	Treatment x Sex	0.81
	PLA F	4.90±0.30	4.91±0.22	4.73±0.31	4.81±0.34	4.85±0.30	Treatment x Time x Sex	0.12
	CNL M	5.09±0.45	4.87±0.43	4.93±0.29	5.11±0.42	5.02±0.42		
	CNL F	5.28±1.02	4.79±0.25	4.71±0.33	4.73±0.47	4.89±0.66		
	CNH M	5.21±0.44	5.03±0.49	5.17±0.33	5.19±0.47	5.15±0.44		
	CNH F	4.77±0.44	4.87±0.24	5.24±1.09	4.83±0.36	4.93±0.66		

Data are means ± SD. Univariate ANOVA p-levels are presented for each variable. p<0.05 is considered significant. Statistical notations. (a) denotes a significant difference from PLA. (b) denotes a significant difference from CNL. (c) denotes a significant difference from CNH. (*) denotes a significant difference from baseline. (†) denotes a significant difference from male.

Table 10: Assessment of blood chemistry changes from baseline to day 6.

			PLA		CNL		CNH		
			N	%	N	%	N	%	Chi-Square
Lipids & Glucose	Total-C	Normal/Normal	25	89%	25	89%	25	89%	0.96
		Normal/High	0	0%	1	4%	1	4%	
		High/High	2	7%	1	4%	1	4%	
		High/Normal	1	4%	1	4%	1	4%	
	HDL-C	Normal/Normal	23	82%	24	86%	22	79%	0.58
		Normal/High	3	11%	2	7%	4	14%	
		High/High	1	4%	0	0%	2	7%	
		High/Normal	1	4%	2	7%	0	0%	
	LDL-C	Normal/Normal	19	68%	15	54%	22	79%	0.32
		Normal/High	3	11%	3	11%	3	11%	
		High/High	1	4%	2	7%	2	7%	
		High/Normal	5	18%	8	29%	1	4%	
	Triglyceride	Normal/Normal	28	100%	28	100%	28	100%	-
		Normal/High	0	0%	0	0%	0	0%	
		High/High	0	0%	0	0%	0	0%	
		High/Normal	0	0%	0	0%	0	0%	
	Glucose	Normal/Normal	27	96%	25	89%	25	93%	0.46
		Normal/High	0	0%	0	0%	1	4%	
		High/High	0	0%	0	0%	0	0%	
		High/Normal	1	4%	3	11%	1	4%	
Liver	ALP	Normal/Normal	20	71%	17	61%	19	68%	0.78
		Normal/High	3	11%	2	7%	3	11%	
		High/High	5	18%	8	29%	6	21%	
		High/Normal	0	0%	1	4%	0	0%	
	ALT	Normal/Normal	24	86%	25	89%	27	96%	0.52
		Normal/High	1	4%	2	7%	0	0%	
		High/High	1	4%	0	0%	1	4%	
		High/Normal	2	7%	1	4%	0	0%	
	AST	Normal/Normal	20	71%	18	64%	25	89%	0.24
		Normal/High	3	11%	3	11%	2	7%	
		High/High	1	4%	0	0%	0	0%	
		High/Normal	4	14%	7	25%	1	4%	
Muscle	CK	Normal/Normal	11	39%	10	36%	11	39%	0.89
		Normal/High	6	21%	6	21%	3	11%	
		High/High	8	29%	7	25%	10	36%	
		High/Normal	3	11%	5	18%	4	14%	
	LDH	Normal/Normal	12	43%	12	43%	13	46%	0.31
		Normal/High	7	25%	1	4%	3	11%	
		High/High	7	25%	10	36%	9	32%	
		High/Normal	2	7%	5	18%	3	11%	
Kidney	BUN	Normal/Normal	23	82%	25	89%	24	86%	0.31
		Normal/High	1	4%	3	11%	2	7%	
		High/High	2	7%	0	0%	0	0%	
		High/Normal	2	7%	0	0%	2	7%	
	Creatinine	Normal/Normal	25	89%	25	89%	27	96%	0.23
		Normal/High	2	7%	0	0%	1	4%	
		High/High	0	0%	0	0%	0	0%	
		High/Normal	1	4%	3	11%	0	0%	

Data are presented as frequency. Statistical significance is detailed from chi-squared analysis.

Table 11: Body water data.

		Day				
	Treatment	0	5	Mean	Interaction	p-Level
Total Body	Overall	34.6±5.3	35.5±6.5	35.1±5.9	Time	0.16
Water	PLA	34.7±5.7	34.9±5.4	34.8±5.5	Treatment	0.60
(L)	CNL	34.8±5.2	36.7±8.5	35.8±7.1	Treatment x Time	0.44
	CNH	34.4±5.1	34.8±4.9	34.6±4.9		
	Male	37.4±3.6	38.4±5.7	37.9±4.8	Sex	0.001
	Female	29.6±4.0	30.2±3.7	29.9±3.9†	Time x Sex	0.66
	PLA M	37.6±3.6	37.9±3.3	37.8±3.4	Treatment x Sex	0.82
	PLA F	29.5±5.1	29.5±4.2	29.5±4.5	Treatment x Time x Sex	0.95
	CNL M	37.7±3.4	39.9±8.7	38.8±6.6		
	CNL F	29.6±3.6	30.9±4.0	30.3±3.8		
	CNH M	36.9±3.9	37.4±3.4	37.2±3.6		
	CNH F	29.8±3.5	30.1±3.3	29.9±3.3		
Intracellular	Overall	19.3±3.5	19.5±3.5	19.4±3.5	Time	0.39
Water	PLA	19.3±3.7	19.3±4.0	19.3±3.9	Treatment	0.77
(L)	CNL	19.4±3.6	19.8±3.4	19.6±3.5	Treatment x Time	0.20
	CNH	19.1±3.4	19.4±3.2	19.2±3.3		
	Male	21.2±2.5	21.4±2.2	21.3±2.3	Sex	0.001
	Female	15.9±2.5	16.0±2.6	15.9±2.6†	Time x Sex	0.61
	PLA M	21.2±2.5	21.6±2.1	21.4±2.3	Treatment x Sex	0.69
	PLA F	16.0±3.2†	14.9±2.9†	15.5±3.0	Treatment x Time x Sex	0.03
	CNL M	21.4±2.3	21.4±2.4	21.4±2.4		
	CNL F	15.8±2.4†	16.9±2.9†	16.4±2.7		
	CNH M	20.8±2.7	21.2±2.1	21.0±2.4		
	CNH F	15.9±2.1†	16.1±1.8†	16.0±1.9		

Table 11: Continued.

		Day				
	Treatment	0	5	Mean	Interaction	p-Level
Extracellular	Overall	15.4±2.1	15.5±2.0*	15.4±2.1	Time	0.04
Water	PLA	15.4±2.3	15.4±2.1	15.4±2.2	Treatment	0.96
(L)	CNL	15.4±2.1	15.6±2.1	15.5±2.1	Treatment x Time	0.96
	CNH	15.3±2.0	15.4±2.0	15.3±2.0		
	Male	16.3±1.7	16.3±1.7	16.3±1.7	Sex	0.001
	Female	13.7±1.7	14.0±1.8	13.9±1.8†	Time x Sex	0.09
	PLA M	16.4±1.7	16.2±1.7	16.3±1.7	Treatment x Sex	0.95
	PLA F	13.5±2.1	13.9±2.0	13.7±2.0	Treatment x Time x Sex	0.27
	CNL M	16.3±1.8	16.4±1.6	16.3±1.7		
	CNL F	13.8±1.7	14.1±2.0	13.9±1.8		
	CNH M	16.1±1.8	16.2±1.8	16.2±1.8		
	CNH F	13.8±1.5	14.0±1.6	13.9±1.5		
Total Body	Overall	47.4±5.0	47.8±5.0	47.6±5.0	Time	0.14
Water	PLA	47.4±5.2	47.7±5.3	47.5±5.2	Treatment	0.82
(%)	CNL	47.7±5.2	48.3±5.4	48.0±5.2	Treatment x Time	0.68
	CNH	47.1±4.9	47.4±4.4	47.3±4.6		
	Male	49.0±4.3	49.2±4.2	49.1±4.2	Sex	0.001
	Female	44.6±5.1	45.2±5.4	44.9±5.2†	Time x Sex	0.54
	PLA M	49.0±4.2	49.5±4.1	49.3±4.1	Treatment x Sex	0.87
	PLA F	44.4±5.7	44.3±5.6	44.3±5.5	Treatment x Time x Sex	0.28
	CNL M	49.4±4.0	49.4±4.7	49.4±4.3		
	CNL F	44.7±5.8	46.4±6.3	45.5±5.9		
	CNH M	48.4±4.7	48.7±3.8	48.6±4.2		
	CNH F	44.7±4.4	45.0±4.5	44.9±4.3		

Table 11: Continued.

		Day				
	Treatment	0	5	Mean	Interaction	p-Level
Intracellular	Overall	55.4±3.2	55.6±3.2	55.5±3.2	Time	0.64
Water	PLA	55.5±3.0	55.5±3.3	55.5±3.2	Treatment	0.94
(%)	CNL	55.5±3.5	55.8±3.5	55.6±3.5	Treatment x Time	0.37
	CNH	55.3±3.2	55.5±2.9	55.4±3.0		
	Male	56.5±3.1	56.8±2.8	56.6±2.9	Sex	0.001
	Female	53.6±2.6	53.5±3.0	53.5±2.8†	Time x Sex	0.35
	PLA M	56.3±2.9	57.1±2.6	56.7±2.8	Treatment x Sex	0.91
	PLA F	53.9±2.8†	52.6±2.4†*	53.3±2.6	Treatment x Time x Sex	0.006
	CNL M	56.7±3.1	56.6±2.9	56.7±3.0		
	CNL F	53.3±3.1†	54.3±4.3†	53.8±3.7		
	CNH M	56.4±3.3	56.6±2.9	56.5±3.0		
	CNH F	53.5±2.1†	53.6±1.7†	53.5±1.8		
Extracellular	Overall	44.6±3.2	44.4±3.2	44.5±3.2	Time	0.64
Water	PLA	44.5±3.0	44.5±3.3	44.5±3.2	Treatment	0.94
(%)	CNL	44.5±3.5	44.2±3.5	44.4±3.5	Treatment x Time	0.37
	CNH	44.7±3.2	44.5±2.9	44.6±3.0		
	Male	43.5±3.1	43.3±2.8	43.4±2.9	Sex	0.001
	Female	46.4±2.6	46.5±3.0	46.5±2.8†	Time x Sex	0.35
	PLA M	43.7±2.9	42.9±2.6	43.3±2.8	Treatment x Sex	0.91
	PLA F	46.1±2.8†	47.4±2.4†*	46.7±2.6	Treatment x Time x Sex	0.006
	CNL M	43.3±3.1	43.4±2.9	43.4±3.0		
	CNL F	46.7±3.1†	45.7±4.3†	46.2±3.7		
	CNH M	43.6±3.3	43.4±2.9	43.5±3.0		
	CNH F	46.5±2.1†	46.4±1.7†	46.5±1.8		

Data are means ± SD. MANOVA analysis revealed overall Wilks' Lambda treatment (p=0.98), time (p=0.04), sex (p=0.001), treatment x time (p=0.007), treatment x sex (p=0.98), time x sex (p=0.15), and treatment x time x sex (p=0.005). Univariate ANOVA p-levels from MANOVA analysis are presented for each variable. p<0.05 is considered significant. Statistical notations. (*) denotes a significant difference from baseline. (†) denotes a significant difference from male.

Side Effects

Table 14 presents the frequency and table 15 present the severity of side effects. Participants were asked to rate the frequency and severity of the following eight symptoms before and after testing each day: dizziness, headaches, tachycardia, heart palpitations, dyspnea, nervousness, blurred vision, and other symptoms. No significant differences for frequency or severity of symptoms were found. These data fail to reject $H_0$4: There will be no significant differences among treatments in reported side effects.

Table 12: Frequency of side effects.

			Rating of Symptom						
Symptom	Day	Treatment	0	1	2	3	4	5	Chi-Square
Dizziness	0 Pre	PLA	28	0	0	0	0	0	-
Frequency	Testing	CNL	28	0	0	0	0	0	
		CNH	28	0	0	0	0	0	
	0 Post	PLA	22	5	1	0	0	0	0.96
	Testing	CNL	20	6	1	1	0	0	
		CNH	19	7	1	1	0	0	
	1 Pre	PLA	27	0	1	0	0	0	0.56
	Testing	CNL	27	1	0	0	0	0	
		CNH	27	1	0	0	0	0	
	1 Post	PLA	25	3	0	0	0	0	0.35
	Testing	CNL	24	1	3	0	0	0	
		CNH	23	3	1	0	1	0	
	5 Pre	PLA	28	0	0	0	0	0	0.60
	Testing	CNL	27	1	0	0	0	0	
		CNH	27	1	0	0	0	0	
	5 Post	PLA	25	1	2	0	0	0	0.83
	Testing	CNL	22	3	3	0	0	0	
		CNH	24	2	2	0	0	0	
	6 Pre	PLA	26	1	0	1	0	0	0.32
	Testing	CNL	26	0	2	0	0	0	
		CNH	27	1	0	0	0	0	
	6 Post	PLA	24	3	0	1	0	0	0.84
	Testing	CNL	23	2	2	1	0	0	
		CNH	22	4	1	1	0	0	

Table 12: Continued.

Symptom	Day	Treatment	Rating of Symptom 0	1	2	3	4	5	Chi-Square
Headache	0 Pre	PLA	26	1	1	0	0	0	0.33
Frequency	Testing	CNL	27	0	1	0	0	0	
		CNH	25	3	0	0	0	0	
	0 Post	PLA	16	7	2	2	0	1	0.99
	Testing	CNL	16	7	2	1	1	1	
		CNH	15	6	4	1	1	1	
	1 Pre	PLA	26	1	1	0	0	0	0.55
	Testing	CNL	26	0	2	0	0	0	
		CNH	27	1	0	0	0	0	
	1 Post	PLA	16	5	2	2	2	1	0.83
	Testing	CNL	18	4	3	2	0	1	
		CNH	18	5	2	3	0	0	
	5 Pre	PLA	25	3	0	0	0	0	0.23
	Testing	CNL	27	0	1	0	0	0	
		CNH	27	1	0	0	0	0	
	5 Post	PLA	18	5	2	2	0	1	0.65
	Testing	CNL	19	4	3	1	0	1	
		CNH	17	6	3	0	2	0	
	6 Pre	PLA	25	2	1	0	0	0	0.55
	Testing	CNL	27	0	1	0	0	0	
		CNH	26	2	0	0	0	0	
	6 Post	PLA	17	5	2	2	1	1	0.99
	Testing	CNL	18	5	2	2	0	1	
		CNH	16	5	2	3	1	1	

Table 12: Continued.

			Rating of Symptom						
Symptom	Day	Treatment	0	1	2	3	4	5	Chi-Square
Tachycardia	0 Pre	PLA	27	1	0	0	0	0	0.12
Frequency	Testing	CNL	27	0	1	0	0	0	
		CNH	24	4	0	0	0	0	
	0 Post	PLA	16	8	4	0	0	0	0.58
	Testing	CNL	17	6	4	1	0	0	
		CNH	14	11	2	0	1	0	
	1 Pre	PLA	25	3	0	0	0	0	0.30
	Testing	CNL	27	0	1	0	0	0	
		CNH	26	2	0	0	0	0	
	1 Post	PLA	14	9	3	2	0	0	0.67
	Testing	CNL	19	4	3	2	0	0	
		CNH	15	9	2	1	0	1	
	5 Pre	PLA	25	3	0	0	0	0	0.55
	Testing	CNL	26	1	1	0	0	0	
		CNH	26	2	0	0	0	0	
	5 Post	PLA	17	7	3	1	0	0	0.87
	Testing	CNL	18	5	4	1	0	0	
		CNH	16	9	3	0	0	0	
	6 Pre	PLA	25	2	1	0	0	0	0.40
	Testing	CNL	26	0	2	0	0	0	
		CNH	27	1	0	0	0	0	
	6 Post	PLA	14	8	3	2	1	0	0.73
	Testing	CNL	18	4	3	3	0	0	
		CNH	17	7	1	3	0	0	

Table 12: Continued.

			Rating of Symptom						
Symptom	Day	Treatment	0	1	2	3	4	5	Chi-Square
Heart	0 Pre	PLA	28	0	0	0	0	0	0.36
Palpitations	Testing	CNL	28	0	0	0	0	0	
Frequency		CNH	28	0	0	0	0	0	
	0 Post	PLA	27	1	0	0	0	0	1.00
	Testing	CNL	27	1	0	0	0	0	
		CNH	27	1	0	0	0	0	
	1 Pre	PLA	28	0	0	0	0	0	-
	Testing	CNL	28	0	0	0	0	0	
		CNH	28	0	0	0	0	0	
	1 Post	PLA	27	1	0	0	0	0	1.00
	Testing	CNL	27	1	0	0	0	0	
		CNH	27	1	0	0	0	0	
	5 Pre	PLA	28	0	0	0	0	0	-
	Testing	CNL	28	0	0	0	0	0	
		CNH	28	0	0	0	0	0	
	5 Post	PLA	26	2	0	0	0	0	0.81
	Testing	CNL	27	1	0	0	0	0	
		CNH	26	2	0	0	0	0	
	6 Pre	PLA	28	0	0	0	0	0	-
	Testing	CNL	28	0	0	0	0	0	
		CNH	28	0	0	0	0	0	
	6 Post	PLA	27	1	0	0	0	0	0.56
	Testing	CNL	27	0	1	0	0	0	
		CNH	27	1	0	0	0	0	

Table 12: Continued.

			Rating of Symptom						
Symptom	Day	Treatment	0	1	2	3	4	5	Chi-Square
Dyspnea	0 Pre	PLA	28	0	0	0	0	0	0.36
Frequency	Testing	CNL	27	1	0	0	0	0	
		CNH	28	0	0	0	0	0	
	0 Post	PLA	23	3	2	0	0	0	0.88
	Testing	CNL	19	5	3	1	0	0	
		CNH	19	5	3	1	0	0	
	1 Pre	PLA	27	0	1	0	0	0	0.40
	Testing	CNL	27	1	0	0	0	0	
		CNH	26	2	0	0	0	0	
	1 Post	PLA	24	4	0	0	0	0	0.38
	Testing	CNL	23	2	3	0	0	0	
		CNH	22	4	1	1	0	0	
	5 Pre	PLA	27	1	0	0	0	0	0.64
	Testing	CNL	26	2	0	0	0	0	
		CNH	26	1	1	0	0	0	
	5 Post	PLA	24	2	1	1	0	0	0.87
	Testing	CNL	21	3	3	1	0	0	
		CNH	23	3	2	0	0	0	
	6 Pre	PLA	26	1	0	1	0	0	0.37
	Testing	CNL	25	1	2	0	0	0	
		CNH	26	2	0	0	0	0	
	6 Post	PLA	23	4	0	1	0	0	0.41
	Testing	CNL	22	1	4	1	0	0	
		CNH	21	2	4	1	0	0	

Table 12: Continued.

Symptom	Day	Treatment	Rating of Symptom 0	1	2	3	4	5	Chi-Square
Nervousness	0 Pre	PLA	26	2	0	0	0	0	0.34
Frequency	Testing	CNL	27	1	0	0	0	0	
		CNH	24	4	0	0	0	0	
	0 Post	PLA	17	5	3	2	0	1	0.98
	Testing	CNL	15	4	3	5	0	1	
		CNH	14	6	3	4	0	1	
	1 Pre	PLA	26	1	1	0	0	0	0.91
	Testing	CNL	26	1	1	0	0	0	
		CNH	27	1	0	0	0	0	
	1 Post	PLA	16	4	3	2	2	1	0.83
	Testing	CNL	17	3	2	5	0	1	
		CNH	17	5	3	2	1	0	
	5 Pre	PLA	24	4	0	0	0	0	0.20
	Testing	CNL	27	1	0	0	0	0	
		CNH	27	1	0	0	0	0	
	5 Post	PLA	18	4	3	1	1	1	0.93
	Testing	CNL	17	5	2	3	0	1	
		CNH	16	7	2	2	1	0	
	6 Pre	PLA	26	1	1	0	0	0	0.66
	Testing	CNL	26	2	0	0	0	0	
		CNH	26	2	0	0	0	0	
	6 Post	PLA	17	3	3	2	2	1	0.64
	Testing	CNL	16	6	3	2	0	1	
		CNH	16	3	1	6	1	1	

Table 12: Continued.

			Rating of Symptom						
Symptom	Day	Treatment	0	1	2	3	4	5	Chi-Square
Blurred	0 Pre	PLA	27	1	0	0	0	0	0.12
Vision	Testing	CNL	27	0	1	0	0	0	
Frequency		CNH	24	4	0	0	0	0	
	0 Post	PLA	17	6	5	0	0	0	0.31
	Testing	CNL	17	2	6	2	1	0	
		CNH	13	9	4	2	0	0	
	1 Pre	PLA	25	3	0	0	0	0	0.30
	Testing	CNL	27	0	1	0	0	0	
		CNH	26	2	0	0	0	0	
	1 Post	PLA	15	5	5	2	1	0	0.59
	Testing	CNL	19	3	2	3	1	0	
		CNH	15	8	3	1	0	1	
	5 Pre	PLA	25	3	0	0	0	0	0.55
	Testing	CNL	26	1	1	0	0	0	
		CNH	26	2	0	0	0	0	
	5 Post	PLA	18	4	5	1	0	0	0.89
	Testing	CNL	17	5	4	1	1	0	
		CNH	17	6	5	0	0	0	
	6 Pre	PLA	25	3	0	0	0	0	0.27
	Testing	CNL	26	0	1	1	0	0	
		CNH	27	1	0	0	0	0	
	6 Post	PLA	15	5	3	3	2	0	0.95
	Testing	CNL	18	3	4	1	2	0	
		CNH	17	3	4	3	1	0	

Table 12: Continued.

			Rating of Symptom						
Symptom	Day	Treatment	0	1	2	3	4	5	Chi-Square
Other	0 Pre	PLA	28	0	0	0	0	0	-
Frequency	Testing	CNL	28	0	0	0	0	0	
		CNH	28	0	0	0	0	0	
	0 Post	PLA	28	0	0	0	0	0	-
	Testing	CNL	28	0	0	0	0	0	
		CNH	28	0	0	0	0	0	
	1 Pre	PLA	28	0	0	0	0	0	-
	Testing	CNL	28	0	0	0	0	0	
		CNH	28	0	0	0	0	0	
	1 Post	PLA	28	0	0	0	0	0	-
	Testing	CNL	28	0	0	0	0	0	
		CNH	28	0	0	0	0	0	
	5 Pre	PLA	27	1	0	0	0	0	0.36
	Testing	CNL	28	0	0	0	0	0	
		CNH	28	0	0	0	0	0	
	5 Post	PLA	27	1	0	0	0	0	0.60
	Testing	CNL	28	0	0	0	0	0	
		CNH	27	1	0	0	0	0	
	6 Pre	PLA	27	1	0	0	0	0	0.36
	Testing	CNL	28	0	0	0	0	0	
		CNH	28	0	0	0	0	0	
	6 Post	PLA	28	0	0	0	0	0	-
	Testing	CNL	28	0	0	0	0	0	
		CNH	28	0	0	0	0	0	

Data are presented as frequency. Statistical significance is detailed from chi-squared analysis. $p < 0.05$ considered significant.

Table 13: Severity of side effects.

			Rating of Symptom						
Symptom	Day	Treatment	0	1	2	3	4	5	Chi-Square
Dizziness	0 Pre	PLA	25	0	2	1	0	0	0.44
Severity	Testing	CNL	23	4	0	1	0	0	
		CNH	23	3	1	1	0	0	
	0 Post	PLA	24	3	0	1	0	0	0.53
	Testing	CNL	22	4	0	1	1	0	
		CNH	20	4	2	2	0	0	
	1 Pre	PLA	23	2	1	2	0	0	0.88
	Testing	CNL	24	2	0	2	0	0	
		CNH	22	3	1	1	0	1	
	1 Post	PLA	23	2	2	1	0	0	0.80
	Testing	CNL	24	2	0	1	1	0	
		CNH	22	2	2	2	0	0	
	5 Pre	PLA	23	1	2	1	0	0	0.45
	Testing	CNL	24	4	0	1	0	0	
		CNH	24	3	0	1	0	0	
	5 Post	PLA	24	2	0	2	0	0	0.64
	Testing	CNL	23	3	1	1	0	0	
		CNH	25	0	1	2	0	0	
	6 Pre	PLA	25	0	2	1	0	0	0.87
	Testing	CNL	23	2	2	1	0	0	
		CNH	25	1	1	1	0	0	
	6 Post	PLA	25	0	1	1	1	0	0.80
	Testing	CNL	23	2	2	1	0	0	
		CNH	24	2	1	1	0	0	

Table 13: Continued.

Symptom	Day	Treatment	Rating of Symptom 0	1	2	3	4	5	Chi-Square
Headache	0 Pre	PLA	28	0	0	0	0	0	-
Severity	Testing	CNL	28	0	0	0	0	0	
		CNH	28	0	0	0	0	0	
	0 Post	PLA	26	1	0	1	0	0	0.54
	Testing	CNL	27	0	0	1	0	0	
		CNH	27	0	1	0	0	0	
	1 Pre	PLA	27	1	0	0	0	0	0.60
	Testing	CNL	27	1	0	0	0	0	
		CNH	28	0	0	0	0	0	
	1 Post	PLA	27	0	1	0	0	0	0.56
	Testing	CNL	27	1	0	0	0	0	
		CNH	27	1	0	0	0	0	
	5 Pre	PLA	28	0	0	0	0	0	-
	Testing	CNL	28	0	0	0	0	0	
		CNH	28	0	0	0	0	0	
	5 Post	PLA	27	0	1	0	0	0	0.56
	Testing	CNL	27	0	1	0	0	0	
		CNH	27	1	0	0	0	0	
	6 Pre	PLA	26	1	1	0	0	0	0.39
	Testing	CNL	28	0	0	0	0	0	
		CNH	28	0	0	0	0	0	
	6 Post	PLA	27	0	1	0	0	0	0.56
	Testing	CNL	27	0	1	0	0	0	
		CNH	27	1	0	0	0	0	

Table 13: Continued.

			Rating of Symptom						
Symptom	Day	Treatment	0	1	2	3	4	5	Chi-Square
Tachycardia	0 Pre	PLA	25	2	0	1	0	0	0.84
Severity	Testing	CNL	23	4	0	1	0	0	
		CNH	23	3	1	1	0	0	
	0 Post	PLA	25	2	1	0	0	0	0.87
	Testing	CNL	23	3	2	0	0	0	
		CNH	23	4	1	0	0	0	
	1 Pre	PLA	24	3	1	0	0	0	0.63
	Testing	CNL	24	3	0	1	0	0	
		CNH	26	2	0	0	0	0	
	1 Post	PLA	25	2	0	1	0	0	0.49
	Testing	CNL	23	3	2	0	0	0	
		CNH	25	2	0	1	0	0	
	5 Pre	PLA	26	1	0	1	0	0	0.52
	Testing	CNL	24	3	1	0	0	0	
		CNH	25	3	0	0	0	0	
	5 Post	PLA	25	1	2	0	0	0	0.44
	Testing	CNL	23	4	1	0	0	0	
		CNH	24	3	0	1	0	0	
	6 Pre	PLA	25	1	1	1	0	0	0.52
	Testing	CNL	25	1	0	2	0	0	
		CNH	28	0	0	0	0	0	
	6 Post	PLA	23	2	3	0	0	0	0.11
	Testing	CNL	24	2	0	2	0	0	
		CNH	27	0	0	1	0	0	

Table 13: Continued.

			Rating of Symptom						
Symptom	Day	Treatment	0	1	2	3	4	5	Chi-Square
Heart	0 Pre	PLA	28	0	0	0	0	0	0.36
Palpitations	Testing	CNL	28	0	0	0	0	0	
Severity		CNH	27	1	0	0	0	0	
	0 Post	PLA	27	1	0	0	0	0	0.73
	Testing	CNL	27	0	1	0	0	0	
		CNH	26	1	1	0	0	0	
	1 Pre	PLA	28	0	0	0	0	0	0.40
	Testing	CNL	27	1	0	0	0	0	
		CNH	27	0	1	0	0	0	
	1 Post	PLA	27	0	0	0	1	0	0.64
	Testing	CNL	26	1	1	0	0	0	
		CNH	25	1	1	1	0	0	
	5 Pre	PLA	27	1	0	0	0	0	0.36
	Testing	CNL	26	2	0	0	0	0	
		CNH	28	0	0	0	0	0	
	5 Post	PLA	27	0	0	1	0	0	0.53
	Testing	CNL	25	2	1	0	0	0	
		CNH	26	1	0	1	0	0	
	6 Pre	PLA	26	2	0	0	0	0	0.13
	Testing	CNL	28	0	0	0	0	0	
		CNH	28	0	0	0	0	0	
	6 Post	PLA	26	1	0	1	0	0	0.67
	Testing	CNL	26	0	1	1	0	0	
		CNH	27	0	0	1	0	0	

Table 13: Continued.

			Rating of Symptom						
Symptom	Day	Treatment	0	1	2	3	4	5	Chi-Square
Dyspnea	0 Pre	PLA	24	1	2	1	0	0	0.66
Severity	Testing	CNL	22	4	1	1	0	0	
		CNH	24	1	1	2	0	0	
	0 Post	PLA	23	3	1	1	0	0	0.68
	Testing	CNL	21	3	1	3	0	0	
		CNH	21	1	3	3	0	0	
	1 Pre	PLA	23	1	1	1	2	0	0.61
	Testing	CNL	24	1	1	2	0	0	
		CNH	22	3	1	1	0	1	
	1 Post	PLA	22	1	3	1	1	0	0.52
	Testing	CNL	23	2	1	2	0	0	
		CNH	21	0	5	2	0	0	
	5 Pre	PLA	23	2	2	1	0	0	0.75
	Testing	CNL	22	5	0	1	0	0	
		CNH	23	3	1	1	0	0	
	5 Post	PLA	23	2	0	3	0	0	0.66
	Testing	CNL	22	3	1	2	0	0	
		CNH	24	0	1	3	0	0	
	6 Pre	PLA	25	1	1	1	0	0	0.83
	Testing	CNL	22	2	1	3	0	0	
		CNH	24	1	2	1	0	0	
	6 Post	PLA	24	1	0	2	1	0	0.66
	Testing	CNL	22	1	2	3	0	0	
		CNH	23	1	3	1	0	0	

Table 13: Continued.

Symptom	Day	Treatment	Rating of Symptom 0	1	2	3	4	5	Chi-Square
Nervousness	0 Pre	PLA	28	0	0	0	0	0	-
Severity	Testing	CNL	28	0	0	0	0	0	
		CNH	28	0	0	0	0	0	
	0 Post	PLA	27	0	0	1	0	0	0.56
	Testing	CNL	27	0	1	0	0	0	
		CNH	27	0	1	0	0	0	
	1 Pre	PLA	27	1	0	0	0	0	0.60
	Testing	CNL	27	1	0	0	0	0	
		CNH	28	0	0	0	0	0	
	1 Post	PLA	27	0	0	1	0	0	0.60
	Testing	CNL	27	0	1	0	0	0	
		CNH	27	0	1	0	0	0	
	5 Pre	PLA	27	1	0	0	0	0	0.36
	Testing	CNL	28	0	0	0	0	0	
		CNH	28	0	0	0	0	0	
	5 Post	PLA	27	0	0	1	0	0	0.56
	Testing	CNL	27	0	0	1	0	0	
		CNH	27	0	1	0	0	0	
	6 Pre	PLA	27	0	1	0	0	0	0.36
	Testing	CNL	28	0	0	0	0	0	
		CNH	28	0	0	0	0	0	
	6 Post	PLA	27	0	0	1	0	0	0.56
	Testing	CNL	27	0	1	0	0	0	
		CNH	27	0	1	0	0	0	

Table 13: Continued.

			Rating of Symptom						
Symptom	Day	Treatment	0	1	2	3	4	5	Chi-Square
Blurred	0 Pre	PLA	25	2	0	1	0	0	0.83
Vision	Testing	CNL	23	3	1	1	0	0	
Severity		CNH	24	2	0	2	0	0	
	0 Post	PLA	25	3	0	0	0	0	0.59
	Testing	CNL	24	3	1	0	0	0	
		CNH	23	5	0	0	0	0	
	1 Pre	PLA	24	3	1	0	0	0	0.64
	Testing	CNL	24	3	0	1	0	0	
		CNH	26	1	1	0	0	0	
	1 Post	PLA	26	2	0	0	0	0	0.58
	Testing	CNL	24	4	0	0	0	0	
		CNH	26	2	0	0	0	0	
	5 Pre	PLA	26	1	0	1	0	0	0.52
	Testing	CNL	24	3	1	0	0	0	
		CNH	25	3	0	0	0	0	
	5 Post	PLA	26	1	1	0	0	0	0.43
	Testing	CNL	24	4	0	0	0	0	
		CNH	25	3	0	0	0	0	
	6 Pre	PLA	25	1	1	1	0	0	0.52
	Testing	CNL	25	2	0	1	0	0	
		CNH	28	0	0	0	0	0	
	6 Post	PLA	24	2	2	0	0	0	0.36
	Testing	CNL	25	2	1	0	0	0	
		CNH	28	0	0	0	0	0	

Table 13: Continued.

			Rating of Symptom						
Symptom	Day	Treatment	0	1	2	3	4	5	Chi-Square
Other	0 Pre	PLA	28	0	0	0	0	0	-
Severity	Testing	CNL	28	0	0	0	0	0	
		CNH	28	0	0	0	0	0	
	0 Post	PLA	28	0	0	0	0	0	0.36
	Testing	CNL	28	0	0	0	0	0	
		CNH	27	1	0	0	0	0	
	1 Pre	PLA	28	0	0	0	0	0	0.40
	Testing	CNL	27	1	0	0	0	0	
		CNH	27	0	1	0	0	0	
	1 Post	PLA	28	0	0	0	0	0	0.52
	Testing	CNL	27	1	0	0	0	0	
		CNH	25	1	1	1	0	0	
	5 Pre	PLA	26	1	1	0	0	0	0.39
	Testing	CNL	26	2	0	0	0	0	
		CNH	28	0	0	0	0	0	
	5 Post	PLA	28	0	0	0	0	0	0.36
	Testing	CNL	26	2	0	0	0	0	
		CNH	27	1	0	0	0	0	
	6 Pre	PLA	25	1	1	1	0	0	0.40
	Testing	CNL	28	0	0	0	0	0	
		CNH	28	0	0	0	0	0	
	6 Post	PLA	27	1	0	0	0	0	0.40
	Testing	CNL	27	0	1	0	0	0	
		CNH	28	0	0	0	0	0	

Data are presented as frequency. Statistical significance is detailed from chi-squared analysis. $p < 0.05$ considered significant.

Secondary Outcome Variables – Performance

Bench Press and Leg Press Performance

Table 14 presents bench and leg press performance. MANOVA analysis revealed overall Wilks' Lambda treatment (p=0.94), time (p=0.001), sex (p=0.001), treatment x time (p=0.11), treatment x sex (p=0.90), time x sex (p=0.001), and treatment x time x sex (p=0.57). Univariate analysis revealed significant time effects for bench press 1RM (p=0.001), maximum number of repetitions at 70% 1RM on bench press (p=0.001), and leg press 1RM (p=0.001), sex effects for bench press 1RM (p=0.001), maximum number of repetitions at 70% 1RM on bench press (p=0.04), and leg press 1RM (p=0.01), time x sex effects for bench press 1RM (p=0.001) and treatment x time x sex effects for maximum number of repetitions at 70% 1RM on leg press (p=0.038). No other significant differences were found among bench press or leg press performance.

Figures 18 through 21 present the mean change from baseline with 95% CI;s in bench press 1RM, maximum number of repetitions at 70% 1RM on bench press, leg press 1RM, and maximum number of repetitions at 70% 1RM on leg press, respectively. Analysis of mean changes from baseline with 95% CI revealed the change in bench press 1RM was significantly lower than baseline for all treatments at day 0 post supplement ($p < 0.05$), significantly higher for only CNH at day 5 pre supplementation ($p < 0.05$), and significantly lower for PLA and CNL, but not CNH, at day 5 post supplementation (PLA: -4.2 [-5.7, -2.7], CNL: -4.2 [-5.7, -2.7], CNH: -1.8 [-3.3, -0.3], p=0.01). The change in leg press 1RM was significantly lower than baseline for all treatments at day 0 post supplement ($p<0.05$) and significantly lower for PLA and CNL,

but not CNH, at day 5 post supplementation (PLA: -13.9 [-23.1, -4.7], CNL: -13.2 [-22.3, -4.0], CNH: -6.0 [-15.2, 3.1], p=0.01). Pairwise comparisons also found a significant difference between CNH and PLA, but not CNL at day 5 pre supplementation (PLA: 0.3 [-0.8, 1.5], CNL: 0.9 [-0.3, 2.1], CNH: 2.8 [1.6, 3.9], p=0.01). No other differences were found among treatments for bench press or leg press performance. These data reject $H_0$5: There will be no significant difference among treatments in bench press or leg press performance.

4-K Time Trial Cycle Ergometer Performance

Table 15 presents 4-K time trial cycle ergometer performance. MANOVA analysis revealed overall Wilks' Lambda treatment (p=0.79), time (p=0.008), sex (p=0.001), treatment x time (p=0.20), treatment x sex (p=0.85), time x sex (p=0.22), and treatment x time x sex (p=0.06). Univariate analysis revealed significant sex (p=0.001) and treatment x time x sex (p=0.02) for time to completion and time (p=0.005) and sex (p=0.001) for mean power. No other significant differences were found in 4-K time trial performance.

Figure 22 presents the change in 4-K time trial time and figure 23 presents the change in 4-K time trial power. Analysis of mean changes from baseline with 95% CI revealed PLA was significantly higher at day 6 post supplement compared to baseline for the change in 4-K time trial power (p = 0.005). No other differences were found for 4-K Time Trial Performance. These data fail to reject $H_0$6: There will be no significant differences among treatments in cycle ergometer performance.

Table 14: Bench Press & Leg Press performance.

		Day						
Variable	Treatment	0 Pre	0 Post	5 Pre	5 Post	Mean	Interaction	p-Level
BENCH PRESS PERFORMANCE								
1-Repetition	Overall	74.0±30.1	68.0±28.5*	75.3±30.4*	70.6±29.0*	71.9±29.4	Time	0.001
Maximum	PLA	74.5±30.7	68.1±28.3	74.8±30.4	70.3±29.2	72.5±29.1	Treatment	0.93
(KG)	CNL	73.2±29.8	67.1±28.3	74.1±30.2	69.0±28.7	70.4±28.8	Treatment x Time	0.46
	CNH	74.2±30.7	68.8±29.7	77.0±31.6	72.4±30.1	73.0±30.7		
	Male	92.6±19.9	84.5±21.2*	93.9±20.6*	87.7±21.1*	89.3±21.0	Sex	0.001
	Female	40.5±7.7†	38.3±8.4†*	41.9±8.9†*	39.7±7.6†	40.0±8.6†	Time x Sex	0.001
	PLA M	92.9±21.2	84.1±21.7	92.9±21.2	87.0±22.1	88.9±21.2	Treatment x Sex	0.95
	PLA F	41.4±9.5	39.3±9.1	42.3±9.9	40.2±8.2	41.0±9.3	Treatment x Time x Sex	0.96
	CNL M	91.5±19.7	83.5±21.1	92.3±20.6	85.9±20.8	87.3±20.8		
	CNL F	40.2±6.8	37.5±7.5	41.4±8.6	38.6±7.1	39.4±7.8		
	CNH M	93.2±20.0	86.0±22.1	96.3±20.8	90.3±21.3	91.7±21.2		
	CNH F	40.0±7.4	38.0±9.2	42.1±8.9	40.2±8.1	39.7±8.6		
Maximum Number	Overall	14.1±5.3	14.1±4.7	14.7±5.0	15.5±5.3*	14.3±5.1	Time	0.006
of Repetitions	PLA	14.8±5.9	14.0±5.0	14.7±5.6	15.6±6.3	14.8±5.8	Treatment	0.55
(70%	CNL	12.9±4.0	14.0±4.4	14.1±4.6	14.9±4.5	13.5±4.1	Treatment x Time	0.76
1-RM)	CNH	14.8±5.6	14.2±4.9	15.3±4.9	15.9±4.9	14.7±5.2		
	Male	13.7±4.8	13.2±4.7	14.1±4.9	14.5±5.2	13.5±4.9	Sex	0.04
	Female	14.9±6.0	15.6±4.4	15.9±5.2	17.2±4.9	15.8±5.3†	Time x Sex	0.34
	PLA M	13.4±4.6	12.7±4.7	12.9±3.9	14.0±5.2	13.0±4.5	Treatment x Sex	0.26
	PLA F	17.2±7.4	16.4±5.0	18.0±6.8	18.6±7.2	18.3±6.5	Treatment x Time x Sex	0.62
	CNL M	12.6±4.4	13.3±5.0	13.7±5.3	14.3±5.2	13.0±4.7		
	CNL F	13.4±3.3	15.1±3.3	14.7±3.1	16.0±2.6	14.2±2.7		
	CNH M	15.2±5.3	13.5±4.8	15.6±5.1	15.3±5.5	14.6±5.3		
	CNH F	14.0±6.3	15.4±5.1	14.9±4.8	17.1±3.7	14.8±5.0		

Table 14: Continued.

Variable	Treatment	Day				Mean	Interaction	p-Level
		0 Pre	0 Post	5 Pre	5 Post			
LEG PRESS PERFORMANCE								
1-Repetition	Overall	408±123	391±121*	417±124*	397±122*	403±119	Time	0.001
Maximum	PLA	411±122	397±119	417±125	397±117	407±115	Treatment	0.66
(KG)	CNL	397±122	379±119	404±122	384±120	387±114	Treatment x Time	0.62
	CNH	417±129	399±128	428±127	411±132	414±127		
	Male	476±96	456±98	483±98	464±97	466±94	Sex	0.001
	Female	286±51	276±50	297±55	278±48	287±54†	Time x Sex	0.38
	PLA M	474±99	457±99	479±105	461±92	464±95	Treatment x Sex	0.88
	PLA F	296±59	288±61	305±63	282±46	299±58	Treatment x Time x Sex	0.97
	CNL M	464±95	443±94	470±95	449±96	448±90		
	CNL F	278±53	262±44	285±53	268±50	275±52		
	CNH M	491±96	467±104	500±96	482±106	485±96		
	CNH F	285±45	276±47	300±52	283±52	286±50		
Maximum Number	Overall	20.8±7.7	20.3±8.1	21.5±7.7	21.8±7.4	21.3±7.7	Time	0.06
of Repetitions	PLA	21.8±9.3	20.5±8.7	21.4±8.2	21.7±8.2	21.8±8.5	Treatment	0.70
(70%	CNL	19.0±7.3	18.9±7.8	21.1±8.3	21.4±7.2	20.2±7.8	Treatment x Time	0.23
1-RM)	CNH	21.6±5.9	21.4±7.9	22.0±6.7	22.4±6.7	21.9±6.7		
	Male	21.6±7.2	20.6±6.3	21.8±6.9	22.5±6.5	21.4±6.9	Sex	0.35
	Female	19.3±8.3	19.7±10.6	20.9±9.0	20.6±8.6	21.2±9.1	Time x Sex	0.56
	PLA M	21.7±9.1	20.2±6.1	20.8±6.4	22.6±7.3	21.2±7.5	Treatment x Sex	0.72
	PLA F	21.9±10.3	21.0±12.4	22.3±11.1	20.1±10.0	22.9±10.2	Treatment x Time x Sex	0.04
	CNL M	19.9±6.5	19.9±6.3	20.7±7.6	21.8±5.4	20.3±6.6		
	CNL F	17.5±8.7	17.0±9.9	21.8±9.7*	20.7±10.1	20.1±9.8		
	CNH M	23.3±5.7	21.6±6.8	23.9±6.4	23.2±7.1	22.7±6.6		
	CNH F	18.5±5.4	21.1±10.0	18.5±6.0	21.1±6.1	20.6±6.8		

Data are means ± SD. MANOVA analysis revealed overall Wilks' Lambda treatment (p=0.94), time (p=0.001), sex (p=0.001), treatment x time (p=0.11), treatment x sex (p=0.90), time x sex (p=0.001), and treatment x time x sex (p=0.57). Univariate ANOVA p-levels from MANOVA analysis are presented for each variable. p<0.05 is considered significant. Statistical notations. (*) denotes a significant difference from baseline. (†) denotes a significant difference from male.

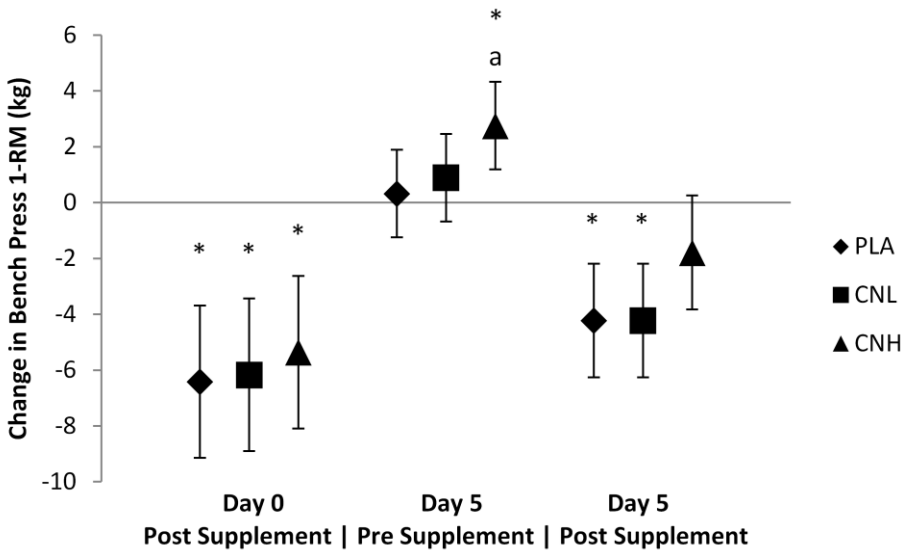

Figure 18. Changes in bench press one repetition maximum. Data are means ± CI. p<0.05 is considered significant. Statistical notations. (a) denotes a significant difference from PLA. (b) denotes a significant difference from CNL. (c) denotes a significant difference from CNH. (*) denotes a significant difference from baseline.

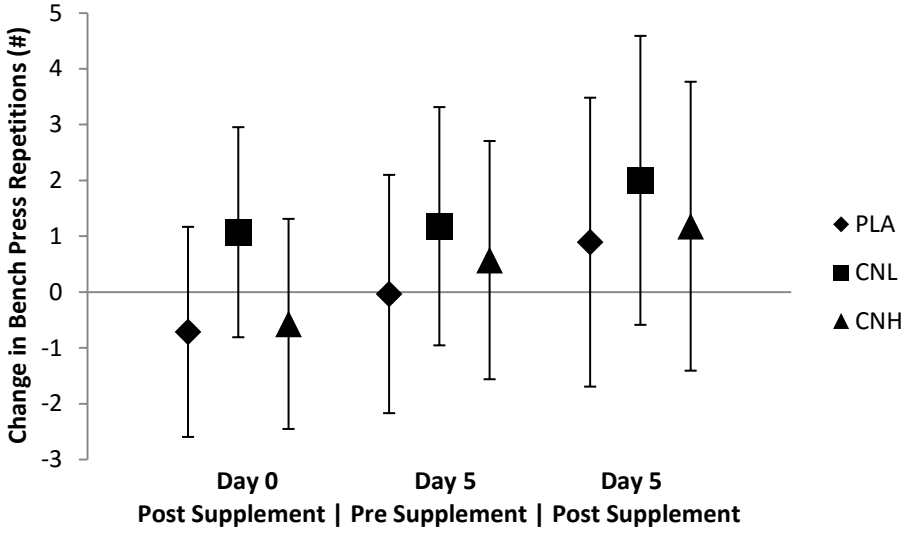

Figure 19. Changes in bench press repetitions to fatigue @ 70% 1RM. Data are means ± CI. p<0.05 is considered significant.

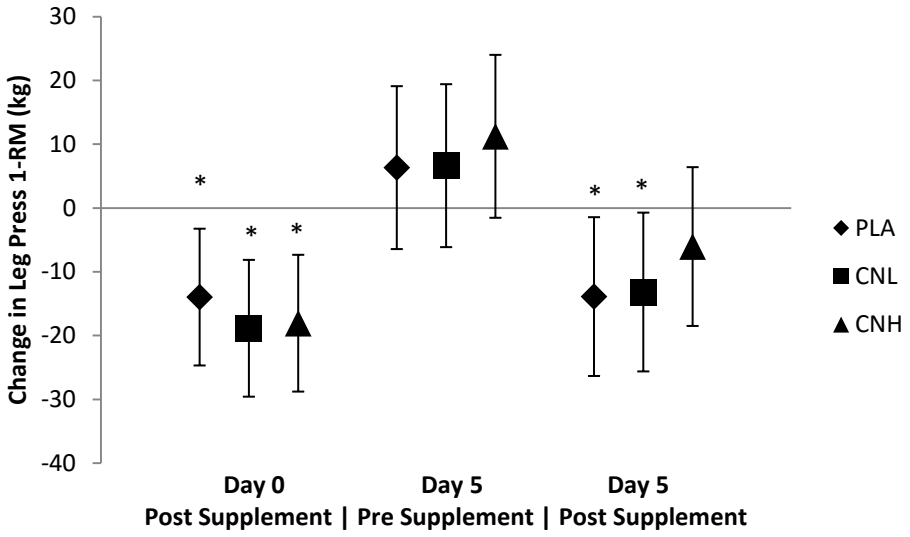

Figure 20. Changes in leg press one repetition maximum. Data are means ± CI. p<0.05 is considered significant. Statistical notations. (*) denotes a significant difference from baseline.

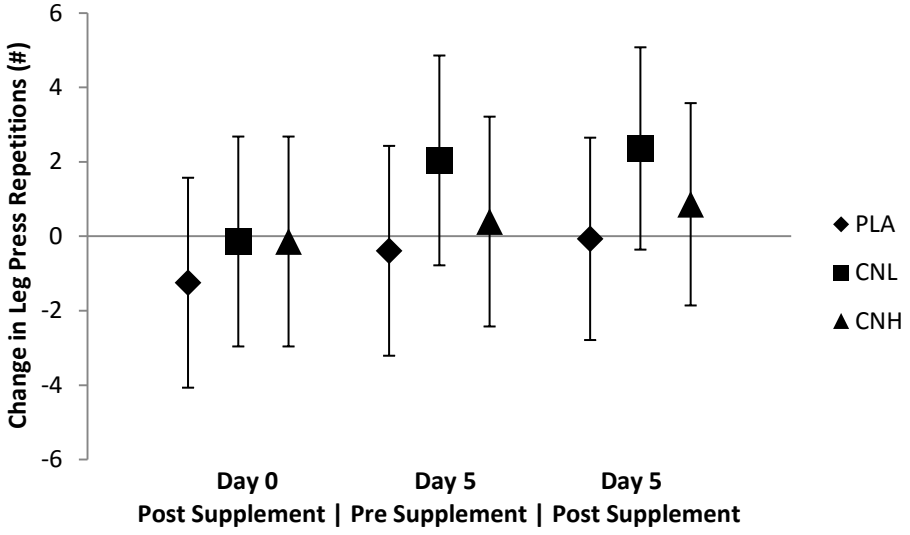

Figure 21. Changes in leg press repetitions to fatigue @ 70% 1RM. Data are means ± CI. p<0.05 is considered significant.

Table 15: 4-K Time Trial Cycle Ergometer Performance.

		Day				
	Treatment	1	6	Mean	Interaction	p-Level
Time	Overall	275±103	270±110	272±106	Time	0.34
(secs)	PLA	271±100	263±105	267±102	Treatment	0.45
	CNL	282±99	286±122	284±110	Treatment x Time	0.07
	CNH	271±113	262±105	267±108		
	Male	210±35	204±36	207±35	Sex	0.001
	Female	391±79	390±98	390±88†	Time x Sex	0.47
	PLA M	212±35	201±36	207±35	Treatment x Sex	0.73
	PLA F	378±89†	374±98†	376±91	Treatment x Time x Sex	0.02
	CNL M	220±37	214±39	217±38		
	CNL F	392±75†	416±114†*	404±95		
	CNH M	198±32	197±31	197±31		
	CNH F	402±80†	381±85†*	392±81		
Mean Power	Overall	245±80	253±86*	249±83	Time	0.005
(W)	PLA	246±79	258±86	252±82	Treatment	0.55
	CNL	237±74	242±85	240±79	Treatment x Time	0.47
	CNH	252±88	260±87	256±87		
	Male	293±55	304±59	299±57	Sex	0.001
	Female	159±27	162±32	160±29†	Time x Sex	0.10
	PLA M	291±57	308±62	300±59	Treatment x Sex	0.72
	PLA F	165±31	168±33	167±31	Treatment x Time x Sex	0.30
	CNL M	282±49	291±61	286±55		
	CNL F	157±26	153±35	155±30		
	CNH M	306±57	313±56	310±56		
	CNH F	154±25	163±30	159±27		

Data are means ± SD. MANOVA analysis revealed overall Wilks' Lambda treatment (p=0.79), time (p=0.008), sex (p=0.001), treatment x time (p=0.20), treatment x sex (p=0.85), time x sex (p=0.22), and treatment x time x sex (p=0.06). Univariate ANOVA p-levels from MANOVA analysis are presented for each variable. p<0.05 is considered significant. Statistical notations. (*) denotes a significant difference from baseline. (†) denotes a significant difference from male.

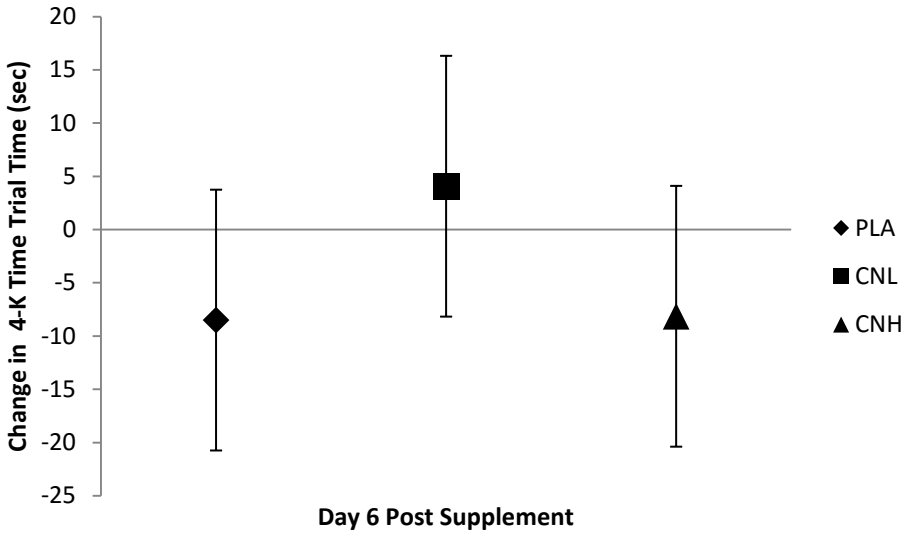

Figure 22. Changes in 4-K time trial time. Data are means ± CI. p<0.05 is considered significant. Statistical notations. (*) denotes a significant difference from baseline.

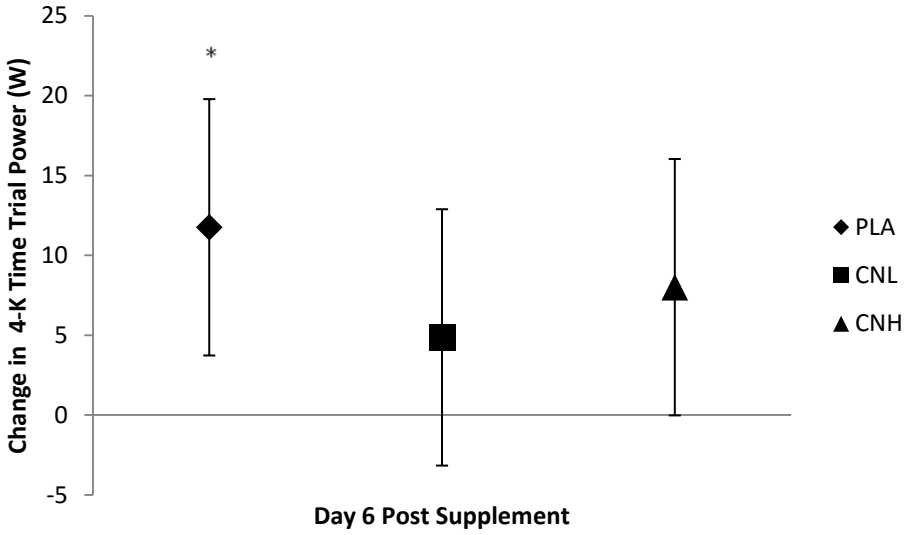

Figure 23. Changes in 4-K time trial power. Data are means ± CI. p<0.05 is considered significant. Statistical notations. (*) denotes a significant difference from baseline.

CHAPTER V

DISCUSSION AND CONCLUSIONS

Creatine and nitrates are popular dietary supplements, but little is known regarding their co-ingestion relative to performance, side effects, and safety. Only two previous studies [42,43] have examined creatine nitrate alone and two other studies [44,45] have examined creatine nitrate as a part of a multiple ingredient supplement. Each of these studies found that creatine nitrate appears to be safe for the dose (1-3 grams per day) and duration (up to 8 weeks) examined. The current study examined low (3 grams per day) and high (6 grams per day) dose creatine nitrate supplementation compared to a placebo (6 grams of glucose per day) over a 7 day period with multiple strenuous exercise bouts on hemodynamic changes, clinical health markers, exercise performance, hydration status, and reported side effects. The results of the present study support 7 days of creatine nitrate supplementation as apparently safe while undergoing strenuous exercise and may provide a performance enhancing benefit.

As expected, significant time effects in diastolic blood pressure, pulse pressure, heart rate, and rate pressure product and the change in DBP, PP, HR, and RPP, but not systolic blood pressure or mean arterial pressure, or the change in SBP or MAP, were observed in this study. Research shows little change in systolic blood pressure, but an increase in diastolic blood pressure and heart rate when comparing supine to standing positions [140]. Prior research has generally shown an increase in heart rate post-exercise compared to pre-exercise [141]. Our findings in this study support both of the

positional changes and the post exercise changes to blood pressure and heart rate. While nitrate supplementation studies have found decreases in blood pressure after supplementation [102,103,106-108], studies examining creatine nitrate, as part of a multi-ingredient supplement [44,45] or independently [42,43] have not found similar results. In a meta-analysis, Siervo et al. [142] found a significant decrease in SBP (-4.4 mm Hg 95% CI [-5.9, -2.8] $p<0.001$), but not DBP (-1.1 mm Hg 95% CI [-2.2, 0.1] $p=0.06$) from beetroot juice and inorganic nitrate supplementation. Sixteen crossover studies were included in the analysis with supplementation durations ranging from 2 hours to 15 days and washout periods ranging from 6 to 28 days. The dose of nitrate ranged from ~150 mg to ~3 grams per day. Nine of the studies asked their participants to not consume foods high in nitrate before the study and six asked their participants to not change their regular diet. Out of the sixteen studies, six (38%) found no change in SBP and 9 (56%) found no change in DBP, equally distributed among the studies which controlled and did not control for dietary nitrate. Neither the duration of supplementation or source of nitrate were found to be associated with decreased blood pressure, but a meta-regression found a significant ($p<0.5$) correlation between dose of nitrate and decrease in SBP. Our study is in agreement with the other creatine nitrate studies, where no treatment effects were found for SBP, DBP, MAP, PP, HR, or RPP. Additionally, no changes in hemodynamic reactivity were found among treatments or over time. While dietary nitrates may relate to an important mechanism of blood pressure regulation in the body, adding additional nitrates through creatine nitrate (up to 2 grams per day for 6 days) on top of those supplied by the diet (0.12 – 1.2 grams per day) appears to not

produce a significant blood pressure lowering effect. These data show high doses of creatine nitrate (6 grams, 2 grams nitrate per day) for 6 days do not pose a risk of hypotension while undergoing strenuous exercise.

In our study, the assessment of blood chemistry changes found no significant differences among treatments changing from normal clinical values to above normal clinical values for any of the blood chemistry parameters measured. Likewise, no differences among treatments were found in hydration status and no differences among treatments were reported for the frequency or severity of any side effect. In agreement with other creatine nitrate studies [42-45] and based on these findings we conclude creatine nitrate supplementation is apparently safe and well-tolerated at 6 grams per day for 6 days.

While significant time effects were found for the performance variables, no significant treatment or treatment x time interactions were found. Although no significant time x treatment interactions were observed, analysis of mean changes from baseline with 95% CI revealed bench press 1RM was significantly lower than baseline for all treatments at day 0 post supplement ($p < 0.05$), significantly higher for only CNH at day 5 pre supplementation ($p < 0.05$) with a significant difference between CNH and PLA, but not CNL (PLA: 0.3 [-0.8, 1.5], CNL: 0.9 [-0.3, 2.1], CNH: 2.8 [1.6, 3.9], $p=0.01$), and significantly lower for PLA and CNL, but not CNH, at day 5 post supplementation (PLA: -4.2 [-5.7, -2.7], CNL: -4.2 [-5.7, -2.7], CNH: -1.8 [-3.3, -0.3], $p=0.01$). The change in leg press 1RM was significantly lower than baseline for all treatments at day 0 post supplement ($p<0.05$) and significantly lower for PLA and CNL,

but not CNH, at day 5 post supplementation (PLA: -13.9 [-23.1, -4.7], CNL: -13.2 [-22.3, -4.0], CNH: -6.0 [-15.2, 3.1], p=0.01). No significant effects among groups were found for the 4K time trial. No significant differences in performance among treatments were expected due to the dose and duration of creatine supplementation. Typical, acute dosages of creatine are ~20 grams per day for 5 days, followed by a maintenance dose of 3-5 grams per day, whereas, our study provided 3 or 6 grams of creatine nitrate per day, which provides 2 and 4 grams of creatine per day, for seven days. Galvan et al [42] demonstrated muscle creatine concentrations were not significantly elevated after 7 days of 6 grams per day of creatine nitrate, but were after 12 grams per day of creatine nitrate. Also, a dose of 3 grams per day of creatine nitrate for 21 days was not sufficient to maintain the elevated creatine concentrations. Based on this data and our findings, the dosage of creatine supplied by the creatine nitrate used in our study was probably too low to induce a performance enhancing effect.

On the other hand, a few studies have shown improvements in power related activities due to nitrate supplementation (0.25 - 0.5 grams) in sub-maximal performance [110,114] and recovery [111,113]. In a double-blind, randomized, crossover study, Thompson et al. [143] gave participants 70 ml of concentrated beetroot juice (400 mg of nitrate) per day for 5 days. After the supplementation period, participants were tested on a series of 5 x 20 m sprints and a repeated sprint interval test. The average split times for the 20 m sprint tests were 2.3% at 5 m, 1.6% at 10 m, and 1.2% at 20 m higher in the beetroot juice treatment compared to placebo and the beetroot juice treatment was able to cover 3.9% more distance in the repeated sprint interval test. Wylie et al. [144] found

similar results, 4.2% improvement in the sprint interval test, while supplementing with 7 x 70 ml doses of beetroot juice (totaling ~1750 mg of nitrate) spread over 24 hours before testing.

In a series of tests, Clifford et al. [111,113] demonstrated beetroot juice could be used to improve recovery from an intense exercise bout. In the first study [111], participants performed 100 drop jumps and immediately consumed either 3 x 250 ml of beetroot juice (250 mg of nitrate), 3 x 125 ml of beetroot juice (125 mg of nitrate), or 250 ml of placebo. At 24 and 48 hours post exercise participants consumed another 2 servings of their assigned supplement. Counter movement jump performance recovered more quickly in the 250 ml beetroot juice group compared to placebo at both 48 (92% vs. 74% of baseline) and 72 (93% vs. 86% of baseline) hours post exercise. In the second study [113], participants performed 20 x 30 m repeated sprints, followed by 2 doses of 250 ml of beetroot juice (250 mg of nitrate) or placebo immediately, at 24 and 48 hours, then another round of 20 x 30 m sprints at 72 hours, immediately followed by 2 more doses of 250 ml of beetroot juice. Both counter movement jump (7.6%) and reactive strength index (13.8%) recovered more quickly for the beetroot juice group compared to placebo at 72 hours.

In a randomized, double-blind, crossover study, Mosher et al. [114] gave participants either 70 ml of concentrated beetroot juice (400 mg of nitrate) or placebo each day for 6 days with a 72 hour washout between treatments. After supplementation, participants performed 3 sets to failure at 60% of their 1RM on bench press. During the

beetroot juice treatment participants performed 19.4% more repetitions and lifted 18.9% more total weight compared to placebo.

Oliveiro et al. [145], in a randomized, double-blind, crossover design, showed a beetroot gel (750 mg of nitrate) can improve the recovery of handgrip maximal voluntary contraction force (MVC) in elderly participants. After baseline MVC was collected participants consumed either the beetroot gel or a placebo and waited 150 minutes, after which, participants performed 1 set at 30% of their MVC for one minute. Twenty minutes post exercise the beetroot gel group significantly recovered more than the placebo group (-18.56±13.8, -26.18±14.6 N; $P<0.05$). Our study is in agreement with these studies showing improved recovery after strenuous exercise, adding creatine nitrate may improve maximal strength after acute supplementation.

In conclusion, 3-6 grams of creatine nitrate consumed while undergoing strenuous physical activity appears to be safe for the durations studied based on hemodynamic and clinical measures. A ~4% improvement in bench press 1RM was found with improved recovery post exercise on bench press and leg press 1RM, with no change in repetitions to fatigue, or cycle ergometry performance. Previous studies have shown chronic creatine nitrate supplementation is effective at improving the benefit induced by strength training (~8% compared to placebo) [42]. More research is needed to determine optimal loading and maintenance doses of creatine nitrate for performance enhancement. While much research has focused on the benefit nitrates may have on endurance performance, further research into the strength enhancing effects of nitrate supplementation is also warranted.

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APPENDIX A

EDUCATION & HUMAN DEVELOPMENT
TEXAS A&M UNIVERSITY

Want to try a new supplement?

Women Needed for a Supplement Study

Researchers in the Exercise & Sport Nutrition Laboratory (ESNL) at Texas A&M University are recruiting approximately 10 recreationally active females between the ages of 18 and 40 to participate in a supplement study. The study will examine the short-term safety and dose effects of different forms of creatine ingestion. Eligible participants will receive $250 for completing the study. 13 total visits throughout approximately a six week period will be required.

For more information call:

Exercise & Sport Nutrition Laboratory (ESNL)
Department of Health & Kinesiology (HLKN)
1700 Research Parkway
Suite # 2500
979- 458-1484
Or email ryanldalton@hlkn.tamu.edu

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EDUCATION
& HUMAN DEVELOPMENT
TEXAS A&M UNIVERSITY

Want to try a new supplement?

Men Needed for a Supplement Study

Researchers in the Exercise & Sport Nutrition Laboratory (ESNL) at Texas A&M University are recruiting approximately 10 recreationally active men between the ages of 18 and 40 to participate in a supplement study. The study will examine the short-term safety and dose effects of different forms of creatine ingestion. Eligible participants will receive $250 for completing the study. 13 total visits throughout approximately a six week period will be required.

For more information call:

Exercise & Sport Nutrition Laboratory (ESNL)
Department of Health & Kinesiology (HLKN)
1700 Research Parkway
Suite # 2500
979- 458-1484
Or email ryanldalton@hlkn.tamu.edu

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APPENDIX B

CONSENT FORM

Project Title: **Short-Term Safety and Dose Effects of Different Forms of Creatine Ingestion**

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 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 the short-term characteristics of ingesting creatine nitrate compared to placebo on blood profiles, heart rate, blood pressure 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 or woman 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 (birth control is allowed); you are pregnant or a lactating female or plan to become pregnant within the next month; you have a history of smoking; you drink excessively (12 drinks per week or more); or you have a recent history of creatine or nitrate supplementation within eight 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 later for potential entry into a similar study with your permission.

How Many People Will Be Asked To Be In This Study?
Approximately 20 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?
We will ask you to not exercise for 48 hours nor eat or drink calorie containing foods or drinks 8 hours before each testing session/visit. Your participation in this study will last approximately six weeks and include 13 visits (visit 1 ~ 1 hour/visit 2-13 ~ 1.5 hours). We will ask you to donate a blood sample up to 13 total times throughout the entire duration of the study and complete one body composition assessment. These visits are detailed below and in Table 1.

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Table 1 - Protocol Overview

Familiarization	Day 1	Day 2	Day 6	Day 7
Phone Screening Familiarization Physical Exam Body Weight DXA Body Composition BIA Body Water Bench Press & Leg Press/Hip Sled 1 RM and 70% 1RM test Practice Bike Test Schedule Testing Refrain from exercise and alcohol 48 hours prior to each testing session Randomized, Double Blind, Crossover Administration of Supplements with at least a 1 week washout period: 1. Placebo (6 g. dextrose) 2. CrN – 3 g. (2 g. creatine, 1 g. nitrate with 3 g. dextrose) 3. CrN – 6 g. (4 g. creatine, 2 g. nitrate) CrN – Creatine Nitrate	Body Mass/Body Water 8 hour fasting blood Side-Effects Questionnaire HR & BP following 15 min supine on tilt table HR & BP following 2 min upright on tilt table Bench Press & Leg Press/Hip Sled 1 RM & 3 sets of 10 @ 70% (of 1 RM at FAM) with 3rd set to failure with 2 m. rest recovery between sets Ingest Supplement and wait 15 m. HR & BP following 15 m. supine on tilt table HR & BP following 2 m. upright on tilt table Bench Press & Leg Press/Hip Sled 1 RM & 1 set of 10 @ 70% (of 1 RM at FAM) to failure Side-Effects Questionnaire	Side-Effects Questionnaire 8 hour fasting blood Ingest Supplement Wait 30 minutes 4 km Time Trial Side-Effects Questionnaire	Body Mass/Body Water 8 hour fasting blood Side-Effects Questionnaire HR & BP following 15 min supine on tilt table HR & BP following 2 min upright on tilt table Bench Press & Leg Press/Hip Sled 1 RM & 3 sets of 10 @ 70% (of 1 RM at FAM) with 3rd set to failure with 2 m. rest recovery between sets Ingest Supplement and wait 15 m. HR & BP following 15 m. supine on tilt table HR & BP following 2 m. upright on tilt table Bench Press & Leg Press/Hip Sled 1 RM & 1 set of 10 @ 70% (of 1 RM at FAM) to failure Side-Effects Questionnaire	Side-Effects Questionnaire 8 hour fasting blood Ingest Supplement Wait 30 minutes 4 km Time Trial Side-Effects Questionnaire

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Visit 1 – Familiarization (T1)
This visit will last about 60 minutes. During this visit, we will explain the details of the study and ask you to sign a consent form, radiation consent form, personal history form and medical history form. We will complete a general physical that may include measurement of blood to determine if you can participate in the study. We may ask you to donate about 5 ml (about 1 teaspoon) of blood from a vein in your arm according to standard procedures. Next, we will measure body weight, body water and body composition. We will then have you perform a warm-up and one repetition maximum test on the bench press and leg press/hip sled followed by three sets of 10 repetitions at 70% of your 1 RM with the final set to failure. Next, we will introduce you to the 4 km bike test. Finally, we will schedule your next visits.

Visit 2 (day1) – This visit will last about 90 minutes. We will first measure your body weight and body water. Next, we will ask you to donate about 20 ml (about 4 teaspoons) of blood from a vein in your arm according to standard procedures. Then we will ask you to complete a side-effects questionnaire. Next, you will lie on a tilt table and we will measure your heart rate and blood pressure after 15 minutes. After we tilt you up, we will measure your heart rate and blood pressure after two minutes. We will next determine your 1 repetition maximum on the bench press and leg press/hip sled and have you perform 3 sets of 10 repetitions on the bench press and leg press/hip sled at 70% 1 RM (from the Familiarization) encouraging you to complete as many repetitions on the 3rd and final set. Next, we will randomize you and ask you to ingest either: 1.) placebo (6 g. dextrose); 2.) creatine nitrate – low (2 g creatine, 1 g nitrate, 3 g dextrose); or 3.) creatine nitrate – high (4 g creatine, 2 g nitrate). After ingesting your supplement, you will rest for 15 minutes and complete the same tilt table test and the same bench press and leg press/hip sled protocol only this time we will ask you to perform one set at 70% 1 RM (from the Familiarization) to failure. Finally, we will ask you to complete the side-effects questionnaire one final time.

Visit 3 (day 2) – This visit will last about 90 minutes. We will first ask you to donate about 20 ml (about 4 teaspoons) of blood from a vein in your arm according to standard procedures. Then we will ask you to complete a side-effects questionnaire. Next, we will have you complete a 4 km time trial on a stationary bike and complete a final side-effects questionnaire.

We will ask you to take your assigned supplement on days 3, 4 and 5 with breakfast every morning and record your supplement intake.

Visit 4 (day 6) – This visit will last about 90 minutes and be just like visit 2.

Visit 5 (day 7) – This visit will last about 90 minutes and be just like visit 3.

We will ask you to repeat these procedures two additional times using an alternate supplement following approximately a one-week washout (i.e., 7-14 days) after visit 5 each time.

Although this is the ideal time line, there may be instances we ask you to ingest the supplement for more than seven but no more than ten days if you miss a scheduled testing session. We will ask you to refrain from using any nonsteroidal anti-inflammatory drugs (NSAIDs) during creatine ingestion. Some potentially nonsteroidal anti-inflammatory drugs include; ibuprofen (Advil, Motrin and Nuprin), indomethacin (Indocin), naproxen (Aleve, Anaprox, Naprelan and Naprosyn) and piroxicam (Feldene). Regardless we will not ask you to complete more testing sessions than previously listed.

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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. You will be exposed to a low level of radiation one time 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 seven times. 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 may donate approximately 5 ml (about 1 teaspoon) of blood during the initial familiarization/screening visit and then approximately 20 ml (about 4 teaspoons) of blood 12 additional times throughout the entire 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. However, only a trained phlebotomist will be performing blood sampling using previously approved sterile procedures. 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. In addition, you may experience stomach pain, nausea, diarrhea and headaches from the supplements. If you are or were to become pregnant, the particular treatment or study procedure might involve risks to the embryo or fetus, which are currently unknown.

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.

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

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experience side effects, particularly "unusual or adverse effects" you will be referred to our given the option of speaking with the Principle Investigator, Dr. Richard Kreider, the ESNL Research Nurse, Amy Heiner, 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 $250 ($10 for the familiarization and $20 for each of the 12 additional testing sessions) in one check at the end of the study. Payment will occur after finishing all testing sessions and after all study materials (questionnaires, 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, to provide input regarding research, or if you have questions, complaints, or concerns about the research, you may call the Texas A&M University Human Subjects Protection Program office by phone at 1-979-458-4067, toll free at 1-855-795-8636, or by email at irb@tamu.edu.

What if I Change My Mind About Participating?

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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 you 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

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APPENDIX C

General Screening Form

Study:_____ **IRB:** _____

Texas A&M, College Station, TX

Subject Initials [ ]

Consent Date [ ] mm [ ] dd [ ] yyyy

Screening Date [ ] mm [ ] dd [ ] yyyy

Personal Data

Visit: SCREENING

Name: _____

Address: _____

Phone #: _____

E-mail: _____

Local PCP: _____ ☐ None

_____

Demographics

Visit: SCREENING

Sex: ☐ M ☐ F

DOB: ____ (mm) ____ (dd) ____ (yyyy) Age at enrollment: _____ y

Race: ***(Mark all which apply)***

- ☐ White
- ☐ Black or African American
- ☐ Native Hawaiian or Other Pacific Islander
- ☐ Asian
- ☐ American Indian/Alaska Native
- ☐ Unknown

Ethnicity: ***(Mark only 1)***

- ☐ Hispanic or Latino
- ☐ Not Hispanic or Latino
- ☐ Unknown

General Health & Physical Exam

Visit: SCREENING

Medications:

PMHx:

Surgical Hx:

Allergies and drug reactions:

Smoking: Duration: _____ PPD x _____ Yrs
Former smoker: when stopped: _____
Duration: _____ PPD x _____ Yrs

EtOH: _____

Vital signs:

HR: _____ m

BP: _____ / _____ mmHg

Anthropometry:

Height: _____ . _____ cm Weight: _____ . _____ kg BMI: _____ . _____ kg/m2

_____ . _____ in _____ . _____ lb

General Health & Physical Exam

Visit: SCREENING

ROS:	fever chills sweats wtΔ fatigue appetite sleep
Skin:	itching rash sores susp. moles/lesions- healing recentΔ
Head:	dizzy fainting HA/LOC trauma
Eyes:	correction Δvision-double tearing itching/redness
Ears:	Δhearing ringing earache vertigo/tinnitus
Nose:	epistaxis rhinorrhea allergies
Mouth/Throat:	bleeding gums sore mouth/throat swollen neck
CV:	angina palpitations DOE orthopnea/PND edema
Pulm:	SOB wheeze cough hemoptysis TB
Hematologic:	bruise /bleed easily transfusion hx
GI:	dysphagia N / V abd pain GERD hematochezia jaundice
GU:	freq urgency hesitancy dys- hematuria incont UTI's stones
Genital:	testicular masses hernias
Endocrine:	polyuria polydipsia skin/hair ? thyroid hx
Vascular:	claudication DVT hx
MSK:	jt pain stiffness arthritis gout
Neuro:	numbness weakness/atrophy seizure/tremor
Psych:	depression anxiety recent memoryΔ
Female:	regular dysmenorrhea pregnancies menopause
Breast:	skinΔ lumps pain discharge

PE:	Gen: Well
Skin:	cap refill: no rash lesions:
Head:	no trauma no bruising no masses
Eyes:	PERRLA EOMI no ptosis sclera clear
Ears:	good acuity TM: nl reflex/intact
Nose:	nl
Mouth/Throat:	nl/pink, moist mucous membranes no lesions
Neurological:	Alert & oriented×3, nl MS via conversation
Cranial Nerves:	II - XII intact/nl
Motor:	5/5 UE/LE's bil
Sensation:	intact LT UE/LE's
DTRs:	symmetric/nl biceps knee ankle
Gait/Station:	nl
Neck:	no LAD no masses no bruits no JVD supple stiff
Chest:	CTA bil equal expansion
Extremities:	no C/C/E Major jts: no swelling full ROM
Heart:	Reg no M/R/G
Pulses Bil:	PT / DP: 2+
Abdomen:	soft, NT/ND BS + no masses / organomegaly

General Health & Physical Exam

Visit: SCREENING

Assessment: _____

Blood Draw: ☐ Y ☒ N _____

Blood draw performed by: N/A

Lab Results Reviewed: ☐ Y ☒ N

Eligible based on General Health and Physical Exam: ☐ Y ☐ N

_____
Signature of staff member performing exam

Date: ____ ____ ____
mm dd yyyy

APPENDIX D

Texas A&M University: Exercise & Sport Nutrition Laboratory
Trial: Short-Term Safety and Dose Effects of Different Forms of Creatine Ingestion

Familiarization

Demographics ESNL Staff Initials: _____

Name: _____
Date: _____
Gender: _____
D.O.B.: _____
Age: _____

Informed Consent: _____
Radiation Consent: _____

General Screening: _____
Height: _____
Weight: _____
DXA: _____
BIA: _____
Lab: _____

Exercise Measures: Strength/Aerobic Testing:

Bench Press: Hand Position: _____
Preceding Weights/Reps:
_____x_____: _____x_____: _____x_____: _____x_____: _____x_____: _____x_____: _____x_____: _____x_____

1 RM: _____
70% 1RM: _____
1st set of 10 at 70% 1RM: _____
2nd set of 10 at 70% 1RM: _____
3rd set of 10 at 70% 1RM to failure: _____

Leg Press: Foot Position: _____ Sled Position: _____
Preceding Weights/Reps:
_____x_____: _____x_____: _____x_____: _____x_____: _____x_____: _____x_____: _____x_____: ____x_____

1 RM: _____
70% 1RM: _____
1st set of 10 at 70% 1RM: _____
2nd set of 10 at 70% 1RM: _____
3rd set of 10 at 70% 1RM to failure: _____

4 km Time Trial Practice: _____

Handle Bar Height: _____ Handle Bar Position: _____
Saddle Height: _____ Saddle Position: _____

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APPENDIX E

Texas A&M University: Exercise & Sport Nutrition Laboratory
Trial: Short-Term Safety and Dose Effects of Different Forms of Creatine Ingestion

Treatment: _____

Demographics ESNL Staff Initials: _____

Name: _____ Date: _____ Group: _____

Day 1: _____

Weight: _____ BIA: _____ Time: _____
Last Meal: _____ Fasted: _____hr. Last Wkout: _____hr.
Lab: _____ (2) SST/(1) EDTA Side-Effects Questionnaire: _____

Post 15 minutes supine on Tilt Table:
HR: _____bpm
BP: _____/_____mmHg
Post 2 minutes upright on Tilt Table:
HR: _____bpm
BP: _____/_____mmHg

Exercise Measures: Strength/Aerobic Testing:

Bench Press: Hand Position: _____
Preceding Weights/Reps:
_____x_____: _____x_____: _____x_____: _____x_____: _____x_____: _____x_____: _____x_____
1 RM: _____ 70% 1RM (from FAM): _____
Set 1-70% 1RM_____x10: Set 2-70% 1RM _____x10: Set 3- 70% 1RM _____x_____(max #)

Leg Press: Foot Position: _____ Sled Position: _____
Preceding Weights/Reps:
_____x_____: _____x_____: _____x_____: _____x_____: _____x_____: _____x_____: ____x_____
1 RM: _____ 70% 1RM (from FAM): _____
Set 1-70% 1RM_____x10: Set 2-70% 1RM _____x10: Set 3- 70% 1RM _____x_____(max #)

Ingest supplement: _____ - Wait 15 minutes

Post 15 minutes supine on Tilt Table:
HR: _____bpm
BP: _____/_____ mmHg
Post 2 minutes upright on Tilt Table:
HR: _____ bpm
BP: _____/_____ mmHg

Bench Press: Hand Position: _____
Preceding Weights/Reps:
_____x_____: _____x_____: _____x_____: _____x_____: _____x_____: _____x_____: _____x_____
1 RM: _____ 70% 1RM (from FAM): _____
Set 1-70% 1RM_____x_____(max #)

Leg Press: Foot Position: _____ Sled Position: _____
Preceding Weights/Reps:
_____x_____: _____x_____: _____x_____: _____x_____: _____x_____: _____x_____: ____x_____
1 RM: _____ 70% 1RM (from FAM): _____
Set 1-70% 1RM_____x_____(max #)
Side-Effects Questionnaire: _____

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Day 2: _____ ESNL Staff Initials: _____

Side-Effects Questionnaire: _____

Time: _____ Last Meal: _____
Fasted: _____hr. Last Wkout: _____hr.
Lab: _____ (2) SST/(1) EDTA

Ingest supplement: _____ - Wait 30 minutes

Exercise Measures: Aerobic Testing:

4 km Time Trial: _____

Handle Bar Height: _____ Handle Bar Position: _____
Saddle Height: _____ Saddle Position: _____

Time to completion: _____ min./sec.
Peak power: _____watts
Mean power: _____watts
Minimal power: _____ watts
Fatigue slope: _____W/sec
Rate of fatigue: _____%
Total work: _____J

Side-Effects Questionnaire: _____

Day 6: _____ ESNL Staff Initials: _____

Weight: _____ BIA: _____ Time: _____
Last Meal: _____ Fasted: _____hr. Last Wkout: _____hr.
Lab: _____ (2) SST/(1) EDTA Side-Effects Questionnaire: _____

Post 15 minutes supine on Tilt Table:
HR: _____bpm
BP: _____/_____mmHg
Post 2 minutes upright on Tilt Table:
HR: _____bpm
BP: _____/_____mmHg

Exercise Measures: Strength/Aerobic Testing:

Bench Press: Hand Position: _____
Preceding Weights/Reps:
_____x_____: _____x_____: _____x_____: _____x_____: _____x_____: _____x_____: _____x_____
1 RM: _____ 70% 1RM (from FAM): _____
Set 1-70% 1RM_____x10: Set 2-70% 1RM _____x10: Set 3- 70% 1RM _____x_____(max #)

Leg Press: Foot Position: _____ Sled Position: _____
Preceding Weights/Reps:
_____x_____: _____x_____: _____x_____: _____x_____: _____x_____: _____x_____: ____x_____
1 RM: _____ 70% 1RM (from FAM): _____
Set 1-70% 1RM_____x10: Set 2-70% 1RM _____x10: Set 3- 70% 1RM _____x_____(max #)

Ingest supplement: _____ - Wait 15 minutes

Post 15 minutes supine on Tilt Table:
HR: _____bpm
BP: _____/_____ mmHg
Post 2 minutes upright on Tilt Table:
HR: _____ bpm
BP: _____/_____ mmHg

Bench Press: Hand Position: _____
Preceding Weights/Reps:
_____x_____: _____x_____: _____x_____: _____x_____: _____x_____: _____x_____: _____x_____
1 RM: _____ 70% 1RM (from FAM): _____
Set 1-70% 1RM_____x_____(max #)

Leg Press: Foot Position: _____ Sled Position: _____
Preceding Weights/Reps:
_____x_____: _____x_____: _____x_____: _____x_____: _____x_____: _____x_____: ____x_____
1 RM: _____ 70% 1RM (from FAM): _____
Set 1-70% 1RM_____x_____(max #)
Side-Effects Questionnaire: _____

Day 7: _____ ESNL Staff Initials: _____

Side-Effects Questionnaire: _____

Time: _____ Last Meal: _____
Fasted: _____hr. Last Wkout: _____hr.
Lab: _____ (2) SST/(1) EDTA

Ingest supplement: _____ - Wait 30 minutes

Exercise Measures: Aerobic Testing:

4 km Time Trial: _____

Handle Bar Height: _____ Handle Bar Position: _____
Saddle Height: _____ Saddle Position: _____

Time to completion: _____ min./sec.
Peak power: _____watts
Mean power: _____watts
Minimal power: _____ watts
Fatigue slope: _____W/sec
Rate of fatigue: _____%
Total work: _____J

Side-Effects Questionnaire: _____

IRB NUMBER: IRB2015-0684F
IRB APPROVAL DATE: 12/10/2015
IRB EXPIRATION DATE: 12/01/2016

APPENDIX F

Texas A&M University: Exercise & Sport Nutrition Laboratory
Trial: Short-Term Safety and Dose Effects of Different Forms of Creatine Ingestion

Radiation Exposure Questionnaire for Women of Child Bearing Age

Radiation exposure may affect fetal development. Although the DXA test will only expose you to a small amount of radiation (1.5mR per scan), you should be aware that there is a possibility that if you become pregnant during the course of the study that the x-ray exposure may be harmful to the fetus. Therefore, it is important to conduct x-ray tests within 10-14 days of the start of a female's menstrual cycle if the she is of child bearing age, sexually active, and/or is not taking birth control pills. The following questionnaire must be completed so that we know when it is an appropriate time to conduct the DXA body composition tests. Please be assured that this information will be kept confidential within the limits permitted by law.

Current Age? _____
Age of first period? _____
Date of last period? _____
Normal length of menstrual cycle? _____
Do you use birth control pills? _____
Are you pregnant or have a desire for pregnancy? _____

Note: If you happen to get pregnant during the course of this study, you must notify research assistants so that appropriate precautions can be made.

I confirm that I have completed this questionnaire honestly and agree to notify researchers within the ESNL of any change in the length of my menstrual cycle and/or pregnancy status.

_____ _____
Name Date

_____ _____
Staff Signature Date

IRB NUMBER: IRB2015-0684F
IRB APPROVAL DATE: 12/10/2015
IRB EXPIRATION DATE: 12/01/2016

APPENDIX G

Side Effects Questionnaire

Texas A&M University: Exercise & Sport Nutrition Laboratory
Trial: Short-Term Safety and Dose Effects of Different Forms of Creatine Ingestion

Participant Name: _____ Date: _____
Participant ID: _____ Treatment: _____

Day	Day 1		Day 2		Day 6		Day 7	
Pre/Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Are you supplementing on schedule?								
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?								
Fast or racing heart rate?								
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?								
Fast or racing heart rate?								
Heart skipping or palpitations?								
Shortness of breath?								
Nervousness?								
Blurred Vision?								
Any other unusual or adverse effects?								

IRB NUMBER: IRB2015-0684F
IRB APPROVAL DATE: 12/10/2015
IRB EXPIRATION DATE: 12/01/2016

APPENDIX H

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

October 7, 2015

CERTIFICATE OF ANALYSIS

Product Name		Creatine Nitrate	
Batch No.	150626	Quantity	3000kg
Packaging	25kg/Drum	Production Date	Jun 26 2015
Test Standard	Enterprise Standard	Expiry Date	Jun 25 2017

ITEM	SPECIFICATION	RESULT	Method
Appearance	Almost white crystalline powder	Almost white crystalline powder	Visual
Loss on drying	≤1.0%	≤1.0%	ChP 2010
Residue on ignition	≤0.6%	0.1%	ChP 2010
Heavy Metals	≤10ppm	<2ppm	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	131 ~138°C	ChP 2010
Bulk Density	415-795g/L	467g/L	USP
Tapped Bulk Density	660-997g/L	741g/L	USP
Total Plate Count	≤1000cfu/g	≤1000cfu/g	ChP 2010
E. Coli	≤10cfu/g	<10cfu/g	ChP 2010
Yeast & Mold	≤100cfu/g	≤100cfu/g	ChP 2010
Coliform Bacteria	≤100cfu/g	≤100cfu/g	ChP 2010
Salmonella	Negative/25g	Negative	ChP 2010
Staph Aureus	≤10cfu/g	<10cfu/g	ChP 2010
Assay	97.0~102.0%	99.4%	Titration
SiO2	0.5%	0.5%	

Kind Regards,

Ron Kramer
ThermoLife International