SHORT-TERM EFFECTS OF A READY-TO-DRINK PRE-WORKOUT BEVERAGE ON EXERCISE PERFORMANCE AND RECOVERY

A Dissertation

by

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ABSTRACT

The effects of ingesting a ready-to-drink pre-workout supplement (RTD) on exercise performance were assessed in this dissertation. Resistance-trained participants (n=25, 24 ± 4 y) ingested in a double-blind, crossover study a: (1) Dextrose placebo (PLA, 12 g) and, (2) RTD containing caffeine (200 mg), β-alanine (2.1 g), niacin (65 mg), folic acid (325 mcg), Vitamin B12 (45 mcg), and arginine nitrate (1.3 g) for 7 d, interspersed by 7 d washout. Data were analyzed by univariate, multivariate, and repeated measures general linear models (GLM), adjusted for gender and relative caffeine intake. Data are presented as mean change (95% CI). An overall multivariate time × treatment interaction was observed on strength performance variables (p = 0.01). Acute RTD ingestion better maintained LP 1-RM (PLA: −0.285 (−0.49, −0.08); RTD: 0.23 (−0.50, 0.18) kg/kg$_{FFM}$, p = 0.30); increased LP RtF (PLA: −2.60 (−6.8, 1.6); RTD: 4.00 (−0.2, 8.2) repetitions, p = 0.031); increased BP lifting volume (PLA: 0.001 (−0.13, 0.16); RTD: 0.03 (0.02, 0.04) kg/kg$_{FFM}$, p = 0.007); and, increased total lifting volume (PLA: −13.12 (−36.9, 10.5); RTD: 21.06 (−2.7, 44.8) kg/kg$_{FFM}$, p = 0.046). Short-term RTD ingestion maintained baseline LP 1-RM (PLA: −0.412 (−0.08, −0.07); RTD: 0.16 (−0.50, 0.18) kg/kg$_{FFM}$, p = 0.30); LP RtF (PLA: 0.12 (−3.0, 3.2); RTD: 3.6 (0.5, 6.7) repetitions, p = 0.116); and, LP lifting volume (PLA: 3.64 (−8.8, 16.1); RTD: 16.25 (3.8, 28.7) kg/kg$_{FFM}$, p = 0.157) to a greater degree than PLA. No significant differences were observed between treatments in cycling TT performance, hemodynamic assessment, fasting blood panels, or self-reported side effects. Within

the confines of this study, the RTD examined appears to be safe and provides an ergogenic benefit for total lifting volume by enhancing muscular endurance; however, it does not appear to be ergogenic to 4 km time trial performance in non-trained cyclists.

DEDICATION

I would like to dedicate this dissertation work to my family and many friends. My wife Kristen has been mostly patient and very supportive with me throughout the program and my 2-year old daughter, Adelynn Aeris, never fails to make me smile even in the stressful times. I also dedicate this dissertation to my parents, Brent & Patricia, and my brothers Logan and Carson as well as Logan's wife Audra.

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The data analyzed in Chapter 4 were collected primarily by the Candidate with help from members of the Exercise and Sports Nutrition Laboratory of the Department of Health and Kinesiology. The analysis completed in Chapter 4 was primarily done by the Candidate with direction from Dr. Earnest as a research associate and Dr. Kreider as principle investigator. All remaining work for the dissertation was completed independently by the Candidate.

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NOMENCLATURE

ESNL	Exercise and Sports Nutrition Laboratory
ISSN	International Society of Sports Nutrition
FAM	Familiarization Session
RTD	Ready-to-Drink pre-workout supplement
PLA	Placebo
TLVC	Total Lifting Volume Combined
TBPV	Total Bench Press Volume
TLPV	Total Leg Press Volume
BP	Bench Press
LP	Leg Press
1-RM	One-repetition maximum
kg/kgFFM	Weight lifted normalized by fat-free mass
RtF	Repetitions to failure
TT	4-km Time Trial
HR	Heart Rate
SBP	Systolic Blood Pressure
DBP	Diastolic Blood Pressure
MAP	Mean Arterial Pressure
RPP	Rate Pressure Product
DXA	Dual x-ray absorptiometry
VO_2	Volume of Oxygen Consumption
PCr	Phosphocreatine, Phosphagen System
MVC	Maximum voluntary contraction
EMG	Electromyography
RPE	Borg Ratings of Perceived Exertion
NO	Nitric Oxide
NO_2	Nitrite
NO_3	Nitrate

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CHAPTER I

INTRODUCTION AND RATIONALE

1.1. Introduction

Many athletes and recreational weightlifters will try almost anything if they perceive a benefit to performance [1]. The estimate as of 2014 yields a range of 48- to 53% of those in the United States utilize some form of nutritional supplementation to enhance well-being and performance [2] with some estimates going as high as 70% [3]. These supplements often contain multiple ingredients and it is important new combinations of ingredients are properly tested for safety and efficacy. Pre-workout drinks containing combinations of caffeine, β-alanine, and vasodilators (e.g., nitrates, L-citrulline, L-arginine) [4-6] have become commonplace to both recreational and competitive lifters with the goal of enhanced mental acuity [7-10] and performance [11-20].

According to the International Society of Sports Nutrition (ISSN), consuming energy drinks primarily containing caffeine, glucose, taurine, or citrulline an improve acute exercise performance, cognitive function, and/or training adaptations [14, 21, 22]. Additionally, ingestion of nitrates prior to exercise has been reported to improve endurance exercise efficiency and high-intensity exercise performance [23-27]. Consequently, there has been interest in examining the individual and synergistic effects of ingesting pre-workout supplements and/or energy drinks prior to exercise

and during training [21, 28]. The acute and short-term safety of adding these types of supplements to the normal diet must be assessed at the absolute doses recommended; as this is the typical way consumers take these supplements. This study examined the use of an RTD version of a market leading pre-workout supplement containing caffeine anhydrous (200 mg), beta-alanine (2.1 g), arginine nitrate (1.3 g), niacin (65 mg), folic acid (325 mcg) and cobalamin (45 mcg) on indices of muscular strength and endurance.

Caffeine acts through multiple mechanisms with the resultant effect of enhancing intercellular cyclic AMP (cAMP) via allosteric modification of adenosine. In practice, caffeine is associated with mental acuity, maximal strength, maximal power, and increased muscular endurance [29, 30]. Various studies have evidenced an ergogenic effect in doses as low as 3 mg/kg up to 9 mg/kg, with the ceiling to the ergogenic effect occurring with 3 mg/kg and 6 mg/kg and a slight decrease at 9 mg/kg [30, 31]. It should also be noted that higher doses also exhibit increases in physiological responses such as heart rate, blood lactate levels, and circulating free fatty acids (FFA) [31, 32] while low to moderate doses provided an ergogenic effect without the aforementioned physiological responses [33].

Nitrates (NO_3) function via reduction to nitrites (NO_2) and further reduction to the vasodilator nitric oxide (NO). The initial conversion takes place in the mouth and absorption into the blood stream occurs in the stomach where nitrites then act on endothelial smooth muscle to promote a vasodilatory response. Hypoxia appears to be the main signal for the action of nitrates/nitrates on endothelial tissue, which fits with

the physiological responses to exercise, particularly in the primarily glycolytic exercises employed in this study [34, 35]. Nitrates are shown to be effective at doses of 5 mmol (310 mg) to as high as 20 mmol (1240 mg) in enhancing exercise efficiency, improving recovery, and maintaining muscular strength and endurance in the later stages of exhaustive exercise [23, 36, 37].

Arginine serves as a precursor to a variety of proteins and cellular signaling molecules, most notably nitric oxide (NO). Given the increased protein synthesis, the conceptual understanding of arginine is that it enhances the recovery of damages tissues. Arginine activates the nitric oxide synthase pathway which results in vasodilation and therefore enhanced blood flow to working tissues. Arginine is a supplement which is shown to enhance nitric oxide activity following acute doses of 3 to 9 mg/d, thereby enhancing nutrient supply to muscles via vasodilation-induced increases in blood flow; however, there is inconclusive evidence of any ergogenic effect in healthy populations [38, 39]. The proposed rationale of combining L-arginine and nitrate postulates the nitrate attached to the arginine enhances the bioavailability of L-arginine to increase the ergogenic effect.

The function of β-alanine is to convert to L-carnitine where it then acts as a pH buffer in skeletal muscle; intramuscular L-carnitine is regulated by the amount of present β-alanine. In practice, chronic, but not acute use, of β-alanine has been shown to enhance muscular strength and power as well as muscular endurance [40, 41]. β-alanine, when taken daily for several in doses of 4 to 6 g/d, has also been evidenced to

be a promising ergogenic aid, being classified as “Apparently Effective” in performance enhancement by the ISSN [14]. Of note, PWS often contain less than the ergogenic amount of some compounds as a “maintenance dose”, primarily for convenience in supplementing normal diet.

1.2. Purpose of the Study

The aim of this study was to assess the effects of consuming a “ready to drink” (RTD) version of a market-leading pre-workout supplement. Our primary outcome was the assessment of exercise performance recovery following acute and short-term supplementation. The secondary outcome was the assessment of safety following acute and short-term use.

1.3. Hypotheses

H_1: RTD ingestion will promote significant improvements in measures of muscular strength and performance in comparison to a ingesting a placebo.

H_2: RTD ingestion will promote significant improvements in measures of 4 km time trial performance compared to placebo.

H_3: There will be no statistically significant differences observed between placebo and RTD in measurements of safety assessed by hemodynamic response or serum and whole blood chemistries.

H_4: There will be no statistically significant differences observed between placebo and RTD in hemodynamic response, blood chemistry, or frequency or severity of self-reported side effects.

1.4. Research Design

This study was conducted in a double-blind, placebo-controlled manner following a randomized crossover model. The independent variable in this work was nutritional supplementation of a RTD caffeine anhydrous (200 mg), beta-alanine (2.1 g), arginine nitrate (1.3 g), niacin (65 mg), folic acid (325 mcg) and cobalamin (45 mcg) and the dependent variables were performance measured as total volume (bench press, leg press, combined), one repetition maximum and repetitions to failure on bench press and leg press, time trial average power and time to completion, hemodynamic response (heart rate, blood pressure, mean arterial pressure, and rate pressure product), blood chemistry, and self-reported side effects.

The primary outcome was the assessment of exercise performance recovery after acute and short-term supplementation, while the secondary outcome was assessment of acute and short-term safety. We hypothesized that the RTD studied would improve muscular strength and endurance as well as 4 km time trial performance without undue alterations in hemodynamic response to a hemodynamic challenge, hepatorenal and muscle enzyme function, or the frequency and severity of self-reported side effects.

Following recruitment and screening, subjects underwent a familiarization session. During the familiarization session, demographic data and anthropometrics including gender, height, weight, race, resting systolic and diastolic blood pressure (SBP, DBP), resting heart rate, and body composition analyzed by dual x-ray absorptiometry (DXA) were recorded. In addition, subjects underwent a familiarization testing protocol on bench press, leg press, and 4 km time trial (TT).

For the strength measurements, subjects were instructed to perform 3 warm-up sets followed by progressively increased weight until a one-rep maximum (1-RM) was determined. Following determination of 1-RM, subjects performed two sets of ten repetitions on each lift followed by one set to volitional fatigue. Following strength measurements, subjects were adjusted for comfort on the bicycle, followed by a short warm-up, and finally the TT. Subjects also provided one 10 mL fasting blood sample at the onset of each testing session in addition to the side effects questionnaire and resting blood pressure and heart rate using a tilt table protocol for added control and safety.

Following the four testing sessions for the initial treatment (or placebo), subjects completed a 7 to 10 d washout period prior to beginning the alternate treatment. The order in which they took treatment or placebo were randomized prior to allocation. Participants were randomized using random number generation in SPSS to provide a random order relative to each participant number (RTD then PLA or PLA then RTD).

The scope of this study involved college-aged males and females from a local Tier 1 University located in East Texas. The results of this study should reasonably be readily extrapolated to similar populations undergoing similar training regimens in other geographical areas.

1.5. Limitations

1. Participants were recruited locally as a function of convenience and logistics. Therefore, there was some limitation to the degree of randomization.
2. Participants were instructed to maintain their current training protocol; however, there did exist the possibility of altering training routines during the three week study period.
3. All laboratory equipment was calibrated according to manufacturer prior to each testing day.
4. Dietary food logs were not collected or controlled for.

1.6. Assumptions

1. Participants answered the screening forms honestly.
2. Participants gave maximum effort during testing.
3. Participants adhered to the supplementation protocol.
4. Participants maintained a consistent dietary intake and exercise regimen throughout the duration of the study.

1.7. Delimitations

The current study was conducted under the following guidelines:

1. IRB approval was for 40 participants consisting of both males and females. Intake was limited to a maximum of 20 males and 20 females.
2. Participants were required to have been involved in a resistance/cardiovascular exercise combination training program for at least the prior six months
3. Eligible participants took part in a familiarization session during which time they were informed of the study protocol, filled out necessary forms including an informed consent form and a general screening form, and established baseline data for leg press, bench press, and 4 km time trial.
4. Participants were advised to maintain a consistent workout and dietary regimen throughout the duration of the study.
5. Participants were instructed to abstain from exercise, caffeine, and use of over-the-counter stimulants for 48- h prior to each testing session.
6. Participants were instructed to be fasted for at least 8 h prior to each testing session.
7. Participants were instructed to perform to their maximal ability on all strength and fatigue tests as well as the time trial.
8. Participants were instructed to consume all supplements and report any disorder(s) in weekly side-effects questionnaires.

CHAPTER II

REVIEW OF THE LITERATURE

2.1. Review of Literature

The literature under review was gathered using the PubMed database and keywords: caffeine, nitrate(s), beta-alanine, L-arginine, exercise, performance, time trial, strength, endurance, and safety. No date restrictions were set. Citations were maintained using EndNote X8 and Journal of Nutrients referencing style.

2.2. Prevalence of Ergogenic Aid Usage

According to the ISSN position stand on caffeine and energy drinks, an energy drink can be defined as having a dose of caffeine between 2 and 6 mg/kg with the addition of carbohydrates, electrolytes, and proprietary mixtures of other compounds [22]. Pre-workout supplements are typically found in powder form and include caffeine, creatine, and other substances with the purpose of improving exercise performance. Conversely, in ready-to-drink pre-workout supplements (RTD) caffeine exists in the ergogenic range and is accompanied by a proprietary blend of proposed ergogenic substances with the exclusion of creatine as creatine becomes the inert creatinine when left suspended in water. These proprietary blends may include vitamins, herbal extracts, and other compounds and are not regulated by the FDA, indicating a need for research to determine efficacy and safety.

2.3. Ergogenic Assessment of Primary Nutrients

In this review, primary focus was placed on the active ingredients found in the present RTD. The RTD in this study contains caffeine (200 mg), arginine nitrate, 1.3 g), and β-alanine (2.1 g). The arginine nitrate compound exists as 73% arginine and 27% nitrate (950 mg and 350 mg, respectively), indicating a nitrate dose of 5.7 mmol. The individual effects of these ingredients were compared with respect to dose, timing, and performance.

2.3.1. Caffeine

Caffeine is structurally similar to adenosine and allosterically modifies adenosine receptors to attenuate the response. Activation of adenosine receptors by their main ligand adenosine has various effects throughout the body including reduction of core temperature and decreases in dopamine release [42-45]. Adenosine Receptor Type 1 (AR1), found throughout the body, initiates a G-protein signaling cascade which results in a decrease in cyclic AMP (cAMP) and ultimately a relaxation effect of the muscle. In the brain, caffeine has been found to antagonize adenosine receptors, leading to increased metabolism at the expense of decreased blood flow [46]. According to Nehlig, et al., [46] caffeine also promotes the release of dopamine via arousal of norepinephrine neurons to facilitate increases in learning, memory, coordination, and memory.

In the heart, adenosine activation of AR1 leads to decreased chronotropy or decreased rate of contraction. By contrast, caffeine activation of AR1 serves an excitatory role to cAMP which results in increased Ca^{++} influx and thus increased chronotropy [42]. AR2a/b, and AR3 also follow this pattern of adenosine and caffeine having opposite effects on cell function via inhibition or excitation of cAMP. In addition to the allosteric modification of adenosine, caffeine also serves to modulate insulin receptor substrate-1 (IRS-1) and impairs insulin signal transduction [47]. These insulin-related mechanisms bay serve to increase fat oxidation and spare glucose [48, 49].

Caffeine is classified by the International Society of Sports Nutrition (ISSN) as "apparently effective" in a variety of areas [22]; it yields measurable improvements most of the time in most populations. Caffeine is purported to not only enhance strength [11, 16-20, 50-54] and endurance [50, 55-59] but also to increase and maintain cognition [7-9, 60] and higher executive functions following a fatiguing bout of exercise. Much of the research has involved moderate to high doses (5 to 13 mg/kg) [30, 32, 61-63] but there is evidence showing low doses of caffeine (<3 mg/kg) to yield similar ergogenic results to those of the moderate and high doses with lower incidence of side effects [22, 30, 64-69]. The key points put forth by the ISSN position stand state caffeine is regarded as ergogenic in doses of 3 to 9 mg/kg, yields a greater effect when consumed as caffeine anhydrous, reduces perceptions of pain, and the evidence is consistent in regards to caffeine and strength [22]. An overview of caffeine's ergogenic potential is found in TABLE 2.1.

In a study by Dekker, et al. [70], twelve caffeine-naïve young males were recruited to analyze the effects of caffeine on insulin, glucose, epinephrine, and response to the glucose tolerance test. Caffeine dose was maintained at 5 mg/kg in unlabeled gel capsules for the duration of the study for a total of 6 total doses. The results of the glucose tolerance test found insulin and blood glucose to be elevated in the caffeine group versus placebo at Day 7 and on Day 14 the caffeine group still yielded elevated insulin concentration however the effect was blunted compared to Day 7. This study found a pertubatory effect of caffeine on glucose homeostasis that slowly corrected after chronic use of caffeine.

Beaudoin, et al. [71] further elucidated the possible mechanism found in Dekker, et al. using a protocol to assess the effects of caffeine on insulin sensitivity. The study sought to further analyze the response of glucose homeostasis as a function of both gender and caffeine dose. This placebo-controlled, double-blind, randomized, crossover study used 24 participants (12 M, 12 F) provided with either 1, 3, or 5 mg/kg caffeine or dextrose placebo. One hour after ingestion, participants underwent a glucose tolerance test in addition to analysis of blood glucose, insulin, and C-peptide. The results yielded no evidence of a gender effect; however, insulin and C-peptide were both increased with increasing doses of caffeine. Despite elevated insulin, glucose also elevated with increasing amounts of caffeine. Caffeine increases fat oxidation, and these data provide a more accurate view of caffeine and glucose sparing.

Table 2.1. Caffeine

Author (year)	Study Design	Sample	Dosing Protocol	Assessment Protocol	Significant Findings
Beaudoin, et al. (2013) [71]	P, R, DB	n = 24 (12 M, 12 F)	1, 3, or 5 mg/kg 1 h prior to testing	Oral glucose tolerance test	Elevated plasma insulin and glucose in dose response manner with return to baseline after 2 weeks; No effect of gender
Hendrix, et al. (2010) [20]	P, R, DB, CO	n = 21 untrained men (23 ± 2.6 y)	400 mg in gel capsule (4.9 mg kg) 1 h prior to exercise, 7 d w/o	1RM, time to exhaustion on CE	No difference noted
Collier, et al (2016) [72]	P, R, DB,	n = 15 healthy participants (26.1 ± 3.5 y)	100 mg (~1.7 mg/kg) caffeine in energy drink 1 h pre-exercise, 7 d w/o	MVC, EMG	Increase MVC in caffeine group with no difference in EMG
Goldstein, et al. (2010) [73]	P, R, DB, CO	n = 15 resistance-trained women (24.6 ± 6.9 y)	6 mg/kg 1 h pre-exercise, 7 d w/o	Bench Press 1RM, RtF at 60%	Increased 1RM in caffeine group; Increased SBP in caffeine group was still within normal limits
Duncan, et al. (2012) [74]	P, R, DB, CO	n = 11 resistance-trained participants (26.4 ± 6.4 y)	5 mg/kg 1 h pre-exercise, 3 d w/o	RtF at 60%	Increased RtF in caffeine group; Decreased RPE in caffeine group
Ivy, et al. (2009) [67]	P, R, DB	n = 12 trained cyclists(6 M, 6 F, 27.3 ± 1.7 y))	~2 mg/kg 40 min pre-exercise	Time trial at 70% W_{max}	Time trial time to completion improved in caffeine group, RPE decreased
Pai, et al. (2015) [66]	P, R, DB, CO	n = 20 students	~2 mg/kg 1 h pre-exercise, 7 d w/o	MVC, EMG	Increased time to fatigue on MVC in caffeine group with no difference in EMG readings
Forbes, et al. (2007) [68]	P, R, DB, CO	n = 15 healthy adults (21 ± 5 y)	~2 mg/kg 30 min pre-exercise, 7 d w/o	RtF, Wingate exercise test	Caffeine group increased RtF with no difference in Wingate performance

Overview of literature reviewed. P = Placebo controlled, R = Randomized, DB = Double-blind, CO = Crossover, w/o = Washout, 1 RM = one repetition maximum, RtF = repetitions to failure, CE = cycle ergometer, MVC = maximal voluntary contraction, SBP = systolic blood pressure, DBP = diastolic blood pressure, RPE = ratings of perceived exertion

Graham and Spriet [75] analyzed a high dose of caffeine in relation to prolonged exercise relative to performance and metabolism. The study analyzed seven competitive runners (28.3 ± 2.3 y, 67.2 ± 3.4 kg) with average 10-km run times of 00:31:36: ± 00:00:36 (n=7) and marathon times of 02:33:00 ± 00:00:03 (n=5). Incremental VO_{2max} tests were conducted on treadmill and cycle ergometer, yielding values of 72.6 ± 1.5 mL/kg/min and 61.9 ± 2.7 mL/kg/min, respectively. Participants were then assigned to either caffeine or placebo group in a double-blind, crossover manner involving a one week washout between treatments. The subjects were administered either a dextrose placebo or caffeine at a dose of 9 mg/kg in an unlabeled capsule 1 h prior to testing. Of the four following visits, separated by 1 w between each visit, two visits were completed on the treadmill and two on the cycle ergometer, all four to exhaustion at 85% VO_{2max}. Prior to each test, each participant provided a urine sample as well as establishment of IV access for 15 min blood draws; within 15 min of test completion, a second urine sample was collected.

The caffeine group yielded a greater time to exhaustion, improving from 49.2 ± 7.2 min at baseline to 71.0 ± 11.0 min following treatment on the treadmill and 39.2 ± 6.5 min to 59.3 ± 9.9 min on the cycle ergometer. Urinalysis revealed a caffeine content of 8.7 ± 1.2 μg/ml on the treadmill and 10.0 ± 0.8 μg/ml on the cycle, both below the International Olympic Committee arbitrary ceiling of 12 μg/ml. The caffeine group also yielded an approximate twofold increase in epinephrine in both tests while placebo remained constant. No effect was reported in relation to free fatty acids at

rest or during exercise, though, there was a trend in the caffeine group. No effect was reported on blood lactate or glucose in either group. Furthermore, there was no report of any side effects even at the high dose of 9 mg/kg.

Caffeine has long been thought to be ergogenic to strength and power. In a meta-analysis by Warren, et al. [76], the authors set out with the aim to assess caffeine's effects on maximal strength and muscular endurance. Covering studies from 1939 to 2008, the authors reviewed a total of 34 studies investigating the effects of caffeine on maximal voluntary contraction (MVC) and muscular endurance. There was an overall effect size of 0.19 for caffeine on MVC ($p < 0.001$) with a lower-body specific effect size of 0.37 in the knee extensors. Analyzing studies that focused on percent muscle activation measured by EMG, the overall effect size of caffeine was found to be 0.67 ($p < 0.001$), evidencing a role of caffeine in the central nervous system (CNS). The overall effect size of caffeine on muscular endurance was 0.28 versus placebo ($p < 0.001$) demonstrating a small beneficial effect of caffeine on muscular endurance.

Hendrix, et al. [20] analyzed the effects of caffeine on strength and time to exhaustion in both the bench press, leg extension, and cycle ergometry at a constant work load of 80% VO_2peak. This study involved 21 untrained men (23 ± 2.6 y, 81 ± 12.1 kg, 180.2 ± 4.8 cm) on either a supplement containing 400 mg caffeine and 66.7 mg capsaicin extract or cellulose placebo separated by a one week washout. This amount of caffeine yielded an average dose of 4.9 mg/kg, well within the accepted ergogenic range. One hour following ingestion, participants underwent a one-repetition

maximum (1-RM) protocol on bench press and knee extension prior to undergoing a time to exhaustion protocol on a cycle ergometer. The authors reported no significant difference of the supplement on any of the metrics.

Collier, et al. [72] analyzed the effects of caffeine in the form of an energy drink on MVC, electrically stimulated force production, and percent muscle activation during MVC. The study involved 15 healthy participants (26.1 ± 3.5 y, 70.7 ± 12.1 kg, 174.6 ± 6.0 cm) in a placebo-controlled, double-blind, repeated-measures, crossover design with a 1 w washout between treatments. The energy drink used in this study contained 100 mg caffeine, 29 g carbohydrate, and other non-ergogenic ingredients which equates to 1.7 mg/kg of caffeine. These metrics were followed by EMG recording of electrical activity following MVC testing. The caffeine group yielded an improvement of 5 ± 1.7% versus -0.5 ± 1.5% in placebo on MVC ($p = 0.015$) with no differences in any other metric.

Goldstein, et al. [73] studied the effects of caffeine on upper body strength in resistance-trained women. Fifteen women were recruited for this study (24.6 ± 6.9 y, 63.6 ± 8.3 kg, 166.2 ± 9.0 cm) wherein either caffeine at 6 mg/kg or placebo was consumed for each study session with a 7 d washout period between sessions. Prior to ingestion, heart rate and blood pressure were assessed, as well as at 60 min post-ingestion and following repetitions to failure. One hour post-ingestion, the participants were assessed for 1-RM and repetitions to failure (RtF) at 60% baseline 1-RM, similar to the protocol in the present study. The caffeine group yielded a greater 1-RM compared

to placebo and no difference in RtF. The caffeine group demonstrated higher systolic blood pressure (116.8 ± 5.3 mmHg against 112.9 ± 4.9 mmHg for placebo); however, SBP remained within normal limits.

Duncan, et al. [74] studied 11 resistance trained participants (9 male, 2 female; 26.4 ± 6.4 y) in a double-blind, placebo controlled, randomized study which used a crossover model wherein the treatment and placebo were separated by a 72 h washout. After establishing 1RM on bench press, rows, deadlifts, and back squat at the familiarization session, participants waited 48 to 72 h before performing their first study session. Upon arrival, they ingested a measured dose of either dextrose placebo or 5mg/kg caffeine followed by a 60 min waiting period. After a 5 min warm-up on a cycle ergometer, participants performed RtF at 60% 1-RM on each of the aforementioned lifts with 3 min separating each exercise. Caffeine increased RtF ($p = 0.049$). Across all exercises, caffeine yielded a lower rating of perceive exertion (RPE) ($p = 0.027$).

In a separate study by Bloms, et al. [77], power as a function of jump height was measured in response to acute caffeine ingestion at a dose of 5 mg/kg. No familiarization session was noted by the authors; however, the participants were allowed practice attempts prior to the actual testing. In this study, NCAA Division I athletes were recruited (16 males, 21 ± 1.5 y; 9 females, 20 ± 1.3 y) and study sessions were built around the athletes' training schedules. Each athlete completed two study sessions separated by one week; the study sessions were completed prior to training.

Participants ingested the caffeine anhydrous or placebo, waited a period of 1 h, and then began the exercise protocol. Participants completed three squat jumps and three countermovement jumps with 30 sec rests separating each attempt. Following data collection, there was an improvement in the caffeine group in jump height for both the squat jump (p = 0.001) and the countermovement jump (p = 0.001). Also of interest, there was increased efficiency of movement in the caffeine group (p = 0.017).

In a similar study by Ribeiro, et al. [78], acute caffeine ingestion was analyzed in relation to leg power measured by vertical jumps. In contrast to the multiple single attempts seen in Bloms, et al., Ribeiro, et al. offered continuous jumping to measure both power and endurance. Six male handball athletes were recruited for this study (21.6 ± 2.9 y). Participants ingested the caffeine (6 mg/kg) or placebo treatment, waited 1 h and, after a brief warm-up, completed four sets of 30 sec continuous vertical jumps interspersed by 60 sec rests. Leg power was recorded by a force plate and plotted. After a one week washout, participants returned to the lab to repeat testing. The caffeine group yielded no change from start to finish; however, the placebo group had a lower end power (p < 0.05) and decreased time to fatigue (p < 0.001).

Shearer & Graham [79] completed a review of energy drinks on performance and metabolism. In this review, articles involving human models after 1990; double-blind, placebo controlled, randomized studies; and articles not using coffee as the caffeine source were included in the analysis. The aims of the review were to elucidate the effects of caffeine on timed performance, endurance/performance time, power

related to performance, and glucose tolerance. This amounted to 38 relevant studies which was then reduced to 24 after further review. Many of the studies used set amounts of caffeine of between 75 and 160 mg in pre-packaged products. The authors attempted to extrapolate this number based on an average male weight of 65 kg, providing a dose of between 1.15 to 2.26 mg/kg of caffeine.

The results of the combined analysis showed an overall timed performance increase of 3.6% with caffeine use. Overall, the studies in the review ranged from 2 to 7 mg/kg and closer inspection revealed an increase in performance of 7% for doses of 2 to 5 mg/kg with a maintained improvement of 7% at doses greater than 5 mg/kg. The authors noted a plateau effect to occur at approximately 6 mg/kg of caffeine in hepatic metabolism resulting in an increase of caffeine metabolites; however, the metabolism of caffeine can be affected by a variety of individual factors including liver cytochrome p450 and CYP1A2 as well as variation in adenosine receptors. Caffeine was also found to increase insulin resistance in sedentary populations but this does not appear to hold true in trained populations, suggesting a safety issue and increased risk of the development of Type II Diabetes in sedentary populations. In trained populations, this mechanism appears to enhance reliance on fat oxidation, possibly lending to the ergogenic effect on performance by sparing muscle glycogen and decreasing lactate accumulation.

Souza, et al. [80] analyzed the effects of caffeinated energy drinks on physical performance in a systematic literature review. This review covered studies from 1998

to 2015 (n=34) and calculated overall effect sizes of caffeinated energy drinks on muscle strength and endurance, endurance exercise, sport-specific actions, and sprinting. The authors found an effect size of 0.49 in muscular strength and endurance ($p < 0.001$), 0.53 in endurance testing ($p < 0.001$), 0.29 in jumping actions ($p = 0.01$), and 0.51 in sport-specific actions ($p < 0.001$); however, the 0.14 effect size in sprinting was not significant ($p = 0.06$). When data were adjusted for taurine content, taurine was more closely associated with increased performance than caffeine. The studies under review contained between 40 and 325 mg of caffeine as an absolute dose; the authors did not calculate relative dose. Taurine content ranged from 71.2 to 3105.8 mg and higher content of taurine correlated with performance improvement unrelated to total caffeine content.

Analyzing the lower end of the dosing spectrum, Spriet, et al. [81] conducted a review on low doses of caffeine to analyze for ergogenic effect as well as safety. There does appear to be a ceiling effect to ergogenic potential as doses exceed 5 mg/kg with a concomitant increase in side effects [79]. These side effects include increased catecholamine levels [32], higher blood lactate concentrations [82], and increased free fatty acids [31]. Spriet, et al. [30] hypothesized a similar ergogenic effect from a lower dose without the side effects. In one of the reviewed studies, endurance-trained participants received either 3, 6, or 9 mg/kg of caffeine and were tested using a run to exhaustion protocol at 85% VO_{2max} with a 1 w washout between treatments. Compared to placebo, the 3 and 6 mg/kg groups yielded a 22% increase in time to exhaustion,

whereas the 9 mg/kg showed an 11% increase. Further investigation found a moderate dose of 4.5 mg/kg spread out during exercise provided the largest ergogenic benefit [56]. Returning to the low dose (<3 mg/kg), a study by Jenkins, et al. [83] utilized either placebo or 1, 2, or 3 mg/kg caffeine on trained cyclists. The cyclists ingested the treatment 1 h prior to a 20 min steady state cycle test at 80% VO_{2max}, followed by 5 min of light pedaling, and then a 15 min time trial. Those on placebo of 1 mg/kg yielded no evidence of an ergogenic effect however 2 and 3 mg/kg increased overall work in the 15 min time trial by 4% and 3%, respectively.

A study by Santos, et al. investigated the effects of caffeine on reaction time in simulated Tae-Kwon-Do matches [84]. This study involved 10 competitive Tae-Kwon-Do practitioners (24.9 ± 7.3 y, 77.2 ± 12.3 kg, 175 ± 0.06 cm) whom were recruited due to familiarization with the testing procedures involved. For baseline testing, the athletes received a dose of caffeine at 5 mg/kg or a cellulose placebo. After two consecutive testing days, the athletes completed a 7 d washout period followed by follow-up testing on the other treatment. Fifty minutes post-ingestion, participants completed a reaction time test; at 1 h post-ingestion, a combat drill was completed. At 70 min, the two tests were repeated followed by one final reaction time test at 100 min. Blood was collected at regular intervals between testing.

The reaction time test utilized a series of 5 roundhouse kicks to abdomen-height following a visual stimulus. Of the 5 kicks, the best and worst were excluded and the average of the remaining three was used in the analysis. For the combat drills, athletes

participated in a 2 min round against the other participants under World Tae-Kwon-Do Federation rules, full protective gear, and a referee. Three rounds were completed separated by 1 min rests. During the first reaction time test, the caffeine group yielded an 11.9% lower time compared to placebo (p = 0.004) but there was no significance in the second or third reaction time test. During the first combat drill, the caffeine group committed fewer errors than the placebo group in the first round (p = 0.036) and second round (p = 0.005) with no significance being found in the third round. During the second combat drill, the caffeine group again performed better during the first and second rounds (p = 0.012 and 0.018, respectively); however, there was again no significance in the third round. There were no differences between the first and second combat drills in relation to the treatment; however, overall performance decreased for both groups (p = 0.036). Despite lactate concentration being higher in the caffeine group during first combat drill rounds 2 and 3 (p = 0.29 and 0.014, respectively), there were no differences in RPE or HR. The results of this study demonstrate an ergogenic effect of caffeine on reaction time and increased intensity during the combat drills.

Caffeine has a role in the brain via antagonizing adenosine receptors and promoting an increase in dopamine [46]. The impact on performance is equivocal [22]; however, there are some who believe the ergogenic effect has its foundation in the brain and central nervous system, not the peripheral tissues. The putative effects on fatigue reduction and increased alertness are accepted but the mechanisms need to be expanded upon. Meeusen, et al. [85] sought to further elucidate the mechanisms in a

review article. The authors hypothesized the increase in dopamine enhances the signaling within the motor coordination and somatosensory centers of the brain, providing a potential mechanism by which caffeine may increase performance from a neurological frame.

The nucleus accumbens, the "pleasure and reward center" of the brain, contains an abundance of adenosine receptors. When adenosine is elevated these receptors are activated and fatigue is enhanced, when caffeine allosterically modifies these receptors fatigue is reduced. One article in the review involved direct cranial injections of either placebo, caffeine, an adenosine analog, or a combination of caffeine and adenosine analog [86]. After injection, the rats performed a forced treadmill to exhaustion. The placebo and combination groups completed 80 min, the caffeine only group completed 120 min, and the adenosine only group completed 25 min. These findings support the CNS-mediated ergogenic effect of caffeine.

Caffeine also has a putative role in decreasing perception of pain, allowing for increased time to fatigue or repetitions to failure. Richardson & Clarke [87] found caffeine to significantly improve total volume lifted in bench press and squats, specifically with a significant increase to total squat volume. Nine resistance trained men (24 ± 2 y) participated in a randomized, crossover, double-blind, placebo-controlled study wherein caffeine was administered by itself (5 mg/kg), with decaffeinated coffee, as caffeinated coffee, or none at all. Each participant participated in each condition with a 2 d washout between each condition. At the onset of each

testing session, participants consumed the treatment, waited 45 min, and performed RtF on squat and bench press. Caffeine added to decaffeinated coffee improved RtF compared to decaffeinated coffee ($p < 0.01$), caffeinated coffee ($p < 0.05$), and placebo ($p < 0.05$). Coffee alone performed better than both the decaffeinated coffee and placebo ($p < 0.05$).

Mets, et al. [65] conducted a study on a popular caffeinated energy drink and its effect on cognition. In this study, 24 participants were given Red Bull with or without the active ingredients and put through a simulated driving test. The driving test consisted of a 2 h "highway" drive, a 15 min break and treatment or placebo ingestion, and a second 2 h "highway" drive. The primary endpoint of the study was to assess how well the driver kept the car in a straight path and secondary analyses were conducted to examine the effects of the treatment on subjective driving quality and alertness. Red Bull significantly improved performance over placebo during both Hour Three and Hour Four ($p = 0.046$ and 0.011, respectively) as well as every secondary endpoint (ie. Sleepiness, mental effort, speed maintenance; $p = 0.001$, 0.024, 0.004, respectively). It is likely that the caffeine was responsible for the increase in performance.

In a separate study by Ivy, et al. [67, 88], also investigating Red Bull, twelve trained cyclists were assigned to either a Red Bull or flavored placebo in a randomized, double-blind manner. The participants (n = 12, 6 M, 6 F, 27.3 ± 1.7 y, 68.9 ± 3.2 kg) fasted for 12 h, ingested the supplement, and then after a 40 min wait performed a time trial

at 70% W_{max}. The Red Bull group yielded better performance ($p < 0.01$). However, rating of perceived exertion and metabolism were unaffected by the treatment. To clarify, those in the Red Bull group exhibited enhanced performance with no difference in subjective difficulty compared to placebo.

Red bull is a popular energy drink containing 80 mg of caffeine and assorted B-vitamins in 250 mL of carbonated water. Assuming a 65 kg (143 lb) individual, this equates to a relative caffeine dose of 1.23 mg/kg, well under the putative ergogenic range. Despite the "low" dose, ergogenic effects have been associated with Red Bull by other researchers. Pai, et al. [66] conducted a crossover design using a sample of medical students (n = 20, 10 M and 10 F) which demonstrated a calculated relative caffeine dose of 2 mg/kg. The findings evidence decreased rate of fatigue with no difference in EMG readings or MVC 1 h after ingestion of Red Bull. Goel, et al. [89] conducted a cross over study analyzing a cohort (n = 20, 10 M and 10 F) that yielded a mean relative dose of 2 mg/kg. The findings evidenced an ergogenic role in reaction time but no difference in maximal voluntary contraction. Forbes, et al. [68]analyzed a sample of healthy young adults (n = 15, 11 M and 4 F, 21 ± 5 y) in a randomized, crossover model. The average relative dose of the sample was 2 mg/kg. Participants underwent a modified RtF protocol on bench press at 70% 1-RM as well as a modified Wingate stress test. The modified RtF protocol used the total repetitions of 3 consecutive sets to failure to calculate total volume and the modified Wingate included 3 separate 30 sec Wingate bouts separated by 2 min active recovery. The Red Bull

group demonstrated significantly increased total volume with no effect on the Wingate. Of note, Red Bull contains taurine which has been found to potentially increase the effects of caffeine[90-93]

In the studies reviewed, there were many trends and commonalities. The current understanding is caffeine yields ergogenic benefit in doses of 3 to 9 mg/kg [22, 30, 32, 61, 62]. However, there exists sufficient evidence of low doses (<3 mg/kg) of caffeine having an ergogenic effect on performance with a lower incidence of side effects [56, 65, 66, 71, 72, 79, 81, 83, 89, 94]. The amount of caffeine in the current treatment is 200 mg. Assuming an average mass of 65 kg, an approximate average dose of 3 mg/kg seems to be a fair estimate. Considering the literature reviewed, it seems likely caffeine could still be effective even if the average dose is slightly less than 3 mg/kg, as multiple studies found ergogenic effects as low as 2 mg/kg [68, 72, 83].

2.3.2. Nitrates

Nitrates (NO_3) act by reducing from NO_3 to nitrites (NO_2) and then to nitric oxide (NO) which then induces vasodilation [95, 96]. Aside from its actions within the endothelium, nitrate has also been found to have effects within muscle tissue and have a role in glucose homeostasis [97] leading to it becoming a popular addition in many PWS [25-27, 98-100].

A review by Affourtit, et al. [35] illustrates a general overview of the role of nitrates in skeletal muscle. In one of the reviewed studies, Bailey, et al. [34]

demonstrated VO_2 during low-intensity cycling was lower in the nitrate-rich beetroot treatment group at a given intensity compared to the nitrate-depleted beetroot group. The authors also suggest a dose dependent response of nitrate on the decreased VO_2 cost during low-intensity exercise, showing the decrease to be greater at 4.2, 8.4, and 16.8 mmol NO_3 [101, 102]. Further work in that same study found 4.2 mmol (260 mg) to have no effect on high-intensity exercise whereas 8.4 and 16.8 mmol (521 and 1042 mg, respectively) improved exercise tolerance similar to low-intensity. The review by Affourtit, et al. also suggests the effects of nitrates may be muscle fiber specific, perhaps through increased removal of metabolites or enhancement of GLUT4 as previously discussed.

One other proposed mechanism in this review shows that nitrate ingestion may actually reduce ATP cost of contraction by increasing phosphocreatine (PCr) synthesis, thus providing an avenue for more 'free' ATP production [103]. The authors explicitly state they are unaware of the exact mechanism by which the ATP cost and subsequent decrease in VO_2 cost is employed. In addition, there is some evidence that nitrate supplementation may enhance the contractile properties of skeletal muscle [104].

During incremental exercise, pulmonary VO_2 uptake increases to meet need within a few minutes of arriving at a given intensity. At steady state, ATP use and production are equal, providing a snap shot of metabolism at a given exercise intensity. As was shown in the studies by Bailey, et al. [34, 105], nitrates decrease VO_2 at varying

intensities, indicating a potentially large role of the metabolically active skeletal muscle tissue in the ergogenic effects of nitrates.

Bailey, et al. [106] investigated the effects of dietary nitrate supplementation in muscle contractile efficiency. They hypothesized nitrates may act to reduce the O_2 cost of mitochondrial ATP synthesis, improves the coupling of ATP hydrolysis in the cross-bridge cycle, and/or potentially inhibits mitochondrial ATP production which would shift energy production to other systems.

Seven recreationally active males were recruited for this study (28 ± 7 y, 81 ± 7 kg, 180 ± 2 cm). Participants were randomly assigned to either a nitrate-rich beetroot juice group or a nitrate-depleted beetroot juice group separated by a 10 d washout; the nitrate content was not specified however other studies in the same lab report 10.2 mmol (632 mg) for nitrate-rich and 0.17 mmol (10.5 mg) for nitrate-depleted beetroot juice [34, 37]. Participants underwent seven total testing sessions over the course of four weeks: a familiarization session where plasma nitrite and blood pressure were measured, MVC of the quadriceps followed by an incremental exercise test on the knee extension ergometer on days 1 through 3, and step test on days 4 through 6, and a 7 d washout between 3 and 4. The MVC was conducted for 3 sec and the incremental leg extension was conducted at 40 repetitions per min starting at 4 kg and increasing by 1 kg each increment. Electromyography (EMG) was recorded from the beginning of MVC testing to completion of the incremental knee extension. The step testing involved two

4 min bouts of low cadence stepping and one 6 min bout of high cadence stepping with 6 min rests between bouts. Pulmonary gases were measured throughout.

There were no side effects reported and the nitrates were tolerated. Blood analysis showed an increase of 137% in NO_2 with the nitrate group compared to placebo ($p < 0.05$). There was no difference in blood pressure or heart rate between groups. During low-intensity exercise, there was no difference in the EMG recordings.

Further evidencing the effects of nitrates ergogenic role in skeletal muscle, Haider & Folland [104] recruited 19 healthy untrained men (21 ± 3 y) to participate in a double-blind, randomized, crossover study to elucidate the effects of nitrate supplementation on contractile function. Participants supplemented on either 9.7 mmol (601 mg) of nitrate-rich beetroot juice or nitrate-depleted placebo for a period of 7 d, after which they returned to the lab for testing and subsequent 7 d washout. Testing included voluntary and involuntary contractions as well as measurements of force production. The nitrate group significantly improved maximal twitch, submaximal contractions, and demonstrated a significant 3 to 15% increase on explosive force production evoked by electric stimulation. No change was seen in voluntary force production. The authors speculate enhanced excitation-contraction coupling efficiency may have been the underlying mechanism to increased submaximal voluntary force and increased maximal involuntary force.

With regards to VO_2, the nitrate group yielded lower VO_2 at the peak (Nitrate: 362 ± 30 mL/min, Placebo: 484 ± 41 mL/min) and end points (Nitrate: 778 ± 38 mL/min,

Placebo: 870 ± 42 mL/min) of low-intensity exercise, consistent with previous findings in their lab. During high-intensity exercise, nitrate supplementation revealed lower VO_2 at 360 sec of exercise (1460 ± 54 mL/min, Placebo: 1692 ± 70 mL/min) as well as the slow component following termination of the test (100 ± 26 mL/min, Placebo: 209 ± 30 mL/min) which is indicative of faster recovery. In addition, degradation of phosphocreatine (PCr) during low-intensity exercise was blunted in the nitrates group (5.2 ± 0.8 $mmol_{PCr}$, Placebo: 8.1 ± 1.2 $mmol_{PCr}$) indicating either increased re-synthesis of PCr or reduced energy cost. In addition, the nitrate group had lower increases in inorganic phosphate (3.5 ± 0.8 $mmol_{Pi}$ against 4.4 ± 0.8 $mmol_{Pi}$ for placebo). Free ADP was also decreased in the nitrates group at all time points, indicating increased ATP production from ADP + P_i. Relative to ATP production, the nitrate group had significantly lower total ATP ($p < 0.05$), ATP from oxidative pathways ($p < 0.05$), and ATP from phosphagen system ($p < 0.05$); however, there was no difference in ATP from glycolytic pathways. These data evidence an increased exercise tolerance from the nitrate group. Coupled with the decreased ATP and VO_2, it is a logical leap to assume nitrates reduced the energy requirements of the testing.

Fulford, et al. [37] examined the effect of nitrate supplementation on skeletal muscle metabolism as well as force production. Despite findings showing decreased ATP turnover rate and reduced VO_2 cost, there have been studies showing an ergolytic effect of nitrate supplementation on force production [107, 108].

Table 2.2: Nitrates

Author (year)	Study Design	Sample	Dosing Protocol	Assessment Protocol	Significant Findings
Haider & Folland (2014) [104]	P, R, DB, CO	n = 19 untrained males (28 ± 7 y)	9.7 mmol/d (601 mg/d) NO_3 for 7 d with 7 d w/o	MVC, electrically stimulated force production	Increased force production and twitch speed in nitrate group, lower VO_2
Bailey, et al. (2010) [106]	P, R, DB, CO	n = 7 recreationally active males (28 ± 7 y)	10.2 mmol/d (632 mg/d) NO_3 for 15 d, 10 d w/o	MVC, incremental exercise test, EMG, VO_2	Lower VO_2 at peak and increased metabolic efficiency from oxidative pathways in NO_3 group
Fulford, et al. (2013) [37]	P, R, DB, CO	n = 8 recreationally-active males (24 ± 4 y)	10.2 mmol/d (632 mg/d) NO_3 for 15 d, 14 d w/o	MVC	No difference in MVC
Bailey, et al. (2015) [109]	P, R, DB, CO	n = 7 recreationally-active males (21 ± 2 y)	6.2 mmol/d (384 mg/d) NO_3 for 9 d, 10 d w/o	TTE on step test, VO_2	Lower VO_2 at high intensity and decreased MAP in NO_3 as well as increased oxygenation of myoglobin
Thompson, et al. (2016) [110]	P, R, DB, CO	n = 16 recreationally-active males (24 ± 4 y)	5 mmol (310 mg) NO_3 acute dose, 7 d w/o	Incremental CE, cognitive testing	Increased TTE, decreased cerebral blood flow, increased muscle blood flow, decreased VO_2 at lower intensities, and increased RER in NO_3
Porcelli, et al. (2016) [111]	P, R, DB, CO	n = 7 recreationally-active males (25 ± 2 y)	8.2 mmol/d (508 mg/d) or 2 mmol/d (124 mg/d) NO_3 for 6 d, 20 d w/o	Incremental CE, MVC, RtF, Wingate	Decreased VO_2 during CE, increased RtF, improved maintenance of power during Wingate for NO_3
Thompson, et al. (2015) [112]	P, R, DB, CO	n = 18 recreationally-active males (27 ± 8 y) and females (n = 18, 23 ± 4 y)	6.5 mmol/d (403 mg/d) NO_3 for 5 d, 7 d w/o	Incremental ST and CE, Wingate	Increased TTE and work rate in NO_3 as well as lower VO_2 and lactate at exhaustion with higher pH
Thompson, et al. (2016) [23]	P, R, DB, CO	n = 35 male athletes (	6.4 mmol/d (397 mg/d) NO_3 for 5 d, 7 d w/o	20 m sprints, Yo-Yo shuttle	Decreased sprint times and increased distance in YoYo in NO_3
Callahan, et al. (2016) [113]	P, R, DB, CO	N = 8 trained male cyclists (34 ± 7 y)	5 mmol (310 mg) NO_3	Incremental CE, 4 min TT, 4 km TT	NO_3 and $NaNO_3$ demonstrated increased lactate at exhaustion

P = Placebo controlled, R = Randomized, DB = Double-blind, CO = Crossover, w/o = washout, VO_2 = volume of oxygen consumption, MVC = maximal voluntary contraction, TT = time trial, TTE = time to exhaustion, CE = cycle ergometer, ST = step test

Eight recreationally active males (24 ± 4 y, 76 ± 8 kg) were recruited. Following familiarization, they were divided into either nitrate-rich or nitrate-depleted beetroot juice supplement groups separated by a 14 d washout. They were instructed to drink 0.5 L daily and on testing days to ingest the supplement 2.5 h prior. For each of the six visits, a blood sample was collected at the onset of testing for nitrite analysis. PCr kinetics were analyzed using short bouts of high-intensity exercise and, after a short recovery, repeated measures of MVC were completed. The protocols for PCr and MVC were similar to the incremental knee extension test used previously in this lab [106]. No side effects were reported. The nitrate group showed increased levels of plasma nitrate compared to placebo ($p < 0.01$). but no difference at any time point in force production between nitrates and placebo. The authors noted a trend towards PCr depletion in the nitrate group and acknowledge a larger sample (n = 18) would likely yield significance.

Continuing to investigate the influence of nitrate supplementation on bioenergetics, specifically the role of nitrates in glucose homeostasis [35], Shepherd, et al. [114] analyzed hepatic blood flow and glucose homeostasis. In this study, 31 participants were recruited and divided into a young (11 males, 5 females, 26.6 ± 6 y, 76.2 ± 13 kg, 175 ± 1 cm) and an old group (8 males, 7 females, 59.2 ± 6 y, 75.3 ± 12 kg, 169 ± 1 cm). Following familiarization, participants were randomly assigned to a nitrate-rich beetroot group with 11.91 mmol (738 mg) of nitrate or a nitrate-depleted beetroot group containing 0.01 mmol of nitrate (6.2 mg) with a 7 d washout between

treatments. Supplements were provided with a meal totaling 76 g of carbohydrate. Following ingestion, blood collection, and blood pressure analysis, the participants were positioned in an MRI to evaluate hepatic blood flow. Blood was analyzed for glucose, glucagon-like peptide-1 (GLP-1) and C-peptide. As expected, plasma nitrate and nitrite were higher in the nitrate group ($p < 0.001$). With regards to hepatic blood flow, the older participants yielded a significantly higher flux and velocity at baseline compared to the younger participants; the differences remained throughout the study. Relative to glucose, the older individuals again yielded higher concentrations at baseline with no treatment or time effect from supplementation. There were also no differences reported in GLP-1, C-peptide, or resting blood pressure. The results indicate no effect of dietary nitrate supplementation on glucose homeostasis which further strengthens the findings of Bailey, et al. [106].

The role of nitrates in various muscle fiber types has been mentioned and the underlying mechanisms were further elucidated by Jones, et al. [115]. In this systematic literature review, it was hypothesized that the ergogenic role of nitrates is more pronounced in the less oxidative, high glycolytic, fast twitch Type II fibers. The authors speculate nitrates to locally alter PO_2 in working muscle tissue. Analyzing PO_2 provides a clearer picture of the physiological effects of nitrates. Normally, PO_2 is around 40 mmHg at rest and can be as low as 2 mmHg during intense exercise. Jones, et al. sought to investigate whether the perfusion would be altered in varying fiber types with nitrate supplementation. This aim was based on previous studies in rats

where nitrate supplementation increased blood flow up to 66% in Type II fibers with minimal change in Type I fibers [116-118]. Furthermore, nitrate supplementation has been shown to decrease the O2 cost of exercise by an average of 5.5% [119, 120]. The authors also state much of the ergogenic effect of nitrate supplementation is found in low- to moderately trained individuals with little to no effect in elite athletes. Given that the elite athletes in reviewed studies are primarily endurance trained athletes, they should have a lower proportion of Type II fibers, providing circumstantial evidence that Type II fibers are the primary target of nitrates. De Smet, et al. [121] found the addition of sprint training and hypoxia with ingestion of nitrates (6.45 mmol, 400 mg) produced a favorable shift towards increased Type IIa muscle fibers.

The studies reviewed thus far evidence a robust role of nitrate within the body ranging from endothelial vasodilation to increased efficiency of muscular contraction TABLE 2.2. Bailey, et al. [109] investigated the effect of inorganic nitrate on muscle oxygenation and O2 kinetics at varying intensities. Seven recreationally active healthy male subjects (21 ± 2 y, 86 ± 10 kg, 182 ± 8 cm) were recruited. Over the course of five to seven weeks, participants completed ten visits. During the first two baseline sessions, participants completed a cadence-specific incremental cycle test at 35 and 115 rpm. They were then divided into a beetroot juice (6.2 mmol, 384 mg NO_3) or placebo group separated by a 10 d washout. For days 2,3, 6, and 7, participants completed either the 35 rpm or 115 rpm test wherein they pedaled to exhaustion with exhaustion being defined as dropping >10 rpm under the specified range for > 3 sec.

On days 4, 5, 8, and 9, participants completed a step test using the same cadence as the cycle ergometer. Pulmonary gasses were measured throughout each test and oxygenation was assessed using a near-infrared spectroscopy system applied to the vastus lateralis of the right leg.

Plasma nitrite, SBP, and mean arterial pressure (MAP) were all significantly different in the nitrate group ($p < 0.05$) and there was no difference in DBP. Systolic blood pressure decreased by 8 mmHg and MAP decreased by 4 mmHg in the nitrate group; however, these values were still within normal limits. No difference was noted in oxygen kinetics for any time point during the 35 rpm conditions. In the 115 rpm, the nitrate group yielded a significantly lower resting VO_2 (1.89 ± 0.25 L/min compared to 1.93 ± 0.34 L/min, $p < 0.05$), 120 sec VO_2 (3.68 ± 0.47 L/min compared to 3.65 ± 0.49 L/min, $p < 0.05$), and higher VO_2 at exhaustion (4.32 ± 0.75 L/min compared to 4.24 ± 0.61 L/min, $p < 0.05$). In addition, the nitrate group evidenced an increase in oxygenated myoglobin at 115 rpm at baseline (3.4 ± 4.9 AU compared to -1.1 ± 3.5 AU), 120 sec (-1.0 ± 6.3-AU compared to -6.1 ± 3.7 AU for placebo), and at exhaustion (-0.6 ± 7.8 AU compared to -6.0 ± 5.4 AU for placebo). The authors concluded the reduction of NO_3 to NO to be augmented as pH decreases with strenuous exercise which pulls the reaction towards NO. In addition, there was an increase in proteins associated with mitochondrial [108] and calcium function [122].

In a study by Thompson, et al., [110] the influence of nitrate supplementation on incremental cycle exercise performance as well as cognition were investigated using

a placebo-controlled, double-blind, cross-over study. Sixteen recreationally active healthy males (24 ± 4 y, 75.6 ± 9.2 kg, 177 ± 7 cm) were recruited and, upon completing familiarization, participants were assigned to either a nitrate group (5 mmol or 310 mg from beetroot juice) or a 450 mL placebo developed to match in appearance and flavor. Treatments were separated by a 7 d washout. Prior to exercise testing, muscle/cerebral oxygenation, blood pressure, blood lactate, and pulmonary gas were assessed. Participants completed a cycle ergometer test separated into 20 min increments beginning at 50% VO_{2peak} followed by 70% and 90% $VO_{2peak.}$ Pulmonary gasses and EMG readings were assessed throughout testing as was HR.

In addition, blood lactate was assessed via finger stick at regular intervals. Cognition was assessed using the Computerized Mental Performance Assessment System (COMPASS) at baseline, prior to exercise, during the 50% VO_{2peak} portion, during the 70% VO_{2peak} portion, and 6 min after the exercise test. Finally, RPE were assessed during the exercise protocol.

The nitrate group again yielded higher plasma nitrite compared to placebo ($p < 0.001$) and no differences were reported for SBP or DBP. The change in cerebral blood flow was blunted in the nitrate group ($p < 0.05$) prior to exercise and the effect remained up to the 90% VO_{2peak} portion of the cycle test. Following the exercise test, cerebral blood flow was restored faster in the nitrate group ($p < 0.05$). The change in muscle blood flow was also blunted in the nitrate group ($p = 0.038$) and muscle blood flow increased to a higher degree during exercise in the nitrate group ($p = 0.001$).

VO_2 was reduced in the nitrate group; however, this effect was only seen during the 50% VO_{2peak} portion. Additionally, respiratory exchange ratio was found to be significantly higher in the nitrate group ($p < 0.001$). There was no treatment effect for EMG, HR, cognitive metrics, or blood lactate. Finally, the nitrate group lasted 16% longer during the 90% VO_{2peak} stage to exhaustion while VO_2 at exhaustion remained similar between the two groups.

Porcelli, et al. [111] studied nitrate-heavy diets in seven recreationally active males (25 ± 2 y, 66.3 ± 6.0 kg, 174 ± 5 cm). After informed consent, baseline data was determined using an incremental cycle ergometer test until volitional fatigue with peak oxygen consumption and work rate being recorded. Participants supplied the researchers with a seven-day food log which a nutritionist then used to create standardized diets based on individual normal intake and activity level, with the high-nitrate group having 8.2 mmol/d (508 mg/d) nitrates and the control diet having approximately 2 mmol/d (124 mg/d) nitrates, all from plant sources. This diet was maintained for six days, performance analyzed, and switched to the other diet after a 20 d washout period where the measures were tested once more. The exercise tests conducted in this study included moderate intensity constant-work rate cycling at a frequency of 80 ± 5 rpm and a work rate of 50% peak work rate.

Also, isometric knee extensions were employed to determine MVC as well as submaximal knee extension to volitional fatigue. Finally, repeated sprints were conducted using a Wingate protocol repeated for six bouts at 0.74 N/kg with 24 sec

rests between bouts. VO_2 and VCO_2 were analyzed throughout testing, as were force, EMG, and blood samples to assess for nitrate/nitrite.

The authors did not include *p*-values; however, the authors noted statistically significant difference in nitrate or nitrite concentration between groups at baseline and a significant increase in both plasma nitrate and nitrite following a high nitrate diet. The high nitrate diet yielded lower VO_2 and V_E values during the moderate intensity cycle test with no difference in HR or blood lactate. The nitrate group saw no difference in MVC on the knee extension portion although the high nitrate group did see a significant increase in the number of repetitions prior to fatigue. Additionally, while there was no difference on the repeated sprint bouts for the first or second bout, the high nitrate group yielded significantly higher power output in the third, fourth, and fifth bouts, indicating a role of nitrates in maintaining exercise intensity in line with current research.

Thompson, et al. [112] also investigated the effect of nitrate supplementation on sprint performance. Using a double-blind, randomized crossover design, the authors recruited 16 male athletes. The protocol involved 7 d of supplementation and 7 d washout using either nitrate-rich (12.8 mmol, 794 mg) or nitrate-depleted beet root juice (0.08 mmol, 4.96 mg) mixed in 140 mL of juice. On the last day of the supplementation period, the participants completed a test wherein 20 sprints were done over the course of 40 minutes. Each sprint consisted of a 6 sec sprint, 100 sec active recovery, and 20 sec rest on a cycle ergometer. The 40 min test was completed

twice, with the second time including cognitive testing during exercise. The nitrate-rich group yielded significantly increased total work and higher cognitive function as the exhaustive exercise continued.

The majority of the studies thus far have involved incremental or steady state exercise to exhaustion. Thompson, et al. [112] investigated the role of nitrates in interval training. Eighteen recreationally active young adults (Males n=18, 27± 8 y, 80 ± 13 kg, 179 ± 8 cm; Females n=18, 23 ± 4 y, 65 ± 9 kg, 166 ± 5 cm) were recruited for this investigation. During familiarization, participants completed an incremental treadmill protocol to determine VO_{2peak} and work rates were determined for moderate and severe exercise. Participants were randomly assigned to either nitrate-rich beetroot juice (6.5 mmol, 403 mg) or nitrate-depleted beetroot juice (0.04 mmol, 2.48 mg). Participants then underwent a 4 week standardized exercise and supplementation period followed by follow-up testing before being moved to the other treatment in a crossover manner after a 7 d washout. Blood pressure and HR were assessed at the beginning, middle, and ending of each session followed by calculation of MAP. Blood was collected at baseline, 1 min into exercise, 3 min into exercise, and following exhaustion. Muscle biopsies were collected to analyze muscle metabolites, muscle glycogen, pH, and fiber types prior to testing. In addition, oxygen uptake was measured throughout the testing using indirect calorimetry. Exercise testing included step tests and incremental cycle tests similar to prior research from this lab [123] and the standardized training involved repeated Wingate tests against a resistance of 7.5%

body mass (in kg). Wingate bouts were separated by 4 min rest periods and were done four times, three times a week during the first two weeks and five bouts, four days per week during the final two weeks.

Plasma nitrites were elevated in the nitrate group at all time points. Blood pressure decreased in the nitrate group by 6 ± 4 mmHg by the fourth week while MAP was reduced by 3 ± 5 mmHg. Work rate on the incremental test was significantly higher for the nitrate group ($p < 0.001$) following four weeks of treatment. Consistent with other studies under review, VO_2 at exhaustion was lower in the nitrate group ($p < 0.05$). Time to failure was higher in the nitrate group (170 ± 90 sec) compared to placebo (163 ± 144 sec, $p < 0.05$). The trained nitrate group also yielded lower blood lactate than placebo or sedentary supplemented group at both 1 min and 3 min ($p < 0.05$). There were no changes in ATP, pH, or PCr at baseline; however, the trained nitrate group yielded lower blood/muscle lactate and higher pH at 3 min of severe exercise ($p < 0.05$). The trained nitrate group also showed evidence of a decrease of Type IIx fibers and increase in Type IIa fibers, indicating a potential effect of nitrates in training adaptations. The results of this study showed that nitrate supplementation in conjunction with sprint training, but not nitrate alone, caused favorable changes to pH, fiber types, and performance. Nitrate supplementation also caused lower VO_2 at exhaustion in addition to increased work rate.

With focus on time trial performance, Callahan, et al. [124] recruited eight trained male cyclists (34 ± 7 y, 73.8 ± 10.1 kg) were enrolled in a placebo-controlled,

double-blind, randomized, crossover study. In this study, participants were randomized into four groups: nitrate in the form of beet root crystals (5 mmol, 310 mg), nitrate + sodium bicarbonate, sodium bicarbonate, and placebo. Diets were standardized throughout the study in pre-packaged meals and participants were instructed to fast overnight prior to testing sessions. During familiarization, participants completed an incremental cycling protocol beginning at 150 W and increasing 50 W in 5 min intervals until peak power was achieved. In addition, blood samples were taken via the ear lobe during the final 30 seconds of each increment and exercise was terminated when lactate concentration eclipsed 7 mmol/L, indicating the onset of blood lactate accumulation had been passed. Following the incremental test, participants completed a 10 min steady state cycle test at 150 W followed by a 10 min seated rest. Once blood lactate had returned to less than 2.5 mmol/L, participants underwent a 4 km time trial to determine VO_{2peak}. Upon completion of the time trial, blood lactate was again assessed. Following that test, a 4 km time trial was completed. For the entirety of the familiarization testing, participants were analyzed for pulmonary gas exchange.

At 72 h pre-baseline testing, participants presented to the lab for baseline blood draw. At baseline testing, participants ingested the supplement as well as a standardized meal containing 2 g/kg carbohydrate as well as completing a gastrointestinal distress questionnaire. This questionnaire was completed 65 min prior, 5 min prior, 6 min post, and 81 min post-time trial. Sixty minutes post-ingestion, participants began to warm-up on the cycle at 50% (8 min), 70% (5 min), 85% (3 min),

and 100% (3 min) lactate threshold determined from familiarization. Following this test, participants were given a sports gel containing 27 g carbohydrate followed by the 4 km time trial. Blood was collected at 72 h pre-testing and five times during the testing day to analyze for glucose, lactate, and nitrate/nitrite concentration.

There was no effect of treatment order on any metric. The nitrate containing groups (beetroot + bicarbonate, beetroot + placebo) yielded higher nitrate and nitrite concentrations compared to bicarbonate + placebo and placebo alone ($p < 0.01$). The beetroot + bicarbonate and bicarbonate + placebo groups yielded higher blood lactate following the time trial compared to beetroot + placebo and placebo ($p < 0.01$). No significance was found in the responses to the GI questionnaire.

To assess the effects of acute and chronic supplementation of nitrates, Jo, et al. [125] employed a randomized, double blind, placebo-controlled study assessing the effect of nitrates on time trial performance. Participants (n = 29, 15 M, 14 F,) were assigned to either a multi-day nitrate supplementation group or a single pre-exercise dose group. Both groups completed 14 d of supplementation with either a nitrate (MD, 8 mmol NO_3, 500 mg) or placebo (SD) with both consuming nitrates on Day 15 at 180 min prior to exercise testing. The exercise testing days consisted of collection of anthropometric and hemodynamic data, infrared muscle oxygenation and the 8 km time trial. Resistance was not specified. The MD group demonstrated a significant decrease of 5.6% in time to completion ($p = 0.01$) as well as a significant increase of

4.2% in average power (p = 0.04); no acute effect was noted in SD. No differences were noted in hemodynamic responses or muscle oxygenation.

Thompson, et al. [110] opted to analyze nitrates in a pure sprint protocol. Thirty-six male athletes (24 ± 4 y, 80 ± 10 kg, 180 ± 7 cm) involved in team sports such as football, rugby, and hockey were introduced to a sprint based study protocol with the aim of demonstrating a positive benefit in both sprint performance and cognition. After a familiarization session, participants were randomized to receive either nitrate-enriched beetroot juice (6.4 mmol, 397 mg) or nitrate-depleted beetroot juice. Participants ingested the supplement for five consecutive days, followed by the first testing session, 7 d washout, and alteration to the other treatment in a crossover manner. Dietary food logs were maintained throughout the study. During the testing protocol, participants underwent five 20 m sprints as a warm-up, followed by the YoYo Intermittent Recovery Level 1 test. In this test, participants would sprint 20 m, immediately turn around, and sprint back to the start. These 40 m sprints were interspersed by 10 second rest periods and increased in speed until time ceased to decrease. Following the Yo-Yo test, participants took the Stroop Color-Word test for cognition and the study session was complete. In addition to the performance and cognitive tests, blood chemistry and blood pressure were also assessed.

Plasma nitrate increased eight-fold from baseline to testing in the nitrate group compared to no change in placebo (p < 0.01). The nitrate group also boasted a reduction in SBP (117 ± 7 mmHg against 119 ± 8 mmHg, p < 0.05) as well as a trend in

MAP (79 ± 15 mmHg compared to 81 ± 15 mmHg, $p = 0.08$). Despite significance or trend toward significance, these values were still well within normal limits. There also was a decrease in 20 m sprint time of 1.2% in the nitrate group ($p < 0.05$) as well as a 2.3% and 1.6% decrease in sprint time in the 5- and 10 m sprints, respectively. In addition, the results of the Yo-Yo test evidence an increase in distance covered of 3.9% for the nitrate group ($p < 0.05$). Finally, cognition was enhanced in the nitrate group on the Stroop Color-Word test ($p < 0.05$).

In a study by Kramer, et al. [126], twelve male cross-training athletes (23 ± 5 y) were recruited to test the effects of nitrate supplementation on high intensity interval training. After a familiarization period where VO_{2peak} was measured on a graded treadmill test, participants partook in two consecutive study days followed by six days of sodium nitrate (8 mmol NO_3 or 496 mg potassium nitrate) or nitrate-free potassium chloride placebo supplementation, and follow-up testing using the baseline protocol. Supplements were pre-packaged as unmarked gel capsules by a third party and consumed in the morning and in the evening. After a 10 d washout, the protocol was repeated using the alternative treatment in a crossover design. Testing Day 1 included isokinetic testing of the quadriceps and hamstrings, a Wingate cycle ergometer test, and a time trial on a cycle ergometer. Testing Day 2 included a specific cross-training workout wherein time to completion was measured.

There was no difference in quadriceps or hamstring isokinetic force. There was a significant increase in peak power on the Wingate protocol for the nitrate group (p =

0.01). Time to completion for the workout was not different between groups. In addition, supplementation yielded complaints of nausea and gastrointestinal discomfort in both the nitrate group (n=5) and placebo group (n=3), indicating something other than the nitrates may have had an effect.

Similar to Kramer, et al., Nyakayiru, et al. [25] investigated the effects of nitrate supplementation on high-intensity interval training (HIIT). This study involved 32 amateur league soccer players (23 ± 1 y, 77 ± 1 kg, 181 ± 1 cm) in a double blind, randomized, placebo-controlled, crossover design investigating the effects of short-term (6 d) use of nitrates on high intensity interval training. At baseline, participants completed the Yo-Yo intermittent exercise test, followed by random allotment of either nitrate-rich beetroot juice (12.9 mmol NO_3, 800 mg) or depleted beetroot juice. Participants were instructed to consume the assigned treatment at approximately the same time daily and at 3 h pre-exercise at follow-up testing.

Short-term treatment resulted in a significant elevation in plasma nitrate and nitrite at all time points in the nitrate group. Performance on the Yo-Yo was significantly improved in the nitrate group compared to PLA (p = 0.027), demonstrating an average increase in distance completed of 3.4 ± 1.3%. Additionally, HR was significantly lower in the nitrate group compared to PLA during the Yo-Yo test (p = 0.014). Finally, there were no differences in side effects reported or RPE.

There is some debate regarding differing effects related to training status [127-131]. Muggeridge, et al. [26] conducted a double-blind, placebo-controlled,

randomized study analyzing the effects of nitrates on adaptation to sprint training in untrained males. In this study, untrained males (n = 27, 28 ± 7 y, 82.3 ± 17.1 kg, 177 ± 5 cm). At familiarization, baseline measurements on an incremental exercise test on a cycle ergometer were determined as VO_{2max}, ventilatory threshold, and maximal work rate. The cycle test began at 50 W for 1 min and increased by 15 W/min until volitional fatigue. Participants were then randomly assigned to an exercise condition (normal activity, sprint interval training) and a treatment condition (no treatment, placebo, nitrates). The nitrate group received 8 mmol/d NO_3 (496 mg/d). Sprint interval training (SIT) took place on a cycle ergometer over 9 training sessions across 3 weeks of supplementation prior to follow-up testing.

The PLA + SIT and NIT + SIT yielded significant ergogenic effects compared to the control group; however, there were no differences between PLA and NIT in regards to VO_{2max}. Analysis of 95% confidence intervals showed NIT to significantly improve VO_{2max}, while both PLA and NIT significantly improved ventilator threshold and maximal work rate. Interestingly, PLA demonstrated increased power in sprint bouts 2 through 9 compared sprint bout 1 whereas NIT demonstrated no difference in power in any sprint bout.

A review by Lara, et al. [132] analyzed studies involving humans over the age of 18 with or without any co-morbidities to assess the difference, if any, of organic and inorganic nitrates. This review specifically analyzed studies utilizing flow-mediated dilation, pulse wave velocity, and augmentation index to assess blood flow. The review

began with 9,684 articles and was eventually narrowed down to 12 articles that fit the criteria. Of these 12 studies, five assessed young and healthy participants, two assessed participants with hypertension, and one assessed patients with Type 2 diabetes. Beetroot juice (organic nitrate, 1.1 to 22 mmol/dose or 6.82 to 1364 mg/dose) was used in nine of the studies whereas inorganic nitrate salts were used in four (8.0 to 24 mmol/dose or 496 to 1488 mg/dose). Lara, et al. concluded inorganic and organic nitrates to not be different with regards to efficacy (p = 0.001).

In a balanced crossover study by Wylie, et al. [133], the dose response of nitrates were examined. The study involved 10 healthy men whom received either 4.2 mmol (260 mg), 8.4 mmol (521 mg), or 16.8 mmol (1042 mg) of concentrated beetroot juice or nitrate-depleted beetroot juice as a placebo. The protocol consisted of seven visits over a 4 to 5 week period (separated by 3 d w/o) where acute supplementation of the randomly assigned treatment was analyzed relative to hemodynamics, blood chemistries, oxygen kinetics and work done during the cycle ergometer testing. Participants arrived, provided fasting blood and ingested the treatment, followed by a 2.5 h wait and a second blood draw. After the blood draw, participants completed a graded exercise test on the ergometer, beginning at 20 W and increasing to severe intensity.

Plasma NO_3 was significantly higher in the NO_3 groups, with the 16.8 mmol group being significantly higher than 4.2 and 8.4 and no difference between 4.2 and 8.4. The 4.2 group evidenced an approximately four-fold increase in plasma nitrate, 8.4

was eight-fold, and 16.8 was twenty-fold. In addition, SBP and DBP decreased relative to the dose, with 16.8 showing a significant decrease at all time points, 8.4 showing an initial significant decrease and returning closer to baseline, and 4.2 showing no difference. MAP was significantly decreased in the 8.4 and 16.8 mmol groups but was unchanged in the 4.2 mmol group.

Regarding performance, the 16.8 mmol group significantly decreased end-exercise VO_2 during moderate-intensity exercise by 3%, 8.4 mmol decreased by 2% which was not significant but did evidence a trend, and 4.2 was not significant. No significant differences were noted in response to severe-intensity exercise; however, the time to exhaustion in the final test was significantly greater in the 8.4 and 16.8 mmol groups with no difference in the 4.2 mmol group.

Based on the studies reviewed, nitrates appear to be effective in doses as low as 5 mmol (310 mg) [35, 110, 124, 133]. The pattern noted within the studies reviewed illustrates a decrease in VO_2 at low- and moderate exercise intensities compared to placebo, increased plasma nitrite and nitrate compared to placebo, increased time to exhaustion compared to placebo, and some benefit to cognition. The current work utilized a dose of 5.7 mmol (353 mg) of inorganic nitrate; given the literature reviewed, a significant ergogenic effect in RtF should occur but the literature appears to be inconclusive regarding the severe-intensity 4 km time trial at the present dose.

2.3.3. L-Arginine

As noted previously, L-arginine enhances nitric oxide via enzymatic conversion using nitric oxide synthase (NOS), oxygen, and L-citrulline. Nitric oxide is accepted to promote vasodilation and thus believed to increase nutrient delivery and metabolite removal to and from working tissue [134, 135] which has been associated with recovery following stress [136]. L-arginine is created in the liver as part of the urea cycle and is a conditionally essential amino acid. Following injury and during periods of growth, L-arginine requirements may increase to aid in repair [135, 137]. A review by Bescos, et al. [138] provided an overview of nitric-oxide enhancing supplements in relation to human performance. L-arginine is typically taken in daily doses of 4 to 5 g and can be found in high amounts in seafood, watermelons, and rice or soy protein. The action of L-arginine in endothelial vasodilation appears to be dependent on the presence of oxygen as well as NADP. Bescos, et al. [139] also reports infusion of L-arginine can have a beneficial effect to growth hormone. A summary of the studies reviewed may be found in TABLE 2.3.

Evans, et al. [140] sought to further elucidate the biochemical response(s) of arginine in healthy humans. Twelve healthy untrained individuals (Males n=5, Females n=7, demographics were not clearly defined) were recruited for this dose response study to analyze the biochemical changes in response to 1 g (3 g/d), 3 g (9 g/d), 7 g (21 g/d), and 10 g (30 g/d) of L-arginine taken as a powder mixed with juice three times daily. Doses increased weekly from 1 g during the first week to 10 g for the fourth

week. At the end of each week, participants returned to the lab for analysis and to receive more supplement. Urinalysis, blood chemistry analysis, and blood pressure were analyzed weekly.

At baseline, L-arginine concentration was between 80 and 125 mmol/L, within normal limits. Side effects were reported in the 21 g/d (n = 4) and 30 g/d groups (n = 10). There were no significant differences reported in blood pressure for any dose. There was significance with regards to plasma concentration of L-arginine, glycine, and L-ornithine. Glycine was found to be significantly lower after 3 weeks (p = 0.010) whereas L-ornithine increased by the third week (p = 0.001). The increase in L-arginine plateaued at 9 g/d, indicating a physiological ceiling effect for L-arginine concentration. Finally, at 3 g/d, insulin increased from 137 ± 33 mmol/L at baseline to 176 ± 44 mmol/L and then dropped to 127 ± 32 mmol/L for the remaining weeks. This change, while not significant, showed a trend (p = 0.09); there was no change in blood glucose. Of note, demographics of the participants were not defined in this study, only that they were healthy; therefore, these data can only be used to give a general idea of dosing and biochemical/hormonal response.

Forbes & Bell [141] analyzed fourteen recreationally active males (25 ± 5 y, 78.0 ± 8.5 kg, 179.5 ± 4.7 cm) for the response of nitrate/nitrite, growth hormone (GH), insulin-like growth factor 1 (IGF-1), and insulin. Prior to the first testing session, participants provided a 1 d food recall to adjust for macronutrient intake; total calories and diets were altered to maintain protein at 0.8 g/kg/d. Participants were assigned to

either a flour placebo group, 0.075 g/kg L-arginine (μ = 5.85 g) , or 0.15 g/kg L-arginine (μ = 11.7 g) in a randomized, double-blind manner. For each of the three study days, randomly assigned in a crossover fashion, participants ingested the supplement and rested for a period of 2 h with blood draws at regular intervals. For each testing session, blood was collected at baseline and in 30 min increments following ingestion.

The 10 g dose was accompanied by complaints of gastrointestinal distress (n = 2). There was not a difference in plasma L-arginine between the low- and high dose although both treatments yielded greater concentrations of L-arginine compared to placebo ($p < 0.05$). While there were no differences reported in the GH or IGF-1 markers, the authors did note a difference in insulin concentration between 30, 90, and 180 min ($p < 0.05$) as well as an increase in nitric oxide that was significantly higher than placebo ($p < 0.05$) with no difference between low- and high-dose at 1 h with no significant differences after 1 h. The implications of this study evidenced no difference between an average dose of 5.85 and 11.7 g of L-arginine in terms of biochemistry; however, complaints of side effects were not present at the lower dose [140].

Forbes, et al. [142] found similar results in so far as L-arginine at a dose of 5.8 g yielded higher plasma L-arginine compared to placebo with no change in GH, lactate, glucose, VO_2, VCO_2, RER, or nitrate/nitrite concentrations. This study involved fifteen aerobically trained men (28 ± 5 y, 77.4 ± 9.5 kg, 180.9 ± 7.9 cm) with an average VO_{2max} of 59.6 ± 5.9 mL/kg/min. In addition to blood chemistry and physiological measures of health, this study also utilized submaximal exercise at 80% of the power output

associated with individual ventilatory threshold for a duration of 60 min. There were no differences seen in performance.

Forbes, et al. [143] analyzed fourteen strength trained males (25 ± 4 y, 81.4 ± 9.0 kg, 179.4 ± 6.9 cm) with an average training experience of 6.3 ± 3.4 y. Following baseline testing, participants were randomly assigned to either a dose of 0.075 g/kg (μ = 6.105 g) or flour placebo packaged in a gel capsule. This double-blind, randomized, crossover study utilized 1-RM protocols on bench press and leg press as well as multiple repetition maximum on pulldowns, leg extension/flexion, shoulder press, elbow extension/flexion, and calf raises. In addition to strength testing, blood was collected to analyze for L-arginine concentration, GH, ghrelin, and IGF-1. Finally, HR and RPE were assessed before and after testing.

Concentrations of L-arginine were elevated in the arginine group by 120% compared to placebo ($p < 0.05$). There were significant differences in GH, growth hormone releasing hormone (GHRH), ghrelin, and IGF-1. Immediately following exercise, GH was enhanced in the placebo group immediately following exercise and significance was maintained for 30 min post exercise. GHRH was also elevated in the placebo group immediately following exercise and followed the same trend of maintaining significance for 30 min post-exercise. Ghrelin and IGF-1 were significantly higher in the placebo group at the onset of exercise. Despite the decreased GH and GHRH in the L-arginine group, growth hormone inhibiting protein (GHIH) remained the same between groups. There were no differences related to strength, HR, or RPE at

any time point. These data are in opposition to prior claims of a beneficial effect of L-arginine to anabolic signaling via GH and IGF-1.

In a study by Alvares, et al., [144] fifteen trained runners (Males n=11, Females n=4) were divided into an arginine (36.8 ± 7.1 y, 66.7 ± 14.4 kg, 172.3 ± 10.6 cm) and placebo group (30.6 ± 9.5 y, 68.1 ± 10.6 kg, 175.0 ± 7.5 cm). Following familiarization, participants presented for baseline testing followed by 4 weeks of L-arginine (6 g/d) or cornstarch placebo supplementation and follow-up testing. During testing sessions, thirty minutes post-ingestion, participants underwent two 5 km time trials separated by a 10 min rest. Blood was collected prior to, between, and after the time trials. Blood was analyzed for nitrate/nitrite, L-arginine, L-citrulline, L-ornithine, and hormonal response. Finally, participants were instructed to avoid foods high in nitrate and nitrite on the day prior to the testing session. As expected, plasma L-arginine concentration increased significantly and remained significantly elevated even at 20 min post exercise. No differences were noted regarding nitrate/nitrite, L-citrulline, L-ornithine, GH, lactate, or cortisol. In regard to ergogenic potential, there were no differences in time trial performance between groups. Further work from this lab evidenced L-arginine at 6 g/d to have a beneficial effect on muscle blood volume measured by near-infrared spectroscopy; however, there were no increases in performance compared to placebo [145].

Da Silva et al. [146] developed a study targeting insulin and GH as primary outcomes. The study involved fifteen aerobically trained individuals (Males n=11,

Females n=4) whom were randomly assigned to either an L-arginine (6 g, 36.8 ± 7.1 y, 66.7 ± 14.4 kg, 172.3 ± 10.6 cm) or cornstarch placebo group (30.6 ± 9.5 y, 68.1 ± 10.6 kg, 175.0 ± 7.5 cm). Following baseline fasting blood draws, participants ingested their assigned treatment, waited for 30 min and then underwent two 5 km time trials. Blood samples were analyzed for insulin, GH, IGF-1, and cortisol. Despite the typical increase in GH and IGF-1 associated with exercise, there were no significant differences in hormonal profiles between groups. In addition, there was no significant difference in time trial performance between-groups.

Chen, et al. [147] investigated a population of Tae-Kwon-Do athletes using a cycle ergometer analog to simulate the intensity of a normal Tae-Kwon-Do match. Twelve male athletes (20 ± 0.8 y, 66.9 ± 5.0 kg, 177 ± 4 cm) were recruited to partake in this study. After baseline measures, participants were randomly assigned to a treatment group containing BCAAs, L-arginine (0.05 g/kg, μ_{arg}=3.35 g), and L-citrulline (0.05 g/kg) or a starch placebo pre-packaged in gel capsules. Treatments were separated by a 7 d washout period. Following treatment randomization, participants underwent three "matches" on a cycle ergometer. Each match consisted of 5 sec all-out sprints with 25 sec normal pedaling. Each match consisted of performing this protocol for two minutes for two bouts. Between the first and second match was a 1 h rest period and the rest period between matches 2 & 3 was 2 h, with the treatment being administered at 1 h. Following the matches and supplementation, the athletes were introduced to a reaction test battery wherein they held a button in one hand and

were instructed to press it upon visual cue from a light. In addition, blood was collected prior to breakfast, after each match, and immediately before the third match (p = 0.002). There were no differences in RPE between groups.

Further investigating performance, Wax, et al. [148] tested acute and short-term treatment of L-arginine α-ketoglutarate on performance in resistance training. Sixteen males, eight trained (19.8 ± 1.9 y, 78.1 ± 7.5 kg, 176 ± 9 cm) and eight untrained (21.8 ± 2.4 y, 88.6 ± 22.4 kg, 179 ± 4 cm), were recruited to partake in this study. After a familiarization session where baseline 1-RM was determined for both bench press and leg press, participants were randomized to either the treatment group (3 g/d arginine α-ketoglutarate) or placebo. Participants took the treatment daily for a week and returned to the lab for follow-up testing. During the first testing session, participants ingested the assigned treatment, waited 45 min, and 1-RM was determined on bench press and leg press followed by RtF at 60% 1-RM. Total load was determined by multiplying the weight lifted (60% 1-RM) by RtF for each exercise. After follow-up testing, participants waited 1 week to allow for washout followed by undergoing the same protocol on the alternate treatment/placebo. There was no treatment effect on 1-RM, RtF, total volume, or HR between groups for trained and untrained individuals (p > 0.05).

Table 2.3. L-Arginine

Author (year)	Study Design	Sample	Dosing Protocol	Assessment Protocol	Significant Findings
Evans, et al. (2004) [140]	P, R, DB, CO	n = 12 (5 M, 7 F) healthy untrained individuals	3 g/d at week 1, 9 g/d at week 2, 21 g/d at week 3, 30 g/d L-arginine at week 4	Blood chemistry, hemodynamics, side effects	Gastric distress reported in 21 and 30 g/d groups, plasma L-arginine plateaued at 9 g/d
Forbes & Bell (2011) [141]	P, R, DB, CO	n = 14 recreationally-active males (25 ± 5 y)	0.075 g/kg L-arginine or 0.15 g/kg L-arginine acute dose, 7 d w/o	Blood draws every 30 min following ingestion for 2 h	Gastrointestinal distress reported in high dose (~10 g), no difference in GH or IGF-1, increase NO at 1 h only
Forbes, et al. (2013) [142]	P, R, DB, CO	n = 15 aerobically-trained men (28 ± 5 y)	5.8 g L-arginine 1 h prior to exercise, 7 d w/o	Submax exercise at 80% ventilator threshold	No difference in performance, VO_2, RER, GH
Forbes, et al. (2014) [143]	P, R, DB	n = 14 resistance-trained males (25 ± 4 y)	0.075 g/kg 1 h prior to exercise	1-RM and RtF on BP and LP	GH, ghrelin, and IGF-1 enhanced in placebo group and no differences in performance
Alvares, et al. (2012) [145]	P, R, DB, CO	n = 15 aerobically-trained participants (36.8 ± 7.1 y)	6 g/d L-arginine for 4 weeks. Exercise testing 30 min post-ingestion, 7 d w/o	Two 5 km TT with 10 min rest between	Increase in muscle blood volume in L-arginine group with no difference in performance
Vanhatalo, et al. (2013) [149]	P, R, DB, CO	n = 18 recreationally-active males (22 ± 3 y)	6 g L-arginine 65 min pre-exercise, 7 d w/o	Incremental test on TM	No difference in performance, decreased DBP and MAP in L-arginine (within normal limits)
Yavuz, et al. (2014) [150]	P, R, DB, CO	n = 9 professional wrestlers (24.7 ± 3.8 y)	0.15 g/kg L-arginine, 7 d w/o	Incremental test on CE	Increased TTE in L-arginine group with no difference in lactate
Chang, et al. (2015)	P, R, DB, CO	n = 15 male (21.1 ± 1.0 y) and 7 female handball players (20.3 ± 0.5 y)	Average dose of 3.13 g L-arginine for males and 2.16 g for females 30 min pre-exercise. 24 h w/o	Simulated handball matches using two 30 min blocks of activity and 20 m sprints	L-arginine improved spring performance

P = Placebo controlled, R = Randomized, DB = Double-blind, CO = Crossover, w/o = washout, GH = growth hormones, IGF-1 = insulin-like growth factor 1, NO = nitric oxide, VO2 = volume of oxygen consumption, RER = respiratory exchange ratio, 1-RM = one repetition maximum, RtF = repetitions to failure, BP = bench press, LP = leg press, TT = time trial, TTE = time to exhaustion, TM = treadmill

In a study by Bailey et al. [151], eight recreationally-active healthy males (22 ± 2 y, 79 ± 12 kg, 183 ± 9 cm) were recruited and required to report to the lab for eight testing sessions over the course of 7 to 9 weeks. At familiarization, a ramp incremental exercise test was conducted to determine VO_{2peak} followed by random assignment to a watermelon juice or apple juice placebo. The watermelon juice, which contained 1.39 g/L L-arginine and 11.4 g/L L-citrulline, was consumed at 150 mL/d. During each visit, pulmonary and muscular oxygen kinetics as well as exercise performance were assessed in addition to 5 d dietary recalls. Testing days included analyses of performance on an incremental cycle ergometer test as well as moderate- or severe-intensity step testing, pulmonary gas exchange, muscle oxygenation, and blood chemistry.

Even at the low dose of L-arginine, reports of gastrointestinal discomfort were common. L-arginine and plasma nitrate/nitrite were both higher in the watermelon juice ($p < 0.001$). The watermelon group yielded significant increases in HR, SBP, and MAP ($p < 0.05$) but DBP was unchanged. There were no differences between groups in measures of VO_2 kinetics, blood glucose or lactate, or exercise performance.

The review by Bescos, et al. [138] found seven studies analyzing L-arginine supplementation with no extra compounds. Of these, two studies involved healthy untrained males and one involved healthy post-menopausal women. In the study investigating post-menopausal women (n = 30, Treatment: 54.4 ± 4.1 y, 67.5 ± 13.7 kg, 165.9 ± 7.1 cm; Placebo: 55.3 ± 4.4 y, 61.3 ± 6.9 kg, 165.4 ± 5.4 cm), Fricke, et al. [152]

investigated the effects of L-arginine supplementation on muscle force and power. Of the recruited women, four treatment and three placebo participants were unable to complete mechanographic analyses due to perceived back and knee pain. The treatment group consumed 18 g/d arginine HCl (14.2 g L-arginine, 3.8 g hydrochloride) for 6 months compared to 18 g dextrose placebo. The tests included mechanographic and force analysis of two-leg countermovement jumping, isometric grip to assess MVC, and standard anthropometrics. Over the 6 month study, no differences arose related to anthropometry. Those in the treatment group exhibited a significant increase in peak jump force when adjusted for weight (p = 0.04) and a trend towards significance without adjusting for weight (p = 0.09). No significance was found in any other metric including MVC. There was an ergogenic effect of a high-dose of L-arginine in post-menopausal women in lower body force production.

In contrast to the findings by Evans, et al. and Forbes & Bell, Campbell, et al. [153] found higher dosing of L-arginine did not cause side effects. In this study, 35 resistance-trained men were enrolled in a randomized, double-blind design. The study consisted of four standardized resistance workouts per week for eight weeks and data collection at weeks 0, 4, and 8. During this time, participants consumed a total of 12 g L-arginine α-ketoglutarate daily split over three doses of 4 g. Eight hour fasting blood sample were collected at the onset of each testing session, followed by measurements of 1-RM on bench press, muscular endurance in the quadriceps, Wingate power testing, and aerobic capacity. At 12 g/day, there were no side effects reported. In addition,

significant increases were noted in 1-RM on bench press, peak power on the Wingate, blood glucose, and plasma arginine concentration.

Koppo, et al. [154] analyzed oxygen kinetics in response to L-arginine treatment. Physically active males (n = 7, 21.1 ± 0.6 y, 70.7 ± 5.5 kg, 184 ± 5 cm) were recruited to this double-blind, placebo-controlled, crossover study. Following familiarization and baseline data collection on an incremental exercise test to assess ventilatory threshold and VO_{2peak}, participants were assigned to either an L-arginine (6 g/d) or lactose placebo to be supplemented for a period of 14 d with a 7 d washout separating treatments. In addition, capillary blood samples were obtained to analyze lactate and venous blood was collected to assess L-arginine and other amino acids.

Also, blood pressure and urinary nitrate/creatinine content were assessed. No difference between groups was reported for blood pressure or heart rate prior to and after exercise. There were also no differences in creatinine, lactate, or nitrate. There was, however, a significant difference in the speed of VO_2 response to achieving steady state during the exercise test (p = 0.014). Despite the increased speed of oxygen kinetics, O_2 deficit was similar between the two groups and there were no effects on performance.

Further evidencing a lack of ergogenic benefit, Vanhatalo, et al. [149] investigated the effects of acute L-arginine ingestion on exercise tolerance measured by VO_2 uptake. Eighteen recreationally active males (22 ± 3 y, 75 ± 9 kg, 176 ± 6 cm) were recruited for this study and randomly assigned to either a L-arginine group (6 g L-

arginine) or flavor- and color-matched placebo separated by a 1 week washout. The performance evaluation in this study was a ramp incremental running test on a treadmill at 1% incline. The test began at 4 km/h and was increased 1 km/h each minute to volitional fatigue. Sixty-five minutes post-ingestion, a second test was employed consisting of two 6 min bouts, one at moderate intensity and the other severe. Pulmonary gas exchange and blood chemistry were monitored and recorded. In this study, SBP was found to be lower in the L-arginine group although it was still within normal limits (124 ± 7 mmHg compared to 128 ± 7 mmHg for placebo). DBP and MAP were also decreased in the L-arginine group yet these were still within normal limits (DBP: 67 ± 5 mmHg compared to 69 ± 5 mmHg for placebo; MAP: 86 ± 5 mmHg compared to 89 ± 5 mmHg for placebo). No differences were noted related to VO_2 kinetics or exercise tolerance/performance.

Yavuz, et al [150] demonstrated acute L-arginine supplementation to have a beneficial effect on time to exhaustion. Nine nationally and internationally competitive wrestlers were recruited (24.7 ± 3.8 y, 80.4 ± 4.1 kg, 174.2 ± 5.2 cm). Athletes were randomly assigned to either an L-arginine group (1.5 g per 10 kg, μ_{arg}=12.1 g) or placebo as gel capsules separated by a 7 d washout. The testing session consisted of ingestion of the assigned treatment followed by incremental bicycle ergometer test to exhaustion beginning at 60 rpm for a warm-up. Following the warm-up, a resistance of 90 W was applied which increased by 30 W every 3 min. Gas exchange was measured continuously and blood was collected at the end of each stage. Heart rate was also

measured continuously and remained under recording until lactic acid concentration had decreased by 50%. Lactate, heart rate, and VO_2 were similar between groups. Despite no changes in the physiological indices, the authors did report a 5.8% increase in time to exhaustion for the L-arginine group ($p < 0.001$). Interestingly, although the L-arginine group took significantly longer to achieve fatigue, blood lactate levels were similar between groups.

Chang, et al. [155] investigated the combination of branched-chain amino acids and L-arginine on handball performance. Handball players (Males n = 15, 21.1 ± 1.0 y, 78.3 ± 11.7 kg, 180 ± 7 cm; Females n = 7, 20.3 ± 0.5 y, 53.9 ± 5.0 kg, 161 ± 4 cm) were recruited to this study and randomly assigned to a treatment group (0.17 g/kg BCAA and 0.04 g/kg L-arginine, μ_{arg} = 3.13 g for males and 2.16 g for females) or placebo with a 24 h washout between treatments.

The exercise tests included 60 min simulated handball games on two consecutive days featuring a crossover model between days. The simulation was achieved using thirty 2 min blocks of activity with a 20 m sprint at the end of each block. Sprint time and RPE were recorded and assessed and blood samples were collected to assess ammonia, glycerol, and lactate. The L-arginine group yielded significantly better sprint performance on Day 2 compared to placebo ($p = 0.001$). RPE was also decreased in the L-arginine group ($p = 0.005$) and no difference in HR was reported. The authors propose the combination of L-arginine and BCAA was ergogenic due to attenuation of central fatigue as defined by Blomstrand, et al. [156].

Ergogenic benefits were seen sporadically in the articles reviewed independent of dose, with Chen, et al. [147] demonstrating increased performance in Tae-Kwon-Do athletes at 3 g/d and Fricke, et al. [152] evidencing increased force production in post-menopausal women at 14 g/d. The lowest reported dose of 1.39 g/d, which is higher than the current dose of 0.95 g, did not demonstrate any significant effects on performance [151]. Given the inconsistencies and findings and the low dose of L-arginine in the current RTD, it is unlikely L-arginine would add to the proposed ergogenic effect of the RTD.

2.3.4. Arginine Nitrate

Arginine and nitrates have both been associated with endothelium-independent vasodilation via increasing the concentration of nitric oxide (NO) [138, 157, 158]. Physiologically, shear stress and stretch applied to vasculature elicits the endothelium-dependent release of endothelial nitric oxide synthase (eNOS) which translates to the formation of NO. NO then acts upon the smooth muscle to cause relaxation and the resultant effect is vasodilation. It should be noted the half-life of NO is short, ranging from a few seconds to 5 min [96, 159].

L-arginine converts to NO via a catabolic pathway and enzymatic conversion to NO [160, 161] whereas nitrates (NO_3) convert to NO via reduction to nitrites (NO_2) and further reduction to NO [96]. The rationale behind formulating L-arginine and nitrates into a single *arginine nitrate* compound holds that the dual vasodilatory effect

synergistically enhances the effect and provides a more noticeable ergogenic outcome. It was also hypothesized that the combination would result in increased bioavailability of L-arginine [162, 163] as well as decreasing ATP cost by increasing the removal of metabolites, increasing mitochondrial respiration [108], and maximizing perfusion[138, 164, 165].

Sandbakk, et al. [157] analyzed the combined treatment of L-arginine and nitrate on endurance and sprint performance. Nine college-aged competitive cross-country skiers (18 ± 0 y, 74.2 ± 8.6 kg, 181.0 ± 8.5 cm) were recruited and VO_{2max} was determined by an incremental running test to exhaustion to be 69.3 ± 5.8 mL/ kg/min. Incline began at 10.5% and speed increased 0.5- to 1.0 km/h when VO_2 stabilized for 30 sec. Following ingestion of the assigned treatment participants endured three trials with a one week washout separating each trial and either L-arginine + nitrate (600 mg and 614 mg or 9.9 mmol), nitrate + placebo, or L-arginine + placebo. Each trial had two 5 min submaximal running tests on a treadmill and both a 180 m and 5 km time trial on an indoor track. Heart rate and blood pressure were monitored at regular intervals while blood was drawn at the beginning of each trial and between each test.

There were no differences in the two submaximal tests or the 5 km time trial between any of the three groups. In the 180 m time trial, the L-arginine + nitrate group (24.4 ± 0.8 sec) and L-arginine + placebo group (24.3 ± 0.7 sec) were no different from placebo; however, the nitrate + placebo group completed the 180 m time trial in a faster time (24.1 ± 0.9 sec, $p = 0.04$). L-arginine + nitrate showed a trend toward lower

baseline plasma nitrate concentrations (23 ± 6 mmol/L or 1.426 ± 0.372 mg/dL) than nitrate + placebo (32 ± 10 mmol/L or 1.984 ± 0.62 mg/dL, p = 0.07) or L-arginine + placebo (35 ± 19 mmol/L or 2.17 ± 0.992 mg/dL, p = 0.06). Following treatment, the L-arginine + nitrate (296 ± 77 mmol/L or 18.35 ± 4.77 mg/dL) and nitrate + placebo groups were not significantly different (296 ± 77 mmol/L or 20.77 ± 4.03 mg/dL); however, both treatments yielded significantly higher nitrate concentration compared to L-arginine + placebo (26 ± 16 mmol/L or 1.612 ± 0.992, p < 0.001). The elevation of the two groups was maintained throughout the entirety of the trial ($p < 0.001$). The results showed an increase in nitrate concentration resultant on combination with L-arginine; however, nitrates alone still yielded a significant increase in nitrates. Even so, the increased concentrations did not yield consistent ergogenic outcomes outside of the 180 m time trial.

Given the putative vasodilation associated with L-arginine and nitrate use, there is some concern of safety, particularly in regards to alterations in cardiovascular response [166-169]. Vasodilation decreases systemic vascular resistance, leading to a decrease in venous return and subsequent decrease in diastolic blood pressure and stroke volume. The decrease in stroke volume facilitates a decrease in cardiac output which must be compensated for by an increase in heart rate. In those with cardiovascular abnormalities or diseases, this compensation may be inhibited or blunted and could lead to orthostatic hypotension; as such, only literature pertaining to

safety as well as performance benefits of nitric oxide enhancing supplements were considered.

Bondonno, et al. [96] summarized the effects of dietary nitrate and nitric oxide on cardiovascular health. Cardiovascular disease (CVD) is the leading cause of death world-wide [170]. CVD can be attributed to loss of vascular compliance leading to decreased oxygen (ischemia), 'hardening' of vasculature (atherosclerosis), and increased systemic vascular resistance from a combination of the factors or buildup of plaque. The endothelial aspect was the primary focus as it is most affected by the ingredients within the supplement. CVD can be influenced by a variety of factors ranging from obesity and dyslipidemia to aging and gender; any perturbation to endothelial response can influence how well the heart can compensate and maintain cardiac output.

In compromised endothelium, eNOS may not function optimally or at all, reducing the ability to locally vasodilate. As such, pharmacological treatments were developed but more recently dietary interventions have been seen to have similar efficacy in blood pressure control via alteration of endothelial function, to wit, fruits and green leafy vegetables are promoted to patients with CVD [169, 171, 172]. Green leafy vegetables have been found to be in high in nitrates, leading to more research in the area, specifically with regards to vegetables high in nitrates such as beet roots. The findings by Bondonno, et al. dispute the claims of risk to safety by showing the research

thus far has been inconsistent and potentially misleading relative to drops in SBP and DBP.

Lovegrove, et al., [173] sought to further elucidate the role of dietary nitrates in CVD. This review further investigates the current literature regarding nitrates and provides further evidence of nitrates being beneficial to vascular compliance. L-arginine requires sufficient oxygen catalyze with eNOS to form NO whereas the reduction of nitrates to nitrates and further reduction to NO is oxygen independent, releasing oxygen in the reduction. Since the seminal paper by Larsen, et al. in 2006 of nitrates lowering blood pressure in healthy individuals [169, 174], the mechanisms and actions have been further investigated and extrapolated to more populations and have shown nitrates to be very effective in the treatment of hypertension and other aspects of CVD. Furthermore, increasing nitrate consumption has been evidenced to decrease the symptoms of metabolic syndrome [169, 175] and up-regulate GLUT4 to better utilize glucose [97].

An overview of the studies reviewed regarding nitrates and L-arginine in relation to exercise performance can be found in TABLE 2.2 and TABLE 2.3, respectively. The studies reviewed no significant risk in regards to cardiovascular health; although, multiple studies reported complaints of gastrointestinal discomfort [140, 151, 176]. The review article by Bescos, et al. [138] asserts an average intake of 4 to 5 g/d of L-arginine from normal diet. The studies reviewed ranged from 3 g [147, 155] to over 10 g of L-arginine [150, 152] with most of the studies involving 6 g of L-arginine [145, 146,

154, 176]. Based on the study by Evans, et al., doses greater than 9 g/d appear to increase the likelihood of gastrointestinal discomfort [140] which is further supported by Fricke, et al. and Forbes & Bell [152, 176].

2.3.5. β-Alanine

Beta-alanine (β-alanine) is an endogenous amino acid with roles in skeletal muscle function. β-alanine acts by regulating carnosine synthesis which then serves a buffering effect within the skeletal muscle, blunting the drop in pH associated with high-intensity exercise [177, 178]. During exercise, pH drops from 7.2 to as low as 6.6 which results in perturbations in oxygen kinetics, fat oxidation, glycolysis, and EC-coupling. These perturbations can and do have detrimental effects on exercise; if supplementation results in increased buffering capacity, pH can be maintained closer to 7.2 resulting in longer time to exhaustion during strenuous exercise.

Early examination of carnosine was conducted in horses and found that horses with increased carnosine content in the gluteus medius was associated with greater performance [179, 180]. Carnosine is also found to be higher in Type II muscle fibers [181]. Carnosine in human skeletal muscle ranges between 10 and 40 mmol/ kg (dry weight) with resistance trained athletes boasting higher concentrations [182] and supplementation of β-alanine yielding anywhere from 20 to 80% increases in muscle carnosine [177]. It makes sense that carnosine is elevated in Type II muscle fibers and, taking the increased carnosine of resistance trained athletes into account, the

mechanism of pH buffering fits well with current physiological understanding. β-alanine has been found to increase muscle carnosine in both Type I and Type II fibers, leading to further buffering capacity [183].

In contrast to the findings by Tallon, et al. on resistance trained athletes [182], other studies have investigated the effects of training on carnosine content. Harris, et al. [178] reported no training effect after 8-weeks of supplementation were found [184, 185]; however, supplementation of β-alanine over similar durations using similar training protocols yielded increased muscle carnosine [185, 186]. Additionally, females have lower muscle carnosine content than males [187] and age has a detrimental effect on muscle carnosine [181, 188].

The International Society of Sports Medicine (ISSN) issued a position stand in 2015 recommending chronic daily doses of 4 to 6 g/d to increase muscular carnosine content by 20 to 30% [14]. The overview of ergogenic potential provided in this position stand presents claims of enhanced high-intensity exercise performance [177] and increased performance in tasks lasting 60 to 240 sec but no effect to exercise lasting less than 60 sec [189]. β-alanine also has a beneficial effect on aerobic activity lasting more than 4 min [189]. Additionally, there is evidence for claims of decreasing neuromuscular fatigue and inconclusive evidence related to enhancing strength [14]. These claims are supported in other review articles [177, 189].

Carpentier, et al. [190] assessed the effects of chronic β-alanine usage at 4.0 to 5.6 g/d on high intensity performance. Twenty seven physically active college-aged

individuals were randomly assigned to either β-alanine (BA, Males n=6, Females n=8, 22.1 ± 2.1 y, 63.6 ± 10.0 kg, 168.8 ± 9.4 cm) or placebo (PL, Males n=6, Females n=7, 21.4 ± 2.1 y, 63.7 ± 8.5 kg, 174.7 ± 11.6 cm) for daily supplementation over 8-weeks. Prior to the first treatment, baseline data was gathered using vertical jump tests, five maximal effort jumps and five countermovement jumps. Following those, participants performed a fatigue test of 45 consecutive countermovement jumps. Blood lactate was assessed prior to the fatigue test and every 5 min post-test. Analysis revealed a significant effect of BA treatment on jump height in both the standing and countermovement conditions ($p < 0.01$ and < 0.001, respectively). The mean height of the fatigue test was also greater in the BA group ($p < 0.01$), indicating preservation of force production. No difference was noted in blood lactate.

Another study investigating the effect of BA on less oxidative exercise was conducted by Glenn, et al. [191] with Wingate performance as the primary outcome. In contrast to many exercise studies, this study recruited only females whom were competitive cyclists (n = 12, 26.6 ± 1.3 y, 58.67 ± 1.74 kg, 161.08 ± 1.78 cm). Participants were randomly assigned to either a BA group (1.6 g β-alanine) or dextrose placebo as an acute dose. BA in doses of less than 4 g/d have not been evidenced to have ergogenic effect. During familiarization, anthropometrics were recorded and participants completed one bout on the Wingate stress test. The Wingate protocol was completed 30 min following ingestion of the assigned treatment. Time to completion, power, RPE, lactate, and heart rate were recorded prior to, immediately after, and 2

min post-Wingate. The BA group had lower RPE values ($p < 0.001$) with no difference in performance between groups.

Glenn, et al. [192] utilized a female-only population; however, this study investigated a larger dose of 3.2 g β-alanine compared to the previous study's dose of 1.6 g β-alanine [191]. In addition to the increased dose, this study investigated an older population of competitive cyclists. At familiarization, anthropometrics were recorded and VO_{2peak} was determined. During the second day, baseline time to exhaustion data were gathered on a cycle ergometer cycling at 120% VO_{2peak}. Participants were then randomly assigned to either the BA group (800mg β-alanine, four times daily) or dextrose placebo (PL) for a supplementation period of 28 d (BA n = 11, 54 ± 2 y, 64.01 ± 2.82 kg, 163.00 ± 2.08 cm; PL n = 11, 53 ± 1 y, 68.7 ± 5.64 kg, 162.33 ± 2.5 cm). The time to exhaustion protocol was completed weekly followed by final analysis of anthropometrics and VO_{2peak}. The BA group showed a significant increase in time to exhaustion of 23% compared to 1% for placebo ($p = 0.03$) as well as an increase in total work of 21% compared to 2% for PL ($p = 0.03$). Lactate was not significantly different between groups however there did exist a trend in favor of BA ($p = 0.056$). The data indicate an ergogenic effect on time to exhaustion after 4 weeks of daily supplementation of β-alanine at 3.2 g/d. Of note, the authors did speculate a potential influence of gender on β-alanine efficacy, specifically that females may be more sensitive.

Table 2.4. β-Alanine

Author (year)	Study Design	Sample	Dosing Protocol	Assessment Protocol	Significant Findings
Carpentier, et al. (2015) [190]	P, R, DB	n = 27 recreationally active (6 M, 8 F, 22.1 ± 2.1 y)	4 to 5.6 g/d for 8 weeks	Jump fatigue test	Increased peak and average jump height for BA group and no difference in blood lactate
Glenn, et al. (2015) [191]	P, R, DB	n = 12 trained female cyclists (26.6 ± 1.3 y)	1.6 g/d BA for 28 d	Wingate	Lower RPE in BA with no difference in performance
Glenn, et al. (2015) [192]	P, R, DB	n = 22 trained female cyclists (54 ± 2 y)	3.2 g/d BA for 28 d	TTE on CE at 120% VO_{2peak}	Increased TTE in BA
Glenn, et al. (2016) [15]	P, R, DB	N = 22 trained female cyclist (54 ± 2 y)	3.2 g/d BA for 28 d	Handgrip and leg MVC	Increased lower body MVC in BA with no change in handgrip or anthropometrics
Hoffman, et al. (2015) [193]	P, R, DB, CO	n = 18 elite male soldiers (19.9 ± 0.8 y)	6 g/d BA	2.5 km run, 1 min sprint, 50 m 'casualty carry'	Faster time in 50 m casualty carry in BA with no difference in any other performance aspect; improved cognitive function in BA

P = Placebo, R = Randomized, DB = Double-blind, CO = Crossover, BA = β-alanine, TTE = time to exhaustion, CE = cycle ergometer

This hypothesis as not yet been directly tested yet the ergogenic effect from the lower dose in this study does strengthen the claim; however, the effect may have been resultant of an older population as well.

In a study by Glenn, et al. [15], Master's-level competitive female cyclists were recruited to analyze the effect of chronic β-alanine supplementation on isokinetic strength. Participants were randomly assigned to either BA (n = 11, 54 ± 2 y, 64.01 ± 2.82 kg, 163.00 ± 2.08 cm) or PL (n = 11, 53±1 y, 68.7 ± 5.64 kg, 162.33 ± 2.5 cm) for the 28 d supplementation period. Dosing remained the same as the previous study at 3.2 g/d spread over four doses. Participants were assessed for isometric handgrip strength and dominant leg isometric contraction at baseline, each week of the supplementation period, and following supplementation. The BA group had greater peak torque during the lower body isokinetic test (p = 0.012) as well as total work (p = 0.008). Handgrip strength and anthropometrics were unchanged by treatment. The results evidence an ergogenic effect daily supplementation of β-alanine supplementation at 3.2 g/d on lower body strength.

Hoffman, et al. [13] reviewed the effects of β-alanine supplementation in military personnel. Previous reports have presented evidence of cognitive and physical detriments associated with the intense training in tactical athletes [194, 195] and one report stated an estimated 53% of military personnel use some form of ergogenic aid [196]. Given the stress of military training, the authors speculate a potential increase in performance and combat ability with chronic use of β-alanine, thus, enhancing

survivability [13]. In addition, the authors hypothesized the increased carnosine found in the brain following β-alanine supplementation may enhance brain-derived neurotropic factor (BDNF) and suggest a potential neuroprotective effect as well as a possible avenue of research in the treatment of post-traumatic stress disorder (PTSD). One particular study in the review yielded evidence of β-alanine enhancing peak jump power, speed of target acquisition, and shooting accuracy [193].

Hoffman, et al. [193] investigated eighteen soldiers from an elite combat unit (19.9 ± 0.8 y, 74.2 ± 5.7 kg) for brain and muscle carnosine concentration and a battery of combat-related exercise tests. Magnetic resonance spectroscopy (MRS) was used to evaluate carnitine concentration and exercises tests conducted included 2.5 km run, a 1 min sprint, a 50 m 'casualty carry' of a 60 kg mannequin, and repeated sprints interspersed with shooting performance evaluation. Cognitive function was also evaluated using the Serial Sevens Test following the shooting evaluation. Participants were assigned to either a BA group (6 g/d) or rice powder placebo and supplementation lasted for a period of 30 d. The BA group demonstrated a significant increase in muscle carnosine (p = 0.048) compared to placebo but no change in brain carnosine. No differences were seen in the 2.5 km run or 1 min sprint. The BA group performed a significantly faster time in the 50 m casualty carry (p = 0.044). Interestingly, there was no difference in the shooting drill; however, despite no change in brain carnosine, the BA group yielded significantly greater results on the cognitive function test (p = 0.022). Referring back to the Hobson [189] meta-analysis, β-alanine

appears to exert the most noticeable effect in exercise lasting 60 to 240 sec. The 2.5 km run yielded an average time of approximately 620 sec and the 1 min run only reached the lower limit of the ergogenic zone. Also of note, the lack of increase in brain carnosine is consistent with other research; however, work by Solis, et al. [197] found no increase in cognition which is in contrast to the increased cognitive performance reported by Hoffman, et al.

Aside from paresthesia, no side effects were reported with chronic or acute use of β-alanine. The studies reviewed yielded a range of 1.6 g/d [191] to 6 g/d [193, 197] with varying results dependent on the methodology. In the two studies by Glenn, et al. [15, 192] involving Masters-level competitive female cyclists, chronic daily intake of 3.2 g for 28 d yielded increased time to exhaustion and increased lower body strength. Carpentier, et al. [190] used a dose of 4 g/d and found a significant increase in mean jump height for a jump test to exhaustion and no change in single maximal effort jumps. Hoffman, et al. [193] provided evidence of 6 g/d enhancing performance in a casualty carry test as well as cognition in elite military personnel. The cognitive effect appears to be reliant on training as Solis, et al. [197] found no change in cognition with β-alanine supplementation (6 g/d) alone.

These findings are in line with the ISSN position stand; chronic daily intake of 4 to 6 g β-alanine has an equivocal ergogenic effect on exercise lasting between 60 and 240 sec [14]. The present study investigated a RTD containing 2.1 g of β-alanine. Given the short (<10 d) supplementation period and the low dose, it is unlikely the β-alanine

within this RTD was responsible for any ergogenic benefit in the tests performed. An overview of the studies reviewed can be found in TABLE 2.4.

2.4 Relevant Work from Exercise and Sports Nutrition Laboratory

The Exercise and Sports Nutrition Laboratory (ESNL) has served an independent investigation role for a variety of ergogenic aids, both as independent compounds and as combinations of various nutrients. A study by Campbell, et al. [153] was covered previously regarding L-arginine α-ketoglutarate, finding a dose of 12 g of L-arginine α-ketoglutarate to show improvements in 1-RM on bench press, peak power on the Wingate, blood glucose, and plasma arginine concentration with no side effects.

Roberts, et al [198] investigated the effects of ingestion of a popular coffee-flavored energy drink in a randomized, crossover design study. Ten healthy, recreationally-active males (n = 5, 27.6 ± 4.2 y, 93.2 ± 11.7 kg, 181.6 ± 6.9 cm) and females (n = 5, 29.0 ± 4.6 y, 61.5 ± 9.2 kg, 167.6 ± 6.9 cm) whom were habitual coffee drinkers with a self-reported intake of 223.9 ± 62.7 mg/d of caffeine were recruited. Baseline testing began with assessment of resting hemodynamics followed by a graded exercise test using normal Bruce protocol procedures. Oxygen kinetics were assessed every 3 min during the test and at 3 and 10 min post-test. After a 20 min rest, participants underwent a standard Wingate protocol. After 1 week, participants were given either the supplement (450 mg caffeine, 1200 mg garcinia cambogia, 360 mg citrus aurantium extract, 225 mcg chromium polynicotinate) or a decaffeinated form of

the supplement, waited 15 min, and performed the same baseline tests. After a 1 week washout, participants returned to the lab to complete the same tests on the alternative treatment.

Exercise post-oxygen consumption (EPOC) was significantly increased in the caffeinated group following the Bruce protocol compared to both baseline (p = 0.02) and the decaffeinated group (p = 0.03). Heart rate was also significantly elevated in the caffeinated group (115 ± 6 bpm) compared to decaffeinated (107 ± 7 bpm, p = 0.03) during the graded exercise test; however, *post hoc* analysis showed the significant difference in HR elevation to be at the point of volitional fatigue. There were no significant differences between groups on the Wingate test.

Kresta, et al. [199] investigated the effects of chronic daily use of β-alanine, with and without co-administration of creatine monohydrate, on exercise performance. This study involved 32 recreationally-active, college-aged females (21.5 ± 2.8 y, 60.5 ± 6.1 kg, 40.2 ± 3.8 FFM, 26.7 ± 5.8% body fat) and used a randomized double-blind, placebo-controlled study to assess the effects. Participants were randomly assigned to either a β-alanine only group (BA, 0.1 g/kg/d), creatine monohydrate (CRE, 0.1 g/kg/d), combined BA and CRE (BAC), or maltodextrin placebo. Supplements were ingested four times each day.

Biopsies were obtained from the vastus lateralis on Days 6 and 27 and exercise testing was conducted on Days 0, 7, and 28 of each treatment. At each testing session, anthropomorphic data were collected as well as body composition followed by a

graded exercise test on a cycle ergometer beginning at 50 W at 70 rpm and increasing 25 W every 3 min until participants were unable to maintain 70 rpm. Blood was collected via finger stick during the final minute of each stage and at 5 min post-exercise to determine lactate threshold.

BA, CRE, and BAC boasted significantly higher muscle carnosine concentration compared to PLA with no significance between groups. Peak blood lactate concentration was significantly reduced in the BA group as well as a decreased delta in blood lactate concentration during exercise. There were no significant differences between groups relative to body composition and only statistical trends were noted in regards to the exercise testing in VO_{2peak} and METS. Relative peak power was significantly increased in BA, CRE, and BAC on the Wingate while only BA and BAC demonstrated a reduced rate of fatigue.

Galvan, et al. [100] tested a novel combination of creatine monohydrate with nitrate, creatine nitrate, relative to acute and chronic effects on exercise performance. In Study Arm 1, the acute effect was analyzed in randomized, crossover design. Thirteen recreationally active, college-aged men (22 ± 5 y, 84.1 ± 18.9 kg, 177.8 ± 7.4 cm) were recruited for this study. Following baseline testing, participants were randomly assigned to a placebo group (PLA, 6.5 g dextrose), creatine monohydrate (CrM, 5 g CrM and 1.5 g dextrose), low-dose creatine nitrate (CrN-Low, 1.5 g CrN and 5 g dextrose), or CrN-High (CrN 3 g CrN, 3.5 g dextrose). All treatments were separated by a 1 week washout. Following ingestion of the assigned treatment, participants

waited 30 minutes wherein HR, SBP, DBP, and blood draws were conducted at 0, 30, and 60 min and every 60 min for hours 1-5.

For Study Arm 2, chronic use was assessed. A total of 48 recreationally active, college-aged males were recruited (21 ± 3 y, 77.4 ± 20.9 kg, 176.8 ± 5.8 cm). The groups were slightly altered from Study Arm 1: PLA (5 g dextrose), CrM (3 g CrM, 2 g dextrose), CrN-Low (1.5 CrN, 3.5 g dextrose), and CrN High (3 g CrN, 2 g dextrose). Following a 2 week standardized exercise program after familiarization, testing was conducted at baseline and following 28 d of daily supplementation and biopsies were collected the day before baseline, and at Days 7 and 27. At baseline, 1-RM was assessed on bench press followed by a single bout of the Wingate stress test. The 1-RM protocol, similar to that of the present study, involved assessment of 1-RM followed by two sets of ten repetitions and a third set to volitional fatigue at 70% 1-RM. The Wingate test was modified. Normally, the Wingate protocol involves a single maximum effort of 30 sec against a resistance of 7.5 J/kg/rev; in this study a total of six bouts of 6 sec against a resistance of 7.5 J/kg/rev were performed with a 3 min rest between each bout.

In Study Arm 1, plasma creatine was significantly higher in CrM than PLA (p = 0.001), CrN-Low (p = 0.004), and CrN-High (p = 0.007) with no differences between PLA, CrN-Low, or CrN-High. Conversely, plasma NO_3 was significantly higher in CrN-High than CrM (p = 0.001) and PLA (p = 0.001) but not different from CrN-Low. Relative to the full blood panel, significant overall interaction effects were only seen in creatinine

(p = 0.001) with CrN-Low/High being significantly greater than PLA from 1 to 5 h post-ingestion. Finally, no significant group or time x group interaction effects were noted in regards to hemodynamics.

In Study Arm 2, there were no statistical differences noted in anthropometrics following 28 d of chronic use. There were overall interaction effects in plasma NO_3 (p = 0.001) and creatine (p = 0.01) that followed a similar pattern to that of Study Arm 1. There were no overall group or time x group interaction effects for any of the performance variables; however, analysis of 95% confidence intervals revealed significant improvement on average power on the bench press in CrN-High only. CrN improved significantly more than PLA and CrN-Low and was not different from CrM relative to average power. While there were no significant improvements on total volume for bench press, CrN-High improved significantly more than PLA but was not different from CrM or CrN-Low.

In the first experimental arm of Jung, et al. [99] a randomized, double blind, crossover study involving 25 individuals (n = 20 M, 5 F, 21.7 ± 3.0 y, 78.2 ± 13.0 kg, 176.1 ± 8.2 cm) was conducted to assess the effects of a pre-workout supplement containing β-alanine (3 g), CrN (2 g), L-arginine α-ketoglutarate (2 g), N-Acetyl-L-Tyrosine (300 mg), caffeine (284 mg), *Mucuna pruiriens* extract (15 mg) and vitamins. This PWS came as stated or with the inclusion of *Citrus aurantium* (*p*-synephrine, PWS + S). Placebo (PLA) contained 12 g of flavored maltodextrin. The treatments were blinded and provided as a powder to be mixed with 235 mL of water.

At each testing session, following ingestion, supine HR, SBP, DBP, and ECG were recorded at 10 min intervals during a 30 min REE. At 1 h post-ingestion, blood was collected and participants completed a Stroop Color-Word test. Participants completed a warm-up and proceeded to perform a 1-RM on bench press and leg press followed by completion 2 sets of 10 repetitions and 1 set to volitional fatigue at 70% 1-RM with 2 min rest breaks. After a 5 min rest, participants completed a standard Wingate stress test followed by the Stroop test and one more blood sample. Each testing session for each of the 3 treatments were separated by a 7 d washout.

No significant differences were noted relative to hemodynamics or ECG. There was an overall interaction effect on REE ($p < 0.001$) with PWS and PWS + S showing an increased VCO_2 compared to PLA. Significant interaction effects were also noted on the readiness to perform questionnaire ($p = 0.004$) and Stroop Color-Word test ($p < 0.001$); in both cases, PWS and PWS + S were not significantly different from each other but were both significantly higher than PLA. No significant differences were noted in any of the performance variables.

In the second arm, Jung, et al. [98] investigated the same supplements and the effects of 8 week chronic daily use on training adaptations. Following familiarization, eighty resistance-trained males were randomly assigned to either PLA (n = 27, 22.3 ± 3.9 y, 81.1 ± 13.3 kg, 178.4 ± 6.9 cm), PWS (n = 27, 20.9 ± 3.9 y, 81.5 ± 13.0 kg, 177.0 ± 4.6 cm), or PWS + S (n = 26, 22.0 ± 2.6 y, 80.2 ± 15.8 kg, 177.8 ± 5.6 cm). Participants completed baseline testing followed by an 8 week standardized training program and

follow-up testing at 4 and 8 weeks. Participants were instructed to consume the assigned treatment 15 to 30 minutes prior to exercise or with breakfast on non-training days.

No differences were observed in any measurement of performance; however, analysis of 95% confidence intervals showed significant changes from baseline. On bench press, PWS and PWS + S significantly improved at Week 4 while all three groups significantly improved at Week 8 with no between-groups differences. Leg press yielded a similar pattern; however, PWS improved significantly more than PWS + S and PLA and PWS + S improved significantly more than PLA. 95% confidence interval analysis showed significant improvements in the Stroop Color-Word test. PWS and PWS + S improved at Week 4 and 8 while PLA only improved at Week 8 on the Word portion; there were no significant differences between groups. All three groups significantly improved the Color portion at both Week 4 and Week 8. There were no significant differences between groups at Week 4; however, at Week 8 PWS improved significantly more than PWS + S which improved significantly more than PLA. Only PWS and PWS + S improved on the Color-Word portion at Week 4 with PWS + S improving significantly more than PWS; at Week 8 all three groups significantly improved and there was no difference.

Koozehchian, et al. performed a double-blind, randomized, placebo-controlled, crossover study wherein 19 resistance-trained participants (21 ± 2 y, 83.9 ± 18.1 kg, 175 ± 9 cm) were recruited. Following baseline testing, participants were assigned to either

a PLA group (12 g glucose), pre-workout supplement (PWS) group [β-alanine (3.2 g), arginine α-ketoglutarate (2.0 g), creatine nitrate (2.0 g), *N*-acetyl tyrosine (300 mg), caffeine (300 mg), *Mucuna pruriens* extract standardized for 15% L-Dopa (1.0 g), and B-vitamins], or a PWS150 group containing the ingredients from PWS at 150% the dose. All treatments included baseline and short-term (7 d) supplementation period testing and were separated by a 7 d washout. Participants underwent an REE protocol for 30 min, ingested the supplement, and underwent a second REE protocol for 30 min, followed by cognitive testing and performance testing. Participants were assessed by performance by establishing 1-RM followed by repetitions to failure at 70% 1-RM and Wingate stress test. PWS and PWS150 exhibited significant improvements in cognitive testing and no ergogenic effects were noted in exercise performance or resting energy expenditure.

2.5. Summary of Literature Regarding Ergogenic Potential

Literature related to ergogenic effects to performance from caffeine, nitrates, L-arginine, and β-alanine was examined in detail in this review. To review, caffeine is claimed to enhance strength, endurance, cognition, and enhance fat oxidation to potentially increase glucose sparing [22]. Nitrates have been reported to decrease VO_2 requirement at various exercise intensities through multiple proposed mechanisms [35] including enhancements in PCr synthesis [103]. Similar to nitrate, L-arginine is reported to increase nitric oxide and thus improve nutrient delivery[134]. In addition, it has

been claimed to enhance growth hormone and reduce recovery time [200]. β-alanine is claimed to enhance performance by buffering pH during exercise, thus potentially prolonging the duration of high-intensity exercise [14].

With regards to the claims presented, caffeine is evidenced to be ergogenic, consistently being found to enhance performance in doses of 3 to 9 mg/kg. Nitrates also appear to be ergogenic in doses of 5 mmol/d or more when analyzing VO_2 response to exercise and power production in high-intensity exercises lasting 60 to 240 sec. L-arginine was consistently found to have no effect on hormonal balance, specifically with regard to growth hormone with one study actually showing a decrease [143]. Additionally, L-arginine at any dose consistently yielded no effect on performance unless combined with L-citrulline. Finally, chronic use β-alanine at 3.2 to 6 g or greater was consistently found to increase time to exhaustion as well as maintenance of power output during the later stages of exhaustive exercise.

Martinez, et al. [201] investigated a RTD (Assault ™)containing 2 g of β-alanine, an undisclosed amount of nitrates as arginine nitrate, BCAA nitrate, and beetroot extract, as well as 400 mg caffeine (4.79 mg/kg). Supplementation yielded enhanced peak and mean power on the Wingate stress test. Spradley, et al. [202] investigated the same RTD as Martinez, et al. and found a significant increase in repetitions to fatigue on leg press as well as increased cognitive function and perceived energy. Ormsbee, et al. [203] analyzed a different RTD (NO-Shotgun ™) which contained 190 ± 10 mg of caffeine anhydrous (2.27 mg/kg) in addition to BCAAs, creatine, and β-alanine.

No performance improvement was noted; however, the RTD group exhibited significantly greater increases in fat-free mass compared to placebo. Of note, only caffeine existed in these RTD within the ergogenic range of 3 to 9 mg/kg.

We previously reported the effects of an RTD containing β-alanine (3 g), creatine nitrate (2 g), arginine α-ketoglutarate (2 g), caffeine anhydrous (284 mg, 3.63 mg/kg), and B-vitamins [99]. This study used the same protocol as the present study to assess maximal strength and repetitions to failure on bench press and leg press as well as resting energy expenditure (REE) and Wingate anaerobic performance. The RTD yielded evidence of increased cognition and greater changes in REE; however, despite the ergogenic level of caffeine, there were no differences in the performance measures.

The primary outcome of the current study was the effect of RTD on exercise performance assessed by total lifting volume, maximal strength, muscular endurance, and 4 km time trial performance. Based on the reviewed studies, there was a logical assumption that there would be a significant difference favoring the RTD group, most likely as a function of increased repetitions to failure.

The secondary outcomes in the current study were related to safety. A review of energy drink-related research from 1970 to 2010 was performed by Higgins, et al. [204]. In this review, the authors conclude that, despite the increase in heart rate and blood pressure, caffeine-containing energy drinks are safe in healthy population; however, they assert consumption of energy drinks should be avoided prior to training.

In a separate review by Eudy, et al. [205], the authors make mention of the potential dangers of proprietary blends, asserting a need for further research in new supplement formulations to assess for safety and efficacy. In both reviews, side effects included gastrointestinal distress, cardiovascular perturbations, and alterations to blood lipids and glucose homeostasis. These findings and conclusions are in agreement with the International Society of Sports Nutrition [21].

In the present work, safety was assessed by measuring hemodynamic response to a postural challenge, serum and whole blood chemistry, and self-reported side effects. Based on the literature, a significant increase in plasma nitrate was expected with the nitrate dose of 5.7 mmol as all studies involving nitrates evidenced a significant increase in plasma nitrate but no other differences were expected in regard to blood chemistry. The safety concerns of nitrate use were addressed within this review and, fortunately, the concerns appear to be unfounded. Granted, there were a studies where MAP or blood pressure were significantly lower compared to placebo; however, these were still well within normal limits and not cause for concern [109, 110]. Finally, aside from paresthesia common to β-alanine use, no evidence in the literature suggests a significant difference between groups in regard to side effects as the 1 g dose of L-arginine was well below the 9 g threshold found by Evans, et al. [140].

CHAPTER III

METHODS*

3.1. Study Overview

Prior to starting the study, approval was obtained from the Texas A&M University Institutional Review Board (#2016-754F). Although not required, the study was registered with clinicatrials.gov (#NCT03032549). Additionally, the study has been previously published [206].

Recreationally active men and women between the ages 18–40 years were recruited to participate in this study through the campus email system as well as posting flyers throughout the university. Participants responding to recruitment advertisements were initially screened by phone to determine general eligibility. Inclusion criteria required that each participant have at least six months of resistance training experience immediately prior to entering the study, inclusive of bench press and leg press or squat training. 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; if they were currently using any prescription medication with the exception of birth control; if they were pregnant, lactating, or planned to become pregnant within the next month; if they had a history of smoking; if they drank excessively (>12 drinks per week); or, if

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they had a recent history of consuming dietary supplements or energy drinks containing β-alanine or high amounts of caffeine within eight weeks of the start of supplementation.

Table 3.1. Protocol Overview

	Baseline			Follow-up	
FAM	Day 1	Day 2	Days 3-5	Day 6	Day 7
Physical exam	8 h fasting blood sample	8 h fasting blood sample	Daily supplementation	8 h fasting blood sample	8 h fasting blood sample
DXA Body Composition	Side effects questionnaire	Side effects questionnaire		Side effects questionnaire	Side effects questionnaire
Bench Press & Leg Press 1-RM and 70% 1-RM test	Pre-ingestion Hemodynamic Challenge Test	Ingest supplement		Pre-ingestion Hemodynamic Challenge Test	Ingest supplement
Practice 4 km cycling Time Trial	Initial Strength Testing	Wait 30 min		Initial Strength Testing	Wait 30 min
Schedule Testing	Ingest Supplement	4 km Time Trial		Ingest Supplement	4 km Time Trial
Randomize to treatment	Wait 15 min	Side effects questionnaire		Wait 15 min	Side effects questionnaire
	Post-ingestion Hemodynamic Challenge Test			Post-ingestion Hemodynamic Challenge Test	
	Recovery Strength Testing			Recovery Strength Testing	
	Side effects questionnaire			Side effects questionnaire	

Table 3.1 presents the general study design. Participants meeting initial phone screening conditions were invited to attend a familiarization session. During the

familiarization session, participants signed informed consent statements and had a physical exam inclusive of providing their medical history, determination of resting heart rate and blood pressure, and assessment of body composition via dual-energy X-ray absorptiometry (DXA). Once cleared to participate, participants had bench one-repetition maximum (1-RM) determined, performed 3 sets of 10 repetitions on the bench press at 70% of 1-RM, with the last set completed to failure. Participants followed a similar familiarization on the leg press and then rested for 15 min prior to performing a warm-up and a 4 km TT on an electronically-braked cycle ergometer. Participants were then randomized to initiate the study with their respective treatments.

Baseline testing took place on two days. Day 1 included fasting blood, hemodynamic assessment, and strength testing while Day 2 included the 4 km TT. All fasting blood samples were obtained following an 8 h fast primarily between the hours of 0600–0900. Participants performed a pre-supplementation hemodynamic postural challenge test using a tilt table, performed 1-RM and a muscular endurance test (3 sets of 10 repetitions with the last set to failure) on the bench press and leg press. Participants then ingested their assigned RTD, waited 15 min and were placed in the supine position on the tilt table for 15 min prior to performing the postural hemodynamic challenge test. Participants then repeated the 1-RM test and one-set to failure at 70% of 1-RM on the bench press and leg press to assess recovery Table 3.2. The rationale for this approach was to determine whether ingestion of the RTD would

influence exercise capacity after exhaustive exercise and toward the end of a training session. On Day 2, participants ingested the assigned treatment, waited 30 min, performed a standard warm-up, and performed a 4 km cycling TT Table 3.3. Participants continued the supplementation protocol for Days 3 to 5 with follow-up testing on Day 6 and Day 7 to repeat experiments as described Figure 3.1.

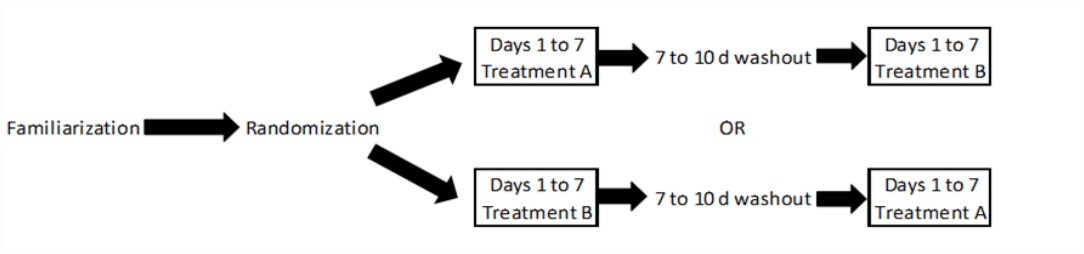

Figure 3.1. Overview of Crossover Design

Table 3.2 Detailed Protocol for Days 1 and 6

Participant arrival
8 h fasting blood sample, anthropomotry, & pre-ingestion side effects questionnaire
Pre-Ingestion Hemodynamic Challenge Test
15 min in supine position
2 min in standing position
Heart rate and blood pressure assessment during final 30 sec of each position
Initial Strength and Endurance
Warmup: 10 x 50%, 5 x 75%, 3 x 90%
1RM attempts interspersed with 2 min rest
Two sets of 10 repetitions at 70% $1RM_{FAM}$ (2 min rests)
One set to volitional fatigue at 70% $1RM_{FAM}$
Ingest treatment and wait 15 minutes
Post-Ingestion Hemodynamic Challenge Test
Same as initial
Recovery Strength and Endurance
Warmup: 10 x 50%, 5 x 75%, 3 x 90%
1-RM attempts interspersed with 2 min rest
One set to volitional fatigue at 70% $1RM_{FAM}$

3.2 Supplementation Protocol

Participants were instructed to maintain normal training, diet, and caffeine intake habits throughout the study. Participants were assigned in a randomized, double-blind, cross-over manner to a placebo (PLA) beverage containing 6.0 g dextrose and non-caloric sweetener or a beverage (RTD) containing caffeine anhydrous (200 mg), _-alanine (2.1 g), niacin (65 mg), folic acid (325 mcg), Vitamin B12 (45 mcg), arginine nitrate (1.3 g providing about 350 mg of nitrates and 950 mg of arginine). A 7 to 10 days washout period was observed between treatment experiments consistent with prior research on caffeine and nitrates using crossover designs. The beverages were prepared by a third party (South East Bottling and Beverage, Dade City, FL, USA) in 10 oz. of purified water matched for color and flavor in indistinguishable bottles. The nutrient contents of the RTD's were analyzed for contaminants and nutrient content by Century Foods International (Sparta, WI, USA). The pre-packaged bottles were received in boxes containing sealed bottles generically labeled as "Treatment A" and "Treatment B" for double-blind administration. The supplement code was maintained in a sealed envelope and was not disclosed to the researchers until the completion of the study for statistical analysis.

3.3. Test Methodology

3.3.1. Anthropometry & 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 measures (excluding cranium) were determined with a Hologic Discovery W Dual-Energy X-ray Absorptiometer (Hologic Inc., Waltham, MA, USA) equipped with APEX Software (APEX Corporation Software, Pittsburg, PA, USA) by using standardized procedures [207, 208]. Mean test-retest reliability studies performed on male athletes in our lab over repeated standardized assessment procedures have demonstrated coefficients of variation 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 [208]. On the day of each test, the equipment was calibrated following the manufacturer's guidelines.

3.3.2. Blood Collection Procedures

Participants provided an 8 h fasted blood sample via venipuncture of an antecubital vein in the forearm in accordance with 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 (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and in one 3.5 mL BD Vacutainer® K2 EDTA tube (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Both tubes sat 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 ^{o}C refrigerated bench top ThermoScientific Heraeus MegaFuge 40R Centrifuge (Thermo Electron North America LLC, West Palm Beach, FL, USA). Both tubes were stored at 4 ^{o}C for 3 to 4 h prior to analysis or storage. Serum was stored at -80 ^{o}C in polypropylene microcentrifuge tubes for later analysis.

3.3.3. 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 daily per manufacturer guidelines. This analyzer has been known to be valid and reliable in previously published reports [209]. The internal quality control for the Cobas® c111 is performed using two levels of control fluids purchased from the manufacturer to calibrate acceptable standard deviation (SD) and coefficient of variation (CV) values for all assays. Samples were re-run if the values observed were outside control values and/or clinical norms according to standard procedures. Prior analysis in our lab has yielded test-to-test reliability of a range of CV

from 0.4 to 2.4% for low control samples and 0.6–1.9% on high controls. Precision has been found between 0.8 and 2.4% on low controls and 0.5–1.7% on high controls.

3.3.4. Hemodynamic Challenge Test

During the strength testing days (Days 1 and 6) participants had hemodynamic response assessed at two time points, prior to initial strength testing measures and following supplementation. Participants were placed on a standard tilt table in a supine position (Gravity 4000 Inversion Table; City of Industry, CA, USA). After 15 min, blood pressure and heart rate were assessed and recorded. Next, the tilt table was adjusted to vertical where the participant rested for 2 min and the metrics were re-assessed. Participants then performed pre-supplementation muscular strength and endurance tests, ingested the assigned treatment, and rested for 15 min prior to be placing on the tilt table in the supine position for 15 min. Heart rate and blood pressure measurements were then taken prior to and after 2 min of being moved to a vertical position. Mean arterial pressure was calculated as ((2 x DBP) + SBP)/3 as an indicator of venous return. Rate pressure product (RPP) was calculated as the product of heart rate and systolic blood pressure to represent an indirect assessment of myocardial oxygen demand. The latter two tests were chosen as they represent a more robust response to a cardiovascular challenge compared to heart rate or blood pressure alone. Hemodynamic response was defined as the change in systolic blood pressure, diastolic

blood pressure, heart rate, mean arterial pressure and rate pressure product from the supine to upright position.

3.3.5. Self-Reported Side Effects

The side effect questionnaires were completed before and after each testing session to access perceived side effects and monitor compliance with the supplementation protocol. The questionnaires were completed a total of 16 times by each participant over the duration of the study: two times each testing day for four testing days per supplement for two different supplements. Participants were asked to rank the frequency and severity of their symptoms—dizziness, headache, tachycardia, heart skipping or palpitations, shortness of breath, nervousness, blurred vision, and unusual or adverse effects. Participants were asked to rank their perception of symptoms using the following scale: 0 (none), 1 (minimal: 1–2/week), 2 (slight: 3–4/week), 3 (occasional: 5–6/week), 4 (frequent: 7–8/week), or 5 (severe: 9 or more/week).

3.3.6. Strength Testing

Participants performed three warm up sets prior to performing 1-RM attempts (i.e., one set of 10 at 50%, one set of 5 at 70%, and one set of 3 at 90% of anticipated 1-RM). Following the warm-up, participants gradually increased weight between 1-RM attempts until they could not lift the load under their own volition. Following

determination of 1-RM, participants performed two sets of 10 repetitions with 2 min rest recovery between sets at the closest bar/leg press weight corresponding to 70% of familiarization session 1-RM. Participants then rested 2 min and performed a third set to failure. After 2 min of rest, participants followed the same procedure to determine leg press 1-RM and leg press muscular endurance. Hand placement on the bench press bar and seat and foot positioning on the leg press were placed in the same position among attempts and testing sessions Table 3.4.

The initial strength tests were performed to pre-fatigue the participant before assessing recovery performance after RTD ingestion. The recovery muscular strength and endurance performance assessment involved performing a 1-RM test and then one set to failure at 70% of the familiarization 1-RM following similar procedures as described above. In this way, the effects of acute RTD ingestion could be assessed on muscular strength and endurance recovery following a standard bout of resistance exercise on Day 1, the effects of 6 days of RTD ingestion could be assessed on initial muscular strength and performance on Day 6, and the effects of acute RTD ingestion on recovery of exhaustive exercise could be assessed after 6 days of supplementation on Day 6. The two sets of 10 repetitions were not conducted during recovery analysis as the initial bout of exercise fatigued the participants; it was unlikely participants could complete all 10 repetitions of these sets and it was unnecessary to assess recovery muscular endurance. Total 1-RM weight lifted in kg and the number repetitions performed each set using 70% of the familiarization weight (rounded to the nearest

2.27 kg or 5 lbs. that could be put on the bar) were recorded. Total lifting volume was calculated by multiplying the 70% of 1-RM weight lifted times the number of repetitions performed each set and summing the total volume performed for all sets. Total combined lifting volume was calculated by adding the bench press and leg press total lifting volumes. Day to day test reliability of performing this performance test in our lab on resistance-trained participants has yielded a CV of 0.34 and an intraclass correlation coefficient of 0.99 for three sets of bench press total lifting volume and an intraclass correlation coefficient of 0.96 for three sets of leg press total lifting volume.

3.3.7. Time-Trial Performance

Time-trial performance was examined on a magnetically braked cycle ergometer (Lode Sport Excalibur, Groningen, The Netherlands) over a distance of 4 km. Participants were allowed a one minute warm up with a gradually increasing load. At the completion of the warm up, a standardized resistance (4 J/kg/rev) was applied and the participant was instructed to complete the distance in as short a time as able. Upon completion, the participant was instructed to continue at a slow pace to facilitate recovery. Data were recorded as time to completion and average power in Watt.

Table 3.4. Strength and Endurance Protocol

	Initial	Rest	Recovery
Warm-up	10 reps @ 50% 5 reps @ 70% 3 reps @ 90%	Ingest supplement Seated rest (15 min)	10 reps @ 50% 5 reps @ 70% 3 reps @ 90%
Strength	1-RM attempts		1-RM attempts
Endurance	10 reps @ 70% 10 reps @ 70% RtF @ 70%	Hemodynamic Challenge Test	RtF @ 70%

* All percentages calculated from 1-RM at familiarization

** All sets separated by 2 min seated rest

3.4. Statistical Analysis

Data were analyzed using IBM® SPSS® Version 24 software (IBM Corp., Armonk, NY, USA). The sample size was determined based on the expectation of a five percent improvement in exercise performance and corresponding power of 0.80. The analysis was initiated by inspecting data for missing values using Little's test for data missing completely at random (MCAR). This analysis showed the data were MCAR (p = 1.0, <1.5%) and subsequently replaced using a multiple imputation algorithm. Data were then analyzed using univariate, multivariate and repeated measures general linear models (GLM) using gender and relative caffeine intake (mg/kg) as covariates using the following models:

Model 1: The cohort was examined for potential gender-by-treatment effects, finding none. Hence, the data were pooled into one cohort instead of reporting gender data separately.

Model 2: Since menstrual cycle, birth control medications, and other gender-related parameters were not controlled, gender was included as a covariate.

Model 3: Given the weight difference between males and females in the study, the analysis was further adjusted for relative caffeine intake (mg/kg). The results are presented for Model 3 with performance-related data expressed in absolute and relative terms to fat free mass.

Data were also examined for a treatment order effect to confirm that randomization procedures were effective. Least significant difference post hoc comparisons were used to compare between-treatment differences when significant time _ treatment interaction effects were observed. Hematological variables were also examined relative to normal clinical limits to examine the frequency of changes in hematology outside of normal, clinical limits from baseline to follow-up a Chi-square and adjusted residual analyses. This analysIs examined the likelihood of excursions outside of clinical limits for each treatment as follows: (1) No change; (2) Normal at Baseline, High at Follow-up; (3) High at Baseline, High at Follow-up; (4) High at Baseline, Normal at Follow-up. Data are reported as mean (SD), mean change from baseline and 95% confidence intervals, and frequency of occurrence according to the chi-square analysis. Data were considered statistically significant when the probability of type I error was 0.05 or less while tendencies towards statistical significance were noted when p-levels were $p > 0.05$ to $p < 0.10$.

CHAPTER IV

RESULTS*

4.1. Results

The results presented are previously published in the Journal of Nutrients [206]. Data, figures, and tables are presented as they are within the published manuscript.

4.2. Participants

Thirty-one participants initially signed informed consent prior to data collection; however, five participants dropped out prior to baseline testing due to schedule or personal reasons. Twenty-six participants began the study, with one male dropping out after the first baseline session due to time constraints. Data from a total of 25 participants were included in statistical analysis. Participant demographic data are presented in Table 4.1.

These data demonstrate that the participants were recreationally active resistance-trained individuals and that participants differed based on gender on a number of variables. No time x gender x treatment interactions were observed on variables evaluated or relative caffeine intake effect. Further, the fully adjusted statistical model did not produce a substantial difference to the unadjusted model.

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Nevertheless, since menstrual cycle and birth control medication were not controlled, results were adjusted via covariate analysis for gender and relative caffeine intake.

Table 4.1. Baseline Demographics

Measurement	Male (*n* = 12)		Female (*n* = 13)		Overall (*n* = 25)		
	Mean	SD	Mean	SD	Mean	SD	*p*-values
Age (y)	23.3	± 4	24.5	± 4	23.9	± 4	0.43
Height (cm)	177	± 7	166	± 5	171	± 8	0.00
Weight (kg)	81.7	± 13	65.1	± 8	73.1	± 13	0.00
Body Mass Index (kg/m^2)	26.0	± 4	23.7	± 3	24.8	± 4	0.12
Body Fat (%)	17.2	± 6	28.4	± 6	23.0	± 8	0.00
Fat Free Mass (kg)	67.1	± 9	47.8	± 8	57.1	± 13	0.00
Bench Press 1-RM (kg)	88.3	± 27	37.9	± 10	62.1	± 32	0.00
Bench Press 1-RM (kg/kg_{FFM})	1.31	± 0.4	0.80	± 0.2	1.05	± 0.4	0.00
Leg Press 1-RM (kg)	455	± 175	284	± 89	366	± 160	0.01
Leg Press 1-RM (kg/kg_{FFM})	6.7	± 2.0	6.0	± 1.6	6.3	± 1.8	0.18

Mean data presented as means ± SD. One-way ANOVA *p*-values listed for each variable. PLA: placebo, RTD: ready-to-drink pre-workout supplement, 1RM: one repetition maximum, FFM: fat free mass, kg/kg_{FFM}: weight relative to participant fat free mass

4.3. Performance

Table 4.2 presents muscular strength and performance results normalized to fat free mass (FFM). Multivariate analysis revealed a significant overall Wilks' Lambda treatment x time interaction effect ($p = 0.01$). Univariate analysis revealed significant treatment x time interactions in bench press ($p = 0.04$) and leg press repetitions to failure ($p = 0.04$) while bench press lifting volume ($p = 0.09$) and total combined lifting volume ($p = 0.09$) evidenced a statistical trend toward significant interaction. Post-hoc analysis revealed that acute RTD ingestion on Day 1 significantly improved recovery bench press muscular endurance to a greater degree than following PLA ingestion.

Table 4.2. Strength and Muscular Endurance Relative to Fat Free Mass

Variable	Treatment	Day 1 Initial			Day 1 Recovery			Day 6 Ingestion			Day 6 Recovery			Treatment				
		Mean		SD	Mean		SD	Mean		SD	Mean		SD	Mean		SE	*p*-values	
BP 1-RM (kg/kg_{FFM})	PLA	1.02	±	0.38	0.94	±	0.36	1.01	±	0.36	0.97	±	0.37	0.99	±	0.06	Time	0.001
	RTD	1.03	±	0.37	0.99	±	0.35	1.04	±	0.35	1.00	±	0.36	1.02	±	0.06	Trt	0.72
	Time	1.02	±	0.37	0.97	±	0.35*	1.03	±	0.35	0.99	±	0.36‡				*I*	0.23
BP Repetitions to Failure @ 70 % 1-RM	PLA	9.96	±	3.23	9.60	±	3.65	10.13	±	3.37	12.27	±	3.22*	10.5	±	0.71	Time	0.38
	RTD	10.28	±	4.37	12.32	±	5.28*†	10.30	±	4.04	13.31	±	4.86*	11.6	±	0.71	Trt	0.27
	Time	10.1	±	3.81	11.0	±	4.70	10.2	±	3.69	12.8	±	4.12				*I*	0.04
BP Lifting Volume (kg/kg_{FFM})	PLA	7.40	±	3.77	7.46	±	4.57	7.46	±	3.73	9.17	±	4.44	7.87	±	0.67	Time	0.36
	RTD	7.45	±	3.75	8.89	±	4.07	7.48	±	3.16	9.45	±	3.54	8.32	±	0.67	Trt	0.64
	Time	7.43	±	3.73	8.17	±	4.34	7.47	±	3.42	9.31	±	3.98				*I*	0.09
LP 1-RM (kg/kg_{FFM})	PLA	6.70	±	1.66	6.41	±	1.58	6.80	±	1.38	6.38	±	1.35	6.57	±	0.26	Time	0.66
	RTD	6.72	±	1.42	6.74	±	1.41	6.83	±	1.29	6.67	±	1.52	6.74	±	0.26	Trt	0.66
	Time	6.71	±	1.53	6.57	±	1.49	6.81	±	1.33	6.53	±	1.43				*I*	0.04
LP Repetitions to Failure @ 70% 1-RM	PLA	21.2	±	10.8	18.6	±	8.4	20.8	±	10.7	20.9	±	11.0	20.3	±	1.81	Time	0.78
	RTD	22.4	±	15.1	26.4	±	13.0†	19.8	±	9.31	25.1	±	14.1	23.9	±	1.80	Trt	0.17
	Time	21.8	±	13.0	22.5	±	11.6	20.3	±	9.94	23.0	±	12.7				*I*	0.11
LP Lifting Volume (kg/kg_{FFM})	PLA	94.3	±	77.9	81.1	±	44.4	88.3	±	49.7	91.9	±	57.9	88.9	±	10.60	Time	0.75
	RTD	96.6	±	66.7	116.3	±	74.9‡	90.7	±	47.6	106.9	±	55.4	102.6	±	10.60	Trt	0.37
	Time	95.5	±	71.8	98.7	±	63.5	89.5	±	48.2	99.4	±	56.6				*I*	
Combined Lifting Volume (kg/kg_{FFM})	PLA	101.7	±	79.0	88.6	±	46.8	95.8	±	50.5	101.1	±	60.2	96.8	±	10.87	Time	0.76
	RTD	104.1	±	66.4	125.2	±	75.5	98.2	±	48.8	116.4	±	57.3	110.0	±	10.87	Trt	0.36
	Time	102.9	±	72.2	106.9	±	64.9	97.0	±	49.2	108.8	±	58.7				*I*	0.09

Values are means ± standard deviations. Multivariate analysis revealed overall Wilks' Lambda treatment ($p = 0.792$), time ($p = 0.010$), and treatment x time ($p = 0.010$). Greenhouse-Geisser *p*-levels are reported with univariate analyses for time, treatment, and time x treatment interactions for each variable. * indicates a significant difference from initial measure, † indicates a significant between-treatment difference, and ‡ indicates a statistical trend between-treatments. BP = bench press, LP = leg press, 1-RM = one repetition maximum, FFM = fat free mass, PLA = placebo, RTD = ready-to-drink pre-workout supplement, Trt = treatment, *I* = time x treatment interaction

Pair-wise differences were also observed between treatments in Day 1 recovery leg press endurance (p = 0.01) and tended to improve leg press lifting volume (p = 0.054). No significant differences were observed between groups in follow-up assessments. Similar findings were observed when analyzing absolute performance results. No significant differences were observed among treatments in cycling performance time or average power output expressed in absolute (W) or relative (W/kg_{FFM}) terms (Table 4.3).

Figures 4.1–4.4 show mean changes from baseline with 95% CI's for 1-RM, repetitions to failure (RtF), lifting volume, and time-trial performance data, respectively. Acute RTD ingestion tended to maintain BP 1-RM to a greater degree (PLA: -0.071 (-0.09, -0.05); RTD: -0.043 (-0.05, -0.01) kg/kg_{FFM}, p = 0.086) and maintained leg press 1-RM performance (PLA: -0.285 (-0.49, -0.08); RTD: 0.23 (-0.50, 0.18) kg/kg_{FFM}, p = 0.30) compared to PLA Figure 4.1. After 6 d of supplementation, recovery LP 1-RM significantly decreased in the PLA but not RTD treatment (PLA: -0.412 (-0.08, -0.07); RTD: 0.16 (-0.50, 0.18) kg/kg_{FFM}, p = 0.30). Recovery RtF on the BP tended to be greater in the RTD versus PLA treatment on Day 1 (PLA: -4.41 (-5.8, -3.0); RTD: -2.59 (-4.0, -1.19) repetitions, p = 0.072) while LP RtF was significantly greater than PLA (PLA: -2.60 (-6.8, 1.6); RTD: 4.00 (-0.2, 8.2) repetitions, p = 0.031) FIGURE 4.2. On Day 6, RtF on the LP was significantly increased above baseline in the RTD group but not PLA (PLA: 0.12 (-3.0, 3.2); RTD: 3.6 (0.5, 6.7) repetitions, p = 0.116). Bench press lifting volume (Figure 4.3) was significantly increased above baseline and was significantly greater than PLA

(PLA: 0.001 (-0.13, 0.16); RTD: 0.03 (0.02, 0.04) kg/kg_{FFM}, p = 0.007) while LP lifting volume tended to be greater (PLA: -13.18 (-36.9, 10.5); RTD: 19.6 (-4.1, 43.3) kg/kg_{FFM}, p = 0.055) in the RTD treatment on Day 1. On Day 6, LP lifting volume was increased above baseline values in the RTD but not PLA treatment (PLA: 3.64 (-8.8, 16.1); RTD: 16.25 (3.8, 28.7) kg/kg_{FFM}, p = 0.157). Recovery total lifting volume was significantly greater for RTD compared to PLA (PLA: -13.12 (-36.9, 10.5); RTD: 21.06 (-2.7, 44.8) kg/kg_{FFM}, p = 0.046) on Day 1 and was increased above baseline while remaining unchanged with PLA treatment on Day 6 (PLA: 5.35 (-7.4, 18.1); RTD: 18.22 (5.5, 30.9) kg/kg_{FFM}, p = 0.157). Finally, cycling performance times and power output improved to a greater degree in the PLA trial from baseline (PLA: -11.48 (-22.3, -1.73); RTD: -5.72 (-15.5, 4.03) s; PLA: 0.289 (0.09, 0.49); RTD: 0.122 (-0.08, 0.32) W/kg_{FFM}) FIGURE 4.4. However, it should be noted that baseline and follow-up performance times were faster in the RTD trials than the PLA trials (see Table 4.3) so it cannot be concluded that the RTD promoted an ergolytic effect. It was hypothesized the RTD would yield significant improvement in muscular strength and endurance compared to PLA (H_1). Based on the findings, there is sufficient evidence to accept this hypothesis. It was hypothesized the RTD would demonstrate significant improvement in time trial performance measured by time to completion and average power (H_2). Based on the collective findings, there is insufficient evidence to accept this hypothesis; the RTD does not improve 4km time trial performance.

Table 4.3. Time Trial Performance

Variable	Treatment	Day 2			Day 7			Treatment				
		Mean		SD	Mean		SD	Mean		SE	*p*-values	
Time (s)	PLA	296	±	105	284	±	104	240	±	11	Time	0.70
	RTD	282	±	94	276	±	95	281	±	11	Trt	0.56
	Time	286	±	99	280	±	99				*I*	0.41
Power (W)	PLA	224	±	82	242	±	93	235	±	10	Time	0.12
	RTD	238	±	85	246	±	95	240	±	10	Trt	0.62
	Time	231	±	83	244	±	82				*I*	0.26
Power (W/kg_{FFM})	PLA	3.87	±	0.89	4.16	±	0.94	4.01	±	0.17	Time	0.26
	RTD	4.17	±	0.87	4.29	±	0.98	4.23	±	0.17	Trt	0.38
	Time	4.02	±	0.89	4.22	±	0.95				*I*	0.25

Values are means ± standard deviations. Multivariate analysis revealed overall Wilks' Lambda treatment (p = 0.62), time (p = 0.036), and treatment x time (p = 0.53). Greenhouse-Geisser p-levels are reported with univariate analyses for time, treatment, and time x treatment interactions for each variable. PLA = placebo, RTD = ready-to-drink pre-workout supplement, FFM = fat free mass, Trt = treatment, *I* = time x treatment interaction

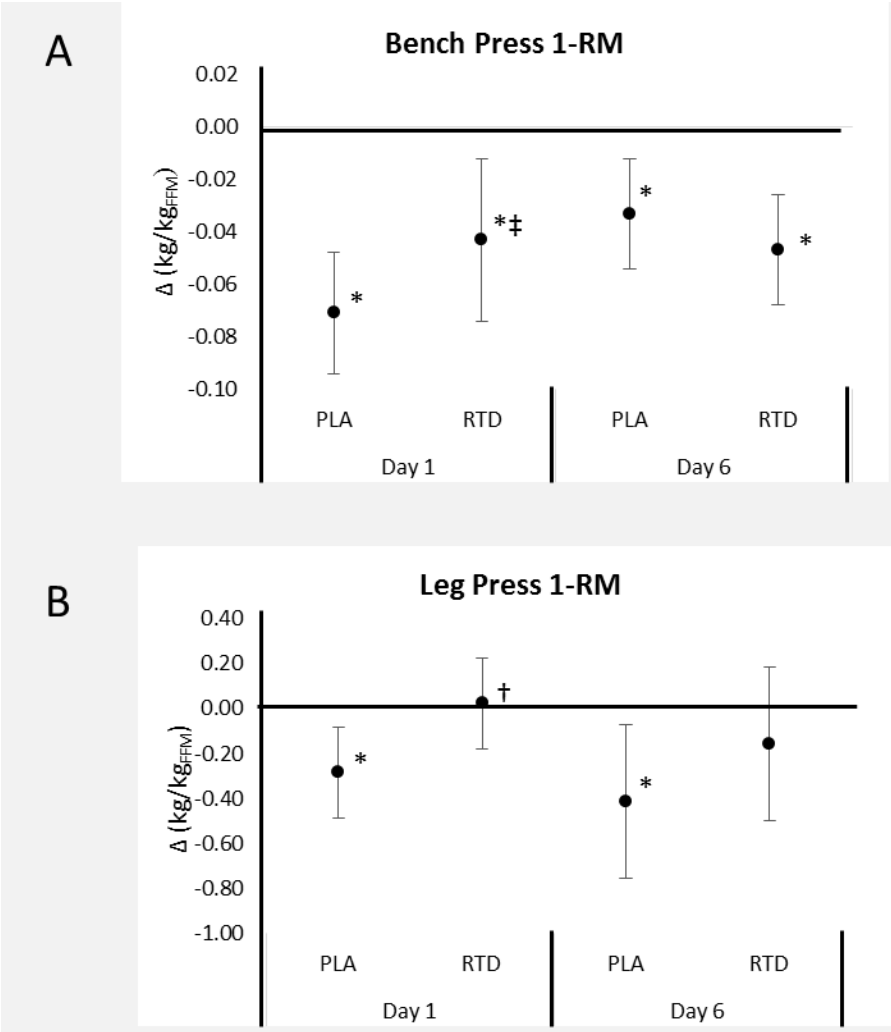

Figure 4.1. Data present mean change (95% CI) in bench press (**Panel A**) and leg press (**Panel B**) one repetition maximum (1-RM) from baseline. Confidence intervals not crossing zero are statistically significant ($p < 0.05$). * represents $p < 0.05$ difference from baseline, † represents $p < 0.05$ between-treatments, ‡ represents $p < 0.05$ to 0.10 trend towards significance between-treatments.

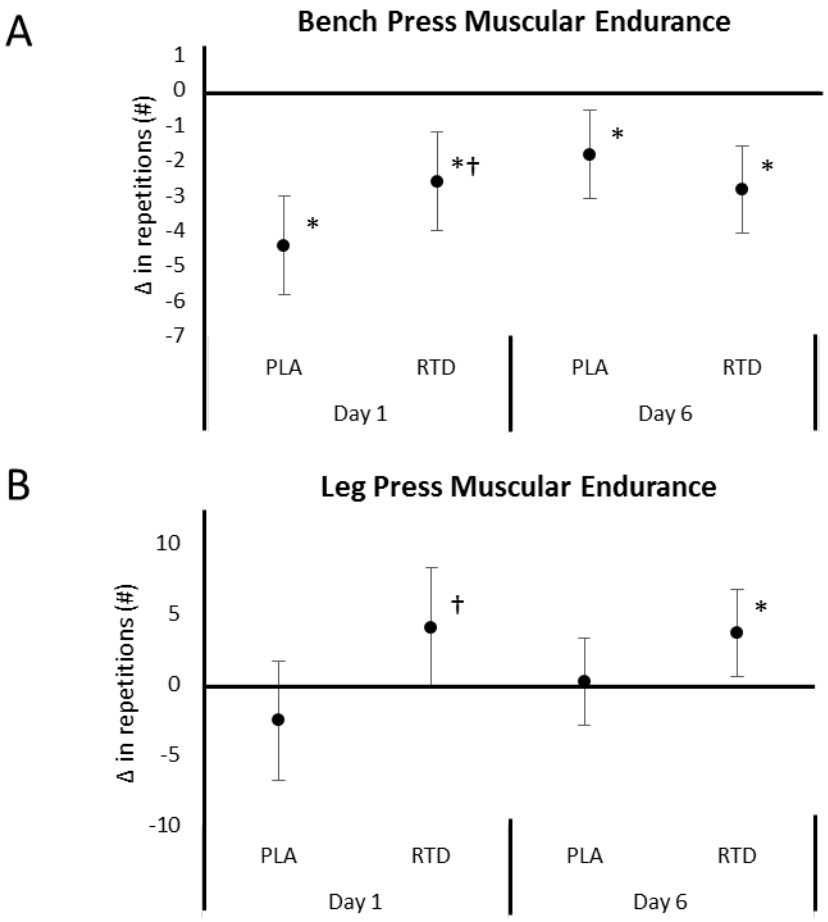

Figure 4.2. Data present mean change (95% CI) in bench press (**Panel A**) and leg press (**Panel B**) muscular endurance repetitions to failure at 70% of one repetition maximum from familiarization (1-RM_{FFM}) from baseline. Confidence intervals not crossing zero are statistically significant ($p < 0.05$). * represents $p < 0.05$ difference from baseline, † represents $p < 0.05$ between-treatments, ‡ represents $p < 0.05$ to 0.10 trend towards significance between-treatments.

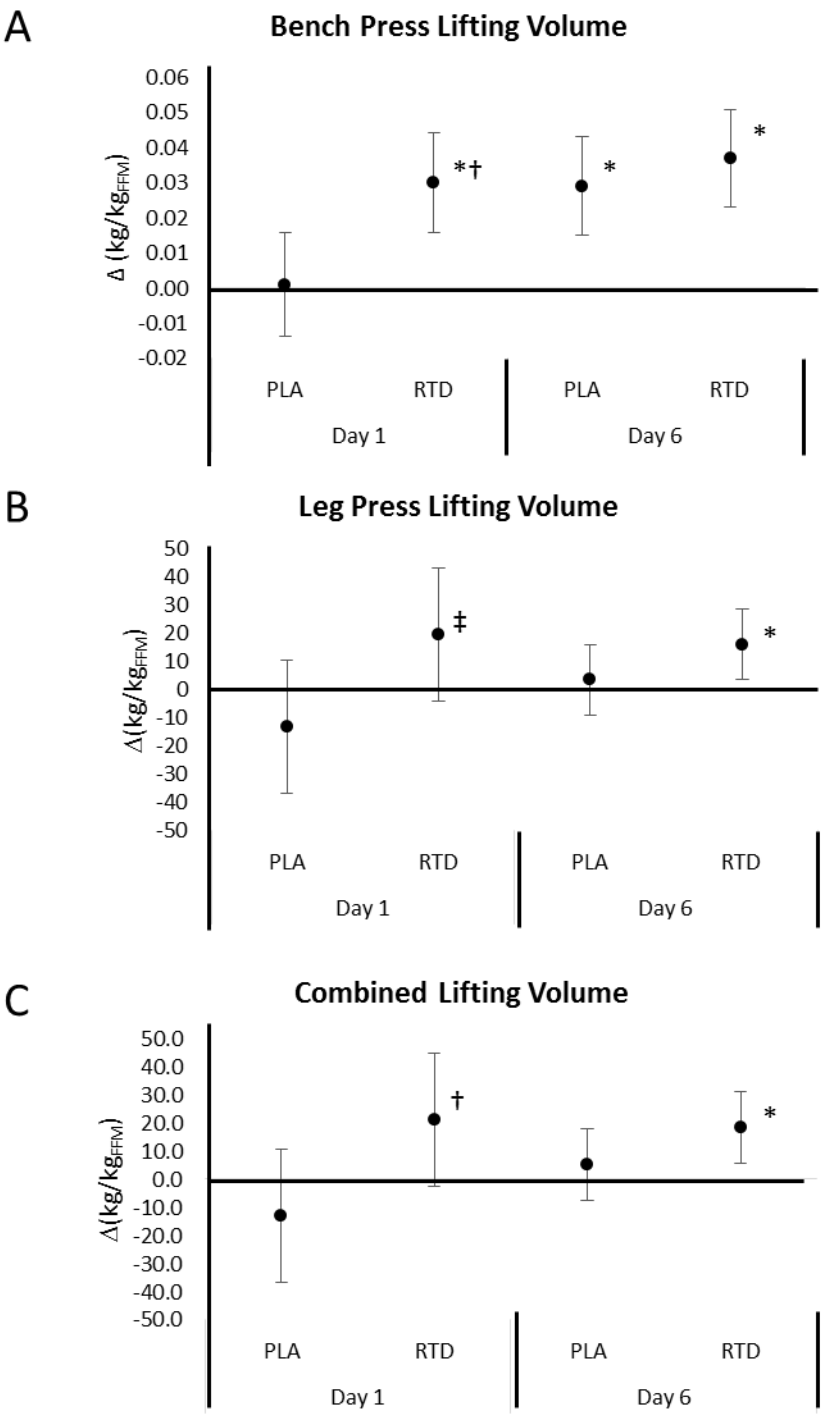

Figure 4.3. Data present mean change (95% CI) in bench press (**Panel A**), leg press (**Panel B**), and total (combined) lifting volume (**Panel C**) from baseline. Confidence intervals not crossing zero are statistically significant ($p < 0.05$). * represents $p < 0.05$ difference from baseline, † represents $p < 0.05$ between-treatments, ‡ represents $p < 0.05$ to 0.10 trend towards significance between-treatments.

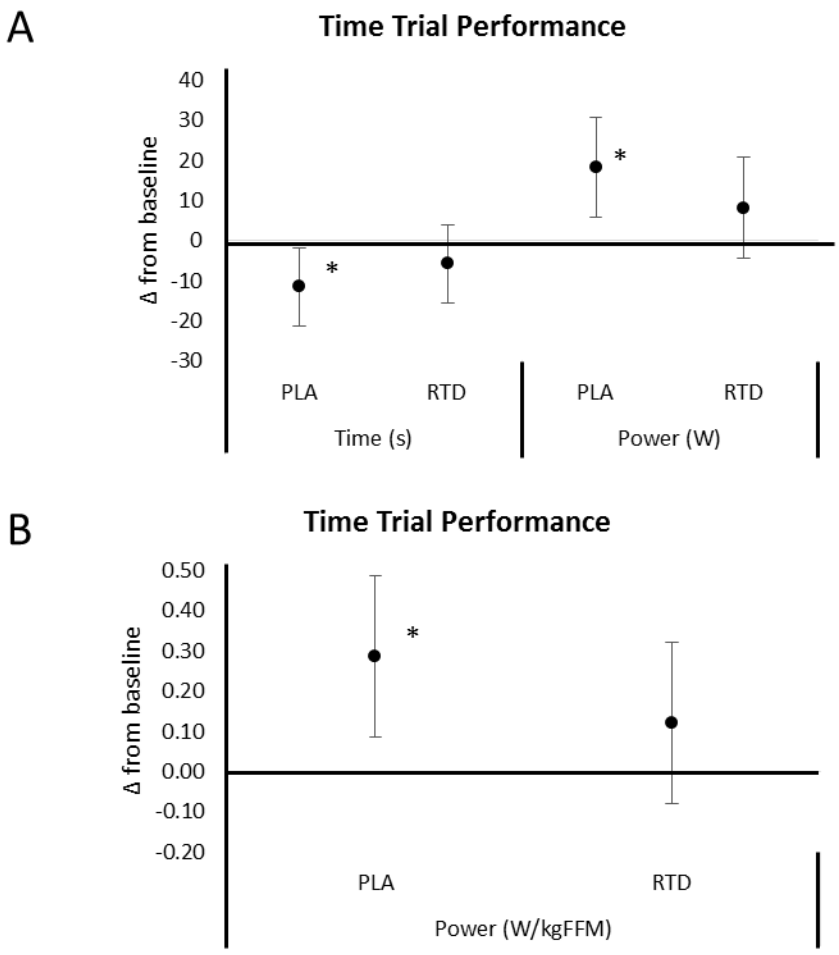

Figure 4.4. Data present mean change (95% CI) in 4 km time trial performance from baseline expressed in completion time and absolute power output (**Panel A**) and relative power output (**Panel B**). Confidence intervals not crossing zero are statistically significant ($p < 0.05$). * represents $p < 0.05$ difference from baseline, † represents $p < 0.05$ between-treatments, ‡ represents $p < 0.05$ to 0.10 trend towards significance between-treatments.

4.4. Safety Analysis

Table 4.4 presents hemodynamic challenge results. Although some time effects were observed as expected when changing postural position, no significant overall multivariate interaction effects ($p = 0.15$) or univariate interaction effects were observed between treatments in HR, SBP, DBP, MAP, or RPP.

Analysis of 95% CI changes from baseline revealed that HR increased from baseline on Day 1 in both groups [PLA: 8.94 (5.36, 12.5); RTD: 8.35 (4.7, 12) bpm]; only PLA yielded a significant increase in HR from supine to standing [6.41 (2.91, 9.92) bpm] compared to RTD post-ingestion [2.4 (-1.2, 6) bpm]. After 6 d of supplementation, both treatments evidenced an increase in HR responses from pre-ingestion [PLA: 10.84 (6.91, 14.8); RTD: 7.08 (3.06, 11.1) bpm]. Following ingestion, HR in the PLA treatment increased [9.35 (5.73, 13) bpm] significantly more than the RTD [3.1 (-0.5, 6.9) bpm] FIGURE 4.5a. PLA increased SBP from supine to standing pre-ingestion after 6 d of supplementation [2.08 (0.05, 4.11) mmHg] compared to RTD [0.5 (-1.57, 2.57) mmHg] with no difference on Day 1 FIGURE 4.5b. DBP significantly increased in PLA pre-ingestion on Day 6 [2.92 (1.12, 4.72) mmHg] and no difference in RTD [0.83 (-1.01, 2.68) mmHg] FIGURE 4.5c. MAP yielded a significant increase in both groups at Day 1 after transitioning from supine to standing [PLA: -13.7 (-17.2, -10.1); RTD: -16.7, (-20.3, -13.1) mmHg]. After 6 d of treatment PLA demonstrated an increase in MAP following ingestion [14.3 (7.35, 21.2) mmHg] and RTD was unchanged [5.25 (-1.8, 12.3) mmHg] FIGURE 4.6a. There were no significant changes for either treatment related to RPP

FIGURE 4.6b. Overall, blood pressure and heart rate values observed remained low and were well within normal values for apparently healthy younger individuals.

Similarly, no overall multivariate or univariate effects were observed among serum or whole blood markers analyzed (Tables 4.5-4.7) or when analyzing the frequency of changes in blood parameters outside of normal clinical ranges (Table 4.8). Finally, as shown in Tables 4.9 and 4.10, no significant differences were observed between treatments in perceived side effects monitored (i.e., headache, dizziness, tachycardia, palpitations, dyspnea, nervousness, or blurred vision). As the data provided no overall significant effect in hemodynamic response, blood chemistry, or frequency and severity of side effects, sufficient evidence exists to accept H_3; RTD did not have a significant effect on indices of safety.

Table 4.4. Hemodynamic Response to Postural Challenge

Variable	Treatment	Day 1 Pre-Ingestion				Day 1 Post-Ingestion				Day 1 Pre-Ingestion				Day 1 Pre-Ingestion				Treatment		*p* -value	
		Supine		Standing		Supine		Standing		Supine		Standing		Supine		Standing					
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SE		
HR	PLA	61.5	± 9.79	70.6	± 12.3	65.0	± 8.00	71.4	± 14.1	59.0	± 7.86	67.7	± 10.1	68.1	± 7.84	77.1	± 15.2	67.9	± 1.40	Time	0.004
	RTD	60.4	± 8.04	68.6	± 10.9	66.6	± 11.7	68.9	± 12.2	60.6	± 7.21	67.8	± 9.50	65.9	± 10.0	69.3	± 12.0	66.0	± 1.42	Trt	0.35
	Time	61.0	± 8.88	69.6	± 11.5	65.8	± 9.95	70.1	± 13.2*	59.8	± 7.51	68.8	± 9.77	67.0	± 9.00	73.2	± 14.3*			I	0.19
SBP	PLA	114	± 8.06	115	± 9.85	116	± 7.80	115	± 9.83	112	± 4.62	114	± 7.29	114	± 5.42	113	± 7.39	114	± 0.76†	Time	<0.001
	RTD	116	± 6.47	116	± 7.41	118	± 7.76	118	± 7.24	116	± 5.56	117	± 5.80	116	± 8.86	116	± 8.68	117	± 0.77†	Trt	0.019
	Time	115	± 7.29	115	± 8.65	117	± 7.65*	116	± 8.69	114	± 5.44*	115	± 6.68	115	± 6.61*	115	± 8.00			I	0.84
DBP	PLA	73.0	± 7.44	72.6	± 9.00	73.0	± 6.88	73.5	± 7.90	73.0	± 6.76	75.9	± 7.41	74.8	± 7.55	75.2	± 7.85	74.0	± 0.78	Time	0.13
	RTD	74.8	± 7.00	74.6	± 6.05	75.6	± 4.69	75.4	± 5.91	72.0	± 7.82	72.9	± 7.66	75.2	± 6.46	75.6	± 6.88	74.6	± 0.80	Trt	0.62
	Time	73.9	± 7.21	73.6	± 7.65	74.3	± 5.97	74.4	± 6.97	72.5	± 7.24	74.4	± 7.61	75.0	± 6.96	75.4	± 7.31			I	0.18
MAP	PLA	86.7	± 6.60	72.6	± 9.00	73.0	± 6.88	73.5	± 7.90	73.0	± 6.76	75.9	± 7.41	74.8	± 7.55	75.2	± 7.85	75.7	± 0.75	Time	0.44
	RTD	88.4	± 5.67	74.6	± 6.04	75.6	± 4.69	75.4	± 5.91	72.0	± 7.82	72.9	± 7.66	75.2	± 6.45	75.6	± 6.88	76.3	± 0.77	Trt	0.63
	Time	87.6	± 6.15	73.6	± 7.65	74.3	± 5.97	74.4	± 6.97	72.5	± 7.24	74.4	± 7.61	75.0	± 6.96	75.4	± 7.31			I	0.18
RPP	PLA	70.2	± 12.5	80.9	± 14.9	75.2	± 70.7	82.1	± 19.3	80.9	± 15.0	79.4	± 12.3	82.1	± 19.3	87.4	± 18.5	77.6	± 1.77	Time	0.001
	RTD	69.8	± 9.69	79.5	± 13.3	78.6	± 15.1	81.4	± 16.5	79.9	± 13.4	79.1	± 12.0	82.3	± 16.2	80.4	± 16.4	77.2	± 1.80	Trt	0.87
	Time	70.0	± 11.04	80.2	± 14.0	76.9	± 13.1	81.7	± 18.0	80.4	± 14.1*	79.2	± 12.0	82.2	± 17.6	83.9	± 17.3 *			I	0.23

Values are means ± standard deviations. Multivariate analysis revealed overall Wilks' Lambda treatment ($p = 0.055$), time ($p < 0.001$), and treatment x time ($p = 0.47$). Greenhouse-Geisser p-levels are reported with univariate analyses for time, treatment, and time x treatment interactions for each variable. * indicates significant change from baseline ($p < 0.05$). HR=Heart rate, SBP=Systolic blood pressure, DBP=Diastolic blood pressure, MAP=Mean arterial pressure, RPP=Rate pressure product, Trt=Treatment, I=Time x Treatment interaction, PLA=Placebo, RTD=Ready-to-drink Pre-workout supplement

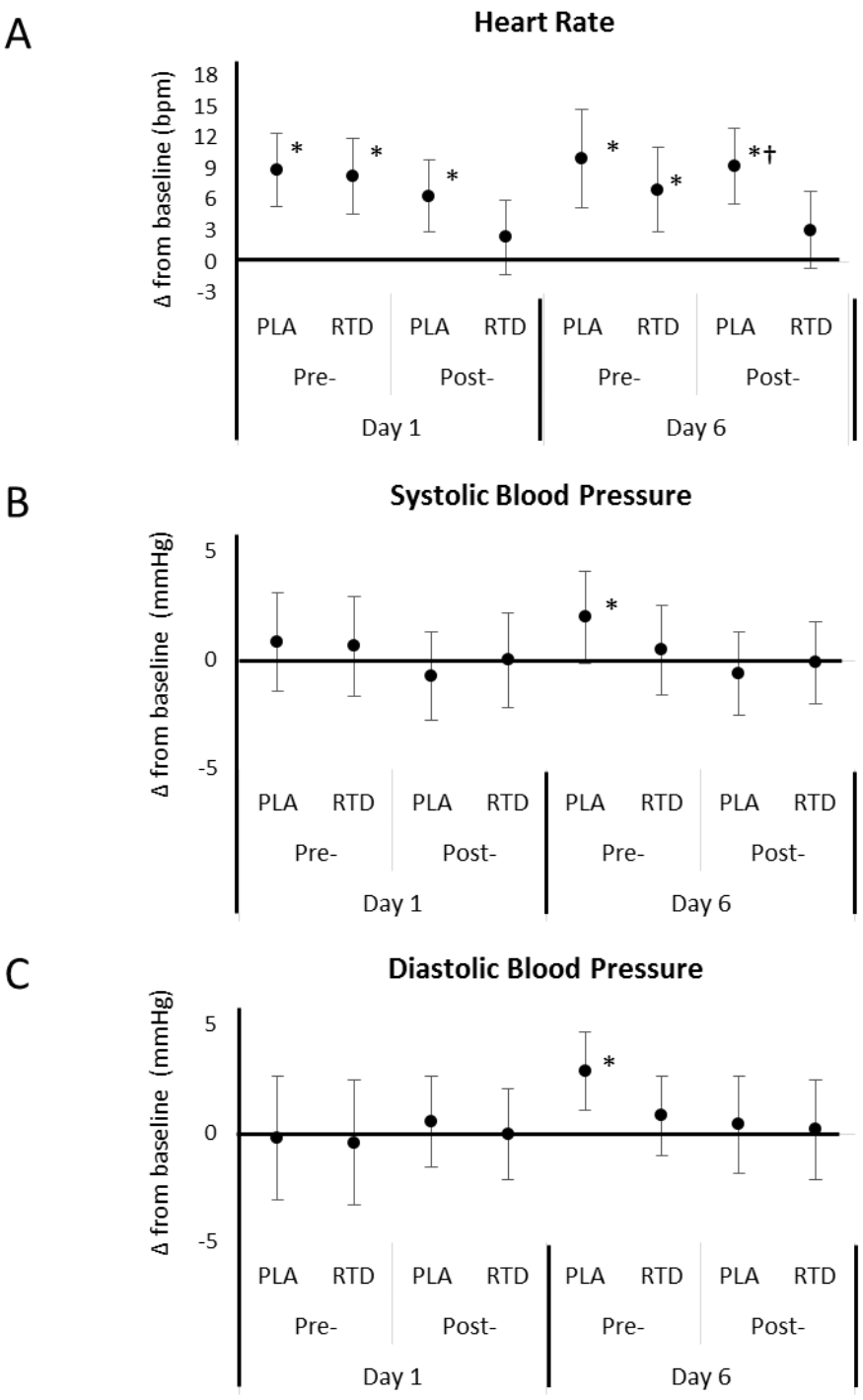

Figure 4.5. Data present mean change (95% CI) in response to the hemodynamic challenge test for heart rate (**Panel A**), systolic blood pressure (**Panel B**), and diastolic blood pressure (**Panel C**) Confidence intervals not crossing zero are statistically significant ($p < 0.05$). * represents $p < 0.05$ difference from baseline, † represents $p < 0.05$ between-treatments, ‡ represents $p < 0.05$ to 0.10 trend towards significance between-treatments.

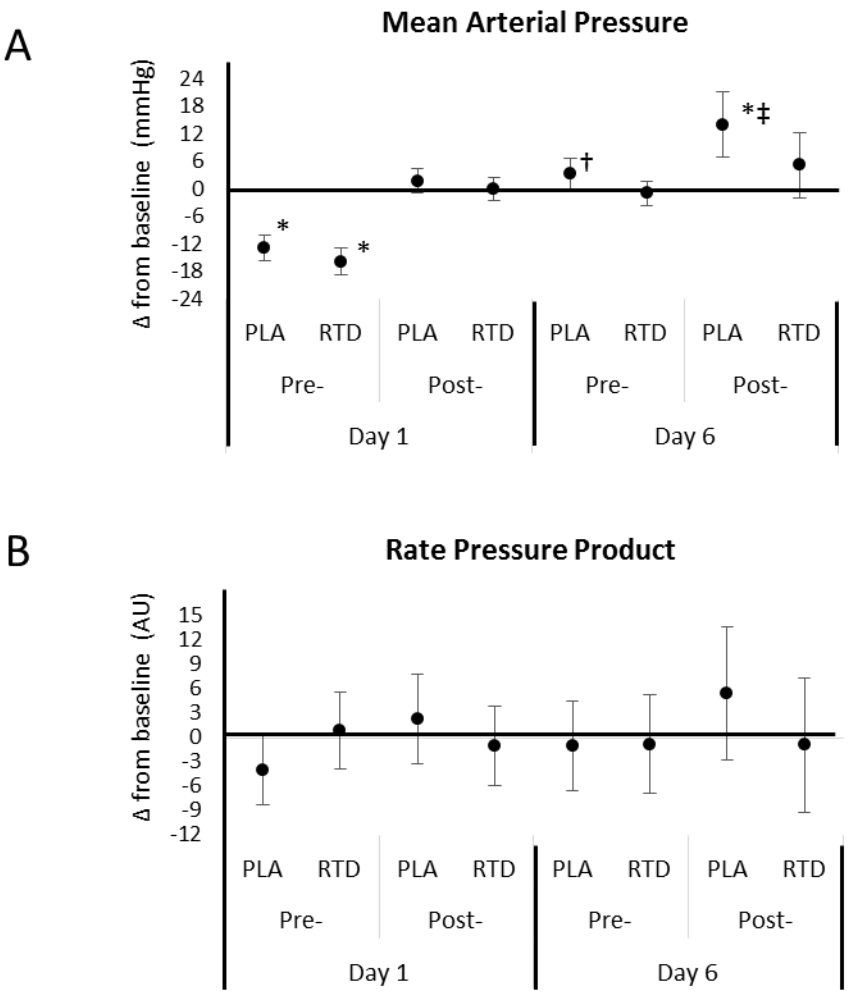

Figure 4.6. Data present mean change (95% CI) in response to the hemodynamic challenge test for mean arterial pressure (**Panel A**) and rate pressure product (**Panel B**). Confidence intervals not crossing zero are statistically significant ($p < 0.05$). * represents $p < 0.05$ difference from baseline, † represents $p < 0.05$ between-treatments, ‡ represents $p < 0.05$ to 0.10 trend towards significance between-treatments.

Table 4.5. Liver, kidney, and muscle data.

	Variable	Treatment	Day 1		Day 2		Day 6		Day 7		Treatment		p-value	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SE		
Liver	AST	PLA	26.4 ±	8.29	24.1 ±	5.17	25.1 ±	8.09	25.6 ±	6.56	25.4 ±	0.99	Time	0.19
	2-50 U/L	RTD	23.5 ±	6.17	29.0 ±	21.4	26.0 ±	6.91	25.6 ±	6.27	26.3 ±	1.01	Trt	0.77
		Time	25.1 ±	7.44	26.4 ±	15.1	25.5 ±	7.49	25.6 ±	6.35			I	0.15
	ALP	PLA	67.1 ±	28.7	63.5 ±	22.2	69.1 ±	18.0	65.6 ±	23.1	66.7 ±	2.22	Time	0.05
	7-60 U/L	RTD	71.9 ±	26.3	68.2 ±	23.2	66.2 ±	28.5	68.6 ±	20.7	67.3 ±	2.26	Trt	0.71
		Time	69.3 ±	27.4	65.7 ±	22.5	67.7 ±	23.3*	67.0 ±	21.8			I	0.59
	ALT	PLA	20.5 ±	8.25	18.4 ±	6.29	24.1 ±	16.6	19.7 ±	6.96	20.8 ±	0.91	Time	0.02
	44-147 U/L	RTD	20.0 ±	6.36	20.4 ±	7.32	20.2 ±	7.18	20.7 ±	6.73	20.3 ±	0.92	Trt	0.78
		Time	20.2 ±	7.35	19.3 ±	6.79	22.3 ±	13.1*	20.2 ±	6.8*			I	0.12
Muscle	CK	PLA	212 ±	171	210 ±	135	208 ±	211	228 ±	225	218 ±	33.1	Time	0.19
	0-200 U/L	RTD	163 ±	105	447 ±	837	216 ±	202	288 ±	281	278 ±	33.7	Trt	0.34
		Time	189 ±	144	321 ±	586	211 ±	205	256 ±	252			I	0.16
	LDH	PLA	190 ±	55.8	171 ±	35.5	180 ±	43.6	189 ±	64.1	182 ±	4.89	Time	0.27
	140-280 U/L	RTD	170 ±	39.2	170 ±	39.5	195 ±	57.6	179 ±	37.6	183 ±	4.98	Trt	0.69
		Time	181 ±	49.4	171 ±	37.0	187 ±	50.7	184 ±	53.0			I	0.11
Kidney	BUN	PLA	5.27 ±	1.57	4.58 ±	1.26	5.60 ±	1.52	4.93 ±	1.52	5.12 ±	0.15	Time	0.68
	2.1-7.1 mmol/L	RTD	5.40 ±	1.65	4.76 ±	1.24	5.14 ±	1.64	5.38 ±	1.76	5.12 ±	0.15	Trt	0.87
		Time	5.33 ±	1.59	4.66 ±	1.24	5.39 ±	1.58	5.14 ±	1.64			I	0.21
	Creatinine	PLA	90.0 ±	19.4	83.9 ±	19.2	89.8 ±	19.4	82.4 ±	17.2	86.9 ±	1.69	Time	0.59
	80-115 µmol/L	RTD	84.1 ±	14.2	84.5 ±	14.6	86.3 ±	20.1	86.3 ±	22.4	85.0 ±	1.72	Trt	0.67
		Time	87.3 ±	17.2	84.1 ±	17.0	88.2 ±	19.6	84.2 ±	19.7			I	0.24

Values are means ± standard deviations with normal clinical values shown for each variable. Multivariate nalysis revealed overall Wilks' Lambda treatment ($p = 0.92$), time ($p = 0.43$), and treatment x time ($p = 0.21$). Greenhouse-Geisser p-levels are reported with univariate analyses for time, treatment, and time x treatment interactions for each variable. * indicates significant difference from prior time point ($p < 0.05$). AST=aminoaspartate transferase, ALP=alkaline phosphatase, ALT=aminoalanine transaminase, CK=creatine kinase, LDH=lactate dehydrogenase, BUN=blood urea nitrogen, PLA=Placebo, RTD=ready-to-drink pre-workout supplement, Trt=Treatment, I=Time x Treatment interaction.

Table 4.6. Blood lipids, macronutrients, and nitrate data.

Variable	Trt	Day 1	Day 2	Day 6	Day 7	Treatment		p-value	
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean	SE		
Glucose	PLA	5.43 ± 0.90	5.25 ± 0.76	5.62 ± 1.09	5.22 ± 0.83	5.38	0.12	Time	0.23
4-6 mmol/L	RTD	5.13 ± 0.55	5.16 ± 0.78	5.16 ± 0.91	5.30 ± 0.93	5.19	0.11	Trt	0.24
	Time	5.29 ± 0.76	5.21 ± 0.76	5.41 ± 1.03	5.26 ± 0.87			I	0.33
Triglycerides	PLA	1.27 ± 0.66	1.03 ± 0.47	1.36 ± 1.23	1.23 ± 0.89	1.22	0.13	Time	0.30
1.7-2.2 mmol/L	RTD	2.97 ± 0.86	1.19 ± 0.65	1.16 ± 0.65	1.10 ± 0.59	1.15	0.13	Trt	0.71
	Time	1.21 ± 0.64	1.11 ± 0.56	1.27 ± 1.00	1.17 ± 0.76			I	0.25
HDL	PLA	1.68 ± 0.62	1.58 ± 0.50	2.15 ± 2.88	1.62 ± 0.59	1.75	0.11	Time	0.74
1-1.55 mmol/L	RTD	1.55 ± 0.49	1.63 ± 0.51	1.68 ± 0.55	1.65 ± 0.47	1.61	0.11	Trt	0.49
	Time	1.62 ± 0.56	1.60 ± 0.50	1.93 ± 2.13	1.63 ± 0.54			I	0.49
Cholesterol	PLA	4.92 ± 1.47	4.36 ± 1.02	4.80 ± 1.33	4.58 ± 1.24	4.66	0.12	Time	0.89
5.18-6.18 mmol/L	RTD	4.63 ± 1.10	4.50 ± 1.01	4.60 ± 1.07	4.56 ± 1.08	4.54	0.12	Trt	0.80
	Time	4.78 ± 1.30	4.43 ± 1.01	4.71 ± 1.21	4.57 ± 1.15			I	0.47
LDL	PLA	2.57 ± 0.96	2.28 ± 0.73	2.56 ± 0.75	2.42 ± 0.69	2.47	0.08	Time	0.25
2.59-4.12 mmol/L	RTD	2.62 ± 1.13	2.40 ± 0.83	2.37 ± 0.81	2.45 ± 0.97	2.45	0.08	Trt	0.94
	Time	2.60 ± 1.03	2.33 ± 0.77	2.47 ± 0.77	2.44 ± 0.82			I	0.42
Nitrates	PLA	0.177 ± 0.10	0.181 ± 0.13	0.197 ± 0.15	0.192 ± 0.18	0.19	± 0.02	Time	0.29
mmol/L	RTD	0.163 ± 0.06	0.201 ± 0.07	0.228 ± 0.15	0.227 ± 0.11	0.21	± 0.02	Trt	0.26
	Time	0.170 ± 0.08	0.191 ± 0.10	0.228 ± 0.15	0.209 ± 0.15			I	0.34

Values are means ± standard deviations with normal clinical values are included with each variable. Multivariate analysis revealed overall Wilks' Lambda treatment (p = 0.82), time (p = 0.44), and treatment x time (p = 0.30). Greenhouse-Geisser p-levels are reported with univariate analyses for time, treatment, and time x treatment interactions for each variable. HDL=high densilty lipoprotein, LDL=low density lipoprotein, PLA=Placebo, RTD=Ready-to-drink Pre-workout supplement

Table 4.7. Whole blood chemistry

Variable	Treatment	Day 1			Day 2			Day 6			Day 7			Treatment			p-value	
		Mean		SD	Mean		SD	Mean		SD	Mean		SD	Mean		SE		
WBC	PLA	6.60	±	1.45	6.04	±	1.34	6.41	±	1.74	5.72	±	1.49	6.19	±	0.15	Time	0.002
($x10^3/\mu l$)	RTD	6.66	±	1.59	6.14	±	1.53	6.22	±	1.36	5.87	±	1.36	6.25	±	0.15	Trt	0.93
	Time	6.63	±	1.51	6.09	±	1.43*	6.31	±	1.55	5.79	±	1.41*				I	0.78
LYM	PLA	2.50	±	0.65	2.33	±	0.72	2.62	±	0.79	2.44	±	0.83	2.47	±	0.12	Time	0.13
(%)	RTD	2.59	±	0.74	2.40	±	1.00	2.61	±	0.80	2.52	±	0.85	2.53	±	0.14	Trt	0.41
	Time	2.55	±	0.69	2.36	±	0.87	2.62	±	0.78	2.48	±	0.84				I	0.92
MID	PLA	0.77	±	0.23	0.66	±	0.22	0.58	±	0.25	0.68	±	0.34	0.67	±	0.04	Time	0.77
(%)	RTD	0.80	±	0.64	0.82	±	0.64	0.79	±	0.54	0.80	±	0.52	0.83	±	0.04	Trt	0.21
	Time	0.83	±	0.48	0.75	±	0.51	0.68	±	0.42	0.74	±	0.44				I	0.91
GRAN	PLA	3.33	±	1.09	3.05	±	0.82	3.21	±	0.93	2.60	±	1.10	3.06	±	0.11	Time	0.01
($x10^3/\mu l$)	RTD	3.18	±	1.43	2.94	±	1.04	2.87	±	1.08	2.56	±	1.10	2.88	±	0.11	Trt	0.47
	Time	3.26	±	1.26	3.00	±	0.95*	3.04	±	1.04	2.58	±	1.09*				I	0.77
RBC	PLA	4.69	±	0.42	4.66	±	0.39	4.73	±	0.57	4.73	±	0.43	4.72	±	0.04	Time	0.56
($x10^6/\mu l$)	RTD	4.83	±	0.53	4.85	±	0.58	4.80	±	0.48	4.84	±	0.54	4.81	±	0.04	Trt	0.06
	Time	4.76	±	0.48	4.76	±	0.50	4.77	±	0.55	4.78	±	0.44				I	0.50
HGB	PLA	13.92	±	1.31	13.91	±	1.14	14.28	±	1.44	14.03	±	1.24	14.1	±	0.12	Time	0.76
(g/dl)	RTD	14.10	±	1.55	14.42	±	1.83	14.12	±	1.43	14.30	±	1.77	14.2	±	0.12	Trt	0.32
	Time	14.16	±	1.53	14.16	±	1.53	14.20	±	1.42	14.16	±	1.50				I	0.16

Values are means ± standard deviations. Multivariate analysis revealed overall Wilks' Lambda treatment ($p = 0.61$), time ($p = 0.052$), and treatment x time ($p = 0.99$). Greenhouse-Geisser p-levels are reported with univariate analyses for time, treatment, and time x treatment interactions for each variable. * represents $p<0.05$ change from baseline. PLA=Placebo, RTD=Ready-to-drink Pre-workout supplement, Trt=Treatment, I=Time x Treatment interaction. WBC=white blood cells leukocyte count, LYM=lymphocytes, MID=mid-range absolute count, GRAN=granulocytes, RBC=red blood counts, HGB=hemoglobin, HCT=hematocrit, MCV=mean cell volume, MCH=mean cell hemoglobin, MCHC=mean corpuscular hemoglobin concentration, RDW=red blood cell distribution width.

Table 4.7. Whole Blood Chemistry

Variable	Treatment	Day 1			Day 2			Day 6			Day 7			Treatment			p-value	
		Mean		SD	Mean		SD	Mean		SD	Mean		SD	Mean		SE		
HCT	PLA	43.97	±	3.72	43.50	±	3.23	45.17	±	4.60	44.14	±	3.77	44.3	±	0.34	Time	0.63
(%)	RTD	45.10	±	4.69	45.38	±	4.87	44.95	±	4.24	44.90	±	4.57	44.9	±	0.34	Trt	0.21
	Time	44.55	±	4.35	44.44	±	4.20	45.06	±	4.40	44.52	±	4.16				I	0.20
MCV	PLA	93.89	±	3.48	93.46	±	3.81	93.95	±	3.25	92.36	±	5.54	93.4	±	0.37	Time	0.09
(fL)	RTD	93.54	±	3.29	93.78	±	3.74	93.82	±	3.43	92.47	±	4.75	93.5	±	0.38	Trt	0.99
	Time	93.72	±	3.35	93.62	±	3.43	93.88	±	3.31	92.41	±	5.11				I	0.84
MCH	PLA	29.72	±	1.32	29.86	±	1.32	29.70	±	1.09	29.70	±	1.29	29.7	±	0.14	Time	0.89
(pg/cell)	RTD	29.61	±	1.37	29.75	±	1.58	29.46	±	1.38	29.58	±	1.42	29.6	±	0.14	Trt	0.68
	Time	29.66	±	1.33	29.81	±	1.44	29.58	±	1.24	29.64	±	1.34				I	0.93
MCHC	PLA	31.65	±	0.82	31.97	±	0.75	31.64	±	0.96	31.78	±	0.76	31.8	±	0.10	Time	0.79
(g/dl)	RTD	31.67	±	1.10	31.73	±	1.32	31.40	±	1.08	31.82	±	1.08	31.6	±	0.10	Trt	0.54
	Time	31.66	±	0.96	31.86	±	1.07	31.52	±	1.01	31.79	±	0.93				I	0.72
RDW	PLA	13.14	±	0.89	13.04	±	0.63	13.29	±	0.85	13.09	±	0.72	13.1	±	0.08	Time	0.66
(%)	RTD	13.32	±	0.86	13.52	±	1.06	13.20	±	0.85	13.42	±	0.76	13.4	±	0.09	Trt	0.20
	Time	13.23	±	0.87	13.28	±	0.90	13.24	±	0.85	13.25	±	0.75				I	0.12

Values are means ± standard deviations. Multivariate analysis revealed overall Wilks' Lambda treatment ($p = 0.61$), time ($p = 0.052$), and treatment x time ($p = 0.99$). Greenhouse-Geisser p-levels are reported with univariate analyses for time, treatment, and time x treatment interactions for each variable. * represents $p<0.05$ change from baseline. PLA=Placebo, RTD=Ready-to-drink Pre-workout supplement, Trt=Treatment, I=Time x Treatment interaction. WBC=white blood cells leukocyte count, LYM=lymphocytes, MID=mid-range absolute count, GRAN=granulocytes, RBC=red blood counts, HGB=hemoglobin, HCT=hematocrit, MCV=mean cell volume, MCH=mean cell hemoglobin, MCHC=mean corpuscular hemoglobin concentration, RDW=red blood cell distribution width.

Table 4.8. Change in blood markers relative to normal clinical limits from Day 1 to Day 7

Marker	Treatment	Change				*n*	p-value	
		No Change	Normal to High	High to Normal	High to High			
AST	PLA	24	0	0	1	25		
2-50U/L	RTD	25	0	0	0	25	Trt	0.31
	Time	49	0	0	1	50		
ALP	PLA	23	0	2	0	25		
7-60U/L	RTD	23	0	1	1	25	Trt	0.51
	Time	46	0	3	1	50		
ALT	PLA	22	0	1	2	25		
44-147-U/L	RTD	24	0	0	1	25	Trt	0.49
	Time	46	0	1	3	50		
CK	PLA	11	8	3	3	25		
0-200 U/L	RTD	13	4	4	4	25	Trt	0.62
	Time	24	12	7	7	50		
LDH	PLA	2	16	4	3	25		
140-280-U/L	RTD	2	12	5	6	25	Trt	0.64
	Time	4	28	9	9	50		
BUN	PLA	24	0	0	1	25		
2.1-7.1-mmol/L	RTD	23	0	1	1	25	Trt	0.60
	Time	47	0	1	2	50		
Creatinine	PLA	18	1	4	2	25		
80-115-umol/L	RTD	24	0	0	1	25	Trt	0.10
	Time	42	1	4	3	50		
Glucose	PLA	25	0	0	0	25		
4.0-6.0-mmol/L	RTD	25	0	0	0	25	Trt	1.00
	Time	50	0	0	0	50		

Data are presented as number of occurrences and were analyzed by Pearson's Chi Square on a marker-by-marker basis. Pearson's Chi Square p-values listed for each variable with significance determined at $p < 0.05$. Data were arranged so that values within normal clinical ranges were labelled 'Normal' and values above normal clinical ranges were labelled 'High'. They were then assessed as 'Normal/Normal' or 'No Change', 'Normal/High', 'High/Normal', or 'High/High' to address values at Day 1 and Day 7. No significant differences were noted. AST=aminoaspartate transferase, ALP=alkaline phosphatase, ALT=aminoalanine transaminase, CK=creatine kinase, LDH=lactate dehydrogenase, BUN=blood urea nitrogen, HDL=high densilty lipoprotein, LDL=low density lipoprotein, PLA=placebo, RTD=ready to drink.

Table 4.8. Change in blood markers relative to normal clinical limits from Day 1 to Day 7

Marker	Treatment	Change: No Change	Change: Normal to High	Change: High to Normal	Change: High to High	*n*	p-value	
Triglycerides	PLA	25	0	0	0	25		
1.7-2.2-mmol/L	RTD	25	0	0	0	25	Trt	1.00
	Time	50	0	0	0	50		
HDL	PLA	6	13	5	1	25		
1.0-1.55-mmol/L	RTD	7	11	1	6	25	Trt	0.09
	Time	13	24	6	7	50		
Cholesterol	PLA	16	5	2	2	25		
5.18-6.18-mmol/L	RTD	19	1	2	3	25	Trt	0.37
	Time	35	6	4	5	50		
LDL	PLA	22	0	2	1	25		
2.59-4.12-mmol/L	RTD	23	0	1	1	25	Trt	0.84
	Time	45	0	3	2	50		

Data are presented as number of occurrences and were analyzed by Pearson's Chi Square on a marker-by-marker basis. Pearson's Chi Square p-values listed for each variable with significance determined at $p < 0.05$. Data were arranged so that values within normal clinical ranges were labelled 'Normal' and values above normal clinical ranges were labelled 'High'. They were then assessed as 'Normal/Normal' or 'No Change', 'Normal/High', 'High/Normal', or 'High/High' to address values at Day 1 and Day 7. No significant differences were noted. AST=aminoaspartate transferase, ALP=alkaline phosphatase, ALT=aminoalanine transaminase, CK=creatine kinase, LDH=lactate dehydrogenase, BUN=blood urea nitrogen, HDL=high densilty lipoprotein, LDL=low density lipoprotein, PLA=placebo, RTD=ready to drink.

Table 4.9. Frequency of Self-Reported Side Effects

Variable & no. of occurrences		Day 1				Day 2				Day 6				Day 7				
		Pre-		Post-		Pre-		Post-		Pre-		Post-		Pre-		Post-		
		PLA	RTD	PLA	RTD	PLA	RTD	PLA	RTD	PLA	RTD	PLA	RTD	PLA	RTD	PLA	RTD	*p*-value
Dizziness	0	22	22	19	18	22	22	20	18	21	19	20	19	22	21	19	16	Trt 0.49
	1	0	0	3	4	0	0	1	3	1	2	1	2	0	1	3	4	
	2	0	0	0	0	0	0	1	1	0	1	1	0	0	0	0	2	
	3	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	
	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Headache	0	20	20	21	21	21	21	21	21	21	20	20	19	21	20	18	18	Trt 0.85
	1	2	2	1	1	1	1	1	1	1	1	1	1	1	1	3	2	
	2	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	
	3	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	1	
	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Tachycardia	0	21	21	18	16	22	21	18	15	21	20	17	16	21	19	17	16	Trt 0.89
	1	1	1	2	3	0	1	1	3	1	2	2	3	0	3	2	2	
	2	0	0	1	1	0	0	2	2	0	0	3	2	1	0	3	3	
	3	0	0	1	1	0	0	1	0	0	0	0	1	0	0	0	1	
	4	0	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	
Palpitations	0	21	22	20	20	21	21	19	20	21	21	20	19	22	21	20	20	Trt 0.24
	1	1	0	2	1	1	1	2	0	1	0	2	1	0	0	2	0	
	2	0	0	0	0	0	0	0	1	0	1	0	2	0	1	0	0	
	3	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	2	
	4	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	
Dyspnea	0	20	20	18	17	21	21	17	14	20	20	17	16	21	19	18	13	Trt 0.56
	1	2	2	1	2	1	1	2	5	2	2	3	3	1	3	2	5	
	2	0	0	2	2	0	0	1	1	0	0	1	3	0	0	1	3	
	3	0	0	1	1	0	0	2	2	0	0	1	0	0	0	1	1	
	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Nervous	0	19	20	19	21	19	20	19	20	21	20	20	21	21	20	20	20	Trt 0.92
	1	1	2	1	1	2	2	2	2	1	2	2	1	1	2	2	2	
	2	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	
	3	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	
	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Blurred	0	22	21	20	20	21	22	21	20	22	21	21	21	22	22	21	21	Trt 0.54
Vision	1	0	1	2	1	1	0	1	2	0	1	1	0	0	0	1	0	
	2	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Other	0	22	22	22	19	22	20	22	19	22	20	22	19	22	21	22	20	Trt 0.54
Side Effects	1	0	0	0	3	0	1	0	3	0	0	0	2	0	0	0	1	
	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	
	3	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	
	4	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	
	5	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	

Data are presented as number of occurrences and were anaylyzed by Pearson's Chi Square on a question-by-question basis. Pearson's Chi Square p-values listed for each variable with significance determined at $p < 0.05$. Values are as follows: 0=No occurrences, 1=1- to 2-occurrences/wk, 2=3- to 4- occurrences/wk, 3=5- to 6-occurrences/wk, 4=7 occurrences/wk, 5=More than 7 occurrences/wk. No significant between-group differences were noted.

Table 4.10. Severity of Self-Reported Side Effects

Variable	Treatment	Day 1 Pre- Mean ± SD	Day 1 Post- Mean ± SD	Day 2 Pre- Mean ± SD	Day 2 Post- Mean ± SD	Day 6 Pre- Mean ± SD	Day 6 Post- Mean ± SD	Day 7 Pre- Mean ± SD	Day 7 Post- Mean ± SD	p-value
Dizziness	PLA	0.00 ± 0.0	0.18 ± 0.4	0.00 ± 0.0	0.14 ± 0.5	0.18 ± 0.7	0.09 ± 0.3	0.00 ± 0.0	0.23 ± 0.7	Time 0.03
	RTD	0.00 ± 0.0	0.32 ± 0.7	0.00 ± 0.0	0.45 ± 1.1	0.27 ± 0.7	0.32 ± 0.9	0.14 ± 0.6	0.59 ± 1.0	Trt 0.14
	Time	0.00 ± 0.0	0.50 ± 1.1 *	0.00 ± 0.0 *	0.59 ± 1.5 *	0.45 ± 1.4	0.41 ± 1.2	0.14 ± 0.6 ‡	0.82 ± 1.7 *	I 0.58
Headache	PLA	0.14 ± 0.5	0.05 ± 0.2	0.05 ± 0.2	0.18 ± 0.7	0.05 ± 0.2	0.09 ± 0.3	0.05 ± 0.2	0.32 ± 0.8	Time 0.46
	RTD	0.09 ± 0.3	0.05 ± 0.2	0.00 ± 0.0	0.00 ± 0.0	0.14 ± 0.6	0.36 ± 1.1	0.14 ± 0.5	0.41 ± 0.9	Trt 0.74
	Time	0.09 ± 0.3	0.05 ± 0.2	0.00 ± 0.0	0.00 ± 0.0	0.14 ± 0.6	0.36 ± 1.1	0.14 ± 0.5	0.41 ± 0.9	I 0.41
Tachycardia	PLA	0.00 ± 0.0	0.45 ± 1.0	0.00 ± 0.0	0.55 ± 1.0	0.05 ± 0.2	0.27 ± 0.7	0.00 ± 0.0	0.18 ± 0.6	Time 0.29
	RTD	0.05 ± 0.2	0.59 ± 1.1	0.00 ± 0.0	0.96 ± 1.5	0.23 ± 1.1	0.59 ± 1.2	0.27 ± 1.1	0.64 ± 1.3	Trt 0.26
	Time	0.05 ± 0.2	0.59 ± 1.1	0.00 ± 0.0	0.96 ± 1.5	0.23 ± 1.1	0.59 ± 1.2	0.27 ± 1.1	0.64 ± 1.3	I 0.64
Palpitations	PLA	0.05 ± 0.2	0.09 ± 0.3	0.05 ± 0.2	0.09 ± 0.3	0.05 ± 0.2	0.09 ± 0.3	0.00 ± 0.0	0.36 ± 1.5	Time 0.34
	RTD	0.00 ± 0.0	0.18 ± 0.7	0.05 ± 0.2	0.36 ± 1.2	0.18 ± 0.9	0.27 ± 0.9	0.23 ± 0.9	0.32 ± 1.0	Trt 0.53
	Time	0.00 ± 0.0	0.18 ± 0.7	0.05 ± 0.2	0.36 ± 1.2	0.18 ± 0.9	0.27 ± 0.9	0.23 ± 0.9	0.32 ± 1.0	I 0.54
Dyspnea	PLA	0.05 ± 0.2	0.41 ± 1.0	0.05 ± 0.2	0.64 ± 1.1	0.09 ± 0.3	0.27 ± 0.7	0.00 ± 0.0	0.32 ± 0.8	Time 0.06
	RTD	0.09 ± 0.3	0.41 ± 0.9	0.00 ± 0.0	0.82 ± 1.3	0.18 ± 0.9	0.55 ± 1.1	0.23 ± 0.9	0.96 ± 1.5	Trt 0.30
	Time	0.09 ± 0.3	0.41 ± 0.9	0.00 ± 0.0	0.82 ± 1.3	0.18 ± 0.9	0.55 ± 1.1	0.23 ± 0.9	0.96 ± 1.5	I 0.24
Nervous	PLA	0.18 ± 0.5	0.05 ± 0.2	0.18 ± 0.5	0.09 ± 0.3	0.05 ± 0.2	0.09 ± 0.3	0.05 ± 0.2	0.09 ± 0.3	Time 0.57
	RTD	0.09 ± 0.3	0.05 ± 0.2	0.05 ± 0.2	0.05 ± 0.2	0.00 ± 0.0	0.05 ± 0.2	0.09 ± 0.3	0.09 ± 0.3	Trt 0.50
	Time	0.09 ± 0.3	0.05 ± 0.2	0.05 ± 0.2	0.05 ± 0.2	0.00 ± 0.0	0.05 ± 0.2	0.09 ± 0.3	0.09 ± 0.3	I 0.45
Blurry Vision	PLA	0.00 ± 0.0	0.09 ± 0.3	0.05 ± 0.2	0.05 ± 0.2	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.05 ± 0.2	Time 0.33
	RTD	0.05 ± 0.2	0.09 ± 0.4	0.00 ± 0.0	0.18 ± 0.7	0.14 ± 0.6	0.23 ± 0.8	0.14 ± 0.6	0.14 ± 0.6	Trt 0.27
	Time	0.05 ± 0.2	0.09 ± 0.4	0.00 ± 0.0	0.18 ± 0.7	0.14 ± 0.6	0.23 ± 0.8	0.14 ± 0.6	0.14 ± 0.6	I 0.51
Other side effects	PLA	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	Time 0.17
	RTD	0.00 ± 0.0	0.32 ± 0.9	0.32 ± 1.0	0.46 ± 0.9	0.27 ± 0.9	0.36 ± 0.9	0.14 ± 0.6	0.41 ± 1.1	Trt 0.06
	Time	0.00 ± 0.0	0.32 ± 0.9	0.32 ± 1.0	0.46 ± 0.9	0.27 ± 0.9	0.36 ± 0.9	0.14 ± 0.6	0.41 ± 1.1	I 0.19

Mean Data presented as Mean ± SD. Multivariate Wilk's Lambda analysis revealed no time ($p = 0.12$), treatment ($p =$), or time x treatment interaction effect ($p = 0.42$). Greenhouse-Geisser p-values are listed for each variable to show time, treatment, and interaction effects with significance determined at $p < 0.05$. A score of 0 means no severity and a score of 4 equals maximum severity. * indicates a significant difference from the prior time point ($p < 0.05$). PLA=Placebo, RTD=Ready-to-drink Pre-workout supplement

CHAPTER V

CONCLUSIONS*

5.1. Discussion

This study has been published previously and this discussion is an extended explanation of published material [206]. The aim of this study was to examine whether acute and/or short-term term ingestion of a commercially available pre-workout RTD beverage would affect workout performance, hemodynamic reactivity, and/or hematological affects during a 7 days intervention period. Overall, there was some evidence of better maintenance of recovery 1-RM and improvement in recovery muscular endurance with acute (Day 1) and short-term (Day 6) RTD supplementation. These findings suggest that acute and/or short-term ingestion of this RTD beverage may provide ergogenic benefit after a short recovery from resistance-training. However, ingestion of this RTD had no effects on 4 km cycling time-trial performance. Additionally, we observed no evidence that acute or short-term ingestion of this RTD negatively affected hemodynamic responses to a standardized hemodynamic challenge, fasting blood makers, or perceived side effects. Based on these findings, the hypotheses that the RTD studied would improve resistance-exercise strength and endurance exhaustive exercise without undue alterations in hepatorenal and muscle enzyme function, hemodynamic responses to a postural challenge, or self-reported side

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effects are accepted. However, there was insufficient evidence that acute and/or short-term ingestion of this RTD affected 4 km cycling time-trial performance in non-trained cyclists. The following discussion provides additional insight as to results observed.

5.2. Performance

Caffeine is well-known for improving exercise performance and has been extensively reviewed elsewhere [22, 32, 76, 210]. Most recently, Souza et al. performed a systematic review and meta-analysis of caffeine containing energy drinks, finding significant improvements in muscular strength and endurance (ES = 0.49), endurance exercise tests (ES = 0.53), jumping (ES = 0.29) and sport-specific actions (ES = 0.51), but not in sprinting (ES = 0.14) [80]. A separate review by Warren, et al. found caffeine to have a greater effect on lower body strength (ES = 0.37) and endurance (ES = 0.28) [76].

The relative caffeine in this study, calculated from the absolute value of 200 mg found in the treatment divided by average weight, was 2.51 ± 0.4 mg/kg for males and 3.1 ± 0.5 mg/kg for females. The dose of 2.5 to 3.1 mg of caffeine is on the lower portion of the ergogenic range of 3 to 9 mg/kg; however, a number of studies have noted ergogenic benefits from as little as 2 mg/kg [22, 30, 66-69, 72, 92, 211]. The data in the present study evidenced acute use as well as short-term (6 d) use promoted better maintenance of strength and endurance following exhaustive exercise. These findings are in agreement with prior research from our lab [98, 99] as well as the literature reviewed showing an ergogenic effect of lower doses of caffeine. There does

exist the possibility that the ergogenic effect of caffeine in this study may have been synergistically enhanced by other ingredients; however, there is conflicting evidence. In a review, Souza, et al. [210] found energy drinks containing both taurine and caffeine to potentially enhance the effects of caffeine whereas Hendrix, et al. [20] found a caffeine supplement containing 400 mg caffeine, 67 mg capsaicin, and 10 mg bioprene had no effect on bench or leg press one repetition maximum or time to exhaustion at 80% maximal power output on cycle ergometer.

Recently, research of nitrates on resistance training and sprint performance has received more interest [23-25, 125, 165, 212-214] compared to those investigating nitrates in relation to endurance oriented performance variables [102, 164, 215]. The literature investigating nitrates and resistance training has consistently evidenced safety as well as an ergogenic effect at doses of approximately 4.8 mmol (300 mg) similar to the 5.7 mmol (353 mg) found in the current work [27, 28, 111, 112, 164]. In the studies found regarding nitrates by themselves, significant increases were seen in repetitions to failure and total lifting volume [26, 111, 126, 216] as well as showing increased muscle firing rates via EMG [217] with no effect on heart rate which is in line with the findings from the current work. These studies generally demonstrate that nitrate supplementation prior to exercise can enhance endurance and high-intensity intermittent exercise performance. For this reason, addition of nitrates to pre-workout supplements has received increasing interest [23, 26, 98-100, 111, 121, 130, 157, 214]. The results of the present work evidenced an ergogenic effect of concomitant use of

caffeine and nitrates to promote maintenance of muscular strength and/or endurance; however, the data do not support an ergogenic effect on time trial performance which is in contrast to recent findings [26, 98, 100, 101, 111, 121, 157]. These conflicting results could potentially be a function of greater variability in non-trained cyclists, differences in the dosages and/or timing of ingestion of the nitrate-containing RTD, or use of arginine nitrate as opposed to other forms of nitrates.

5.3. Safety

Concerns have been presented previously related to the effects of caffeine or nitrates on cardiovascular health and hemodynamic response to exercise [21, 204, 205, 218, 219]; this is the primary reason for assessing responses to the hemodynamic challenge test before and after strength testing. The hemodynamic challenge test was appropriate as a normal exercise bout involves multiple postural changes from supine to standing which may increase the chance of occurrence of orthostatic hypotension, particularly when supplementation with ingredients thought to increase the risk is undertaken. As hypothesized, there was no evidence of a negative effect on hemodynamic response associated with the RTD. In addition, the present work yielded no evidence of negative effects on blood chemistry or side effects. Overall, these data are in agreement with previous work from our lab [98-100] and other studies [220, 221].

5.4. Strengths and Limitations

A strength of the current work was the use of a fairly large cohort comprised of men and women who ingested their respective treatments in addition to their normal diet in a randomized double blind, cross-over manner. While this does not discount the possibility that gender differences may exist when using a larger or single-gender study protocol, gender served as a covariate to account for gender differences. Additionally, the muscular performance protocol was vigorous with regard to the number of exercises performed during testing and applicable as typical resistance-training sessions are comprised of multiple sets of multiple exercises; thus, the design allowed for a practical assessment of the ability of resistance-trained participants to maintain performance throughout a rigorous workout.

Finally, a strength of this study was the concerted effort to examine several parameters associated with safety by examining potential hemodynamic changes accompanying supplementation and exercise as well as a thorough analysis of hepatorenal and muscle enzyme function associated with the supplementation protocol. Assessment of the cardiovascular and hemodynamic responses to a postural challenge represents a similar pattern of movement as would take place during resistance training, as athletes often go from supine to standing positions throughout a workout and may experience orthostatic hypotension. Furthermore, a further strength of this study was an extended reporting schema to include potential changes out of normal clinical ranges, without adverse consequence.

Potential limitations in the present study included the utilization of recommended absolute serving sizes rather than relative doses to body weight or fat-free mass. It is possible that more consistent performance results would have been observed if relative doses were used; however, this is not how these types of supplements are consumed so normal serving sizes were controlled for by using relative caffeine intake as a covariate in the analysis. Additionally, although prior research in our lab has examined the effects of ingesting pre-workout supplements for up to 8 weeks, this study only assessed the acute and short-term effects. It is possible that the ergogenic benefits may lessen with longer periods of supplementation due to habituation, but research in this area is limited. Of note, RTD products are marketed as having an immediate effect on performance without requiring a loading period or alterations in diet; this design was a practical analysis of how individuals may use this type of supplement.

Another potential limitation was the examination of the effects of this RTD on recreationally-active resistance-trained participants. While this population was well-prepared to assess changes in muscular strength and endurance performance, they were not trained cyclists accustomed to performing sprints. Thus, it is conceivable that the lack of effect observed on 4 km cycling time-trial performance may have been affected by a lack of familiarity with cycling, regardless of partaking in a familiarization session. Additional research should examine whether ingestion of this type of RTD may affect sprint and/or high-intensity short-duration sprint performance.

5.5. Conclusions

Based on the collective findings and within the limitations of the current work, the RTD treatment used in this study appears to acutely improve indices of strength training via the observed improvement in bench press, leg press, and total lifting volume, as well as an attenuated decrease in one repetition maximum. Consumption of the RTD did not have an effect on 4 km time trial performance on the cycle ergometer in non-cycling trained participants. The RTD also appears to be safe in resistance-trained, college-aged males and females; however, additional research should assess the safety and efficacy of nutrients found in pre-workout supplements so that active individuals may make an informed decision concerning the use of supplements during training and/or competition.

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APPENDIX A
INFORMED CONSENT

TEXAS A&M UNIVERSITY HUMAN SUBJECTS PROTECTION PROGRAM

CONSENT FORM

Project Title: **Short-Term Safety and Dose Effects of a Ready to Drink Pre-Workout Supplement**

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 a ready to drink (RTD) formula immediately prior to exercise on blood safety, 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 beta alanine, arginine 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 four weeks and include nine visits (visit 1 ~ 1 hour/visit 2-9 ~ 1.5 hours). We will ask you to donate a blood sample up to nine 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 (dextrose and non-caloric flavoring)) 2. Active (2.1 g beta alanine, 1.3 g arginine nitrate, 200 mg caffeine, 65 mg niacin, 325 mcg folic acid, 45 mcg vitamin B12)	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 (dextrose and non-caloric flavoring); or 2.) one serving of the active RTD formula containing beta alanine (2.1 g), arginine nitrate (1.3 g), caffeine (200 mg), niacin (niacinamide, 65 mg), folic acid (325 mcg) and vitamin b12 (methylcobalmin, 45 mcg). 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 one additional time using the 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. Regardless we will not ask you to complete more testing sessions than previously listed.

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

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- 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 five 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 eight 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 parasthesia (tingling of the skin), stomach pain, nausea, diarrhea and jitteriness 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. ***If you are a competing athlete you may test positive for Performance-enhancing drugs (PED) given that caffeine is on the NCAA banned drug list.***

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 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

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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 $175 ($15 for the familiarization and $20 for each of the eight 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.

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

IRB NUMBER: IRB2015-0754F
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APPENDIX B
SIDE EFFECTS QUESTIONNAIRE

Side Effects Questionnaire

Texas A&M University: Exercise & Sport Nutrition Laboratory
Trial: Short-Term Safety and Dose Effects of a Pre-Workout Dietary Supplement

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?								

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APPENDIX C
TESTING FORMS (FAMILIARIZATION)

Texas A&M University: Exercise & Sport Nutrition Laboratory
Trial: Short-Term Safety and Dose Effects of a Ready to Drink Pre-Workout Supplement

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 D
TESTING FORMS (STUDY DAYS)

Texas A&M University: Exercise & Sport Nutrition Laboratory
Trial: Short-Term Safety and Dose Effects of a Ready to Drink Pre-Workout Supplement

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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IRB EXPIRATION DATE: 12/01/2016

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: _____

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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: _____

IRB NUMBER: IRB2015-0754F
IRB APPROVAL DATE: 12/18/2015
IRB EXPIRATION DATE: 12/01/2016

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: IRB2015-0754F
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IRB EXPIRATION DATE: 12/01/2016