ACUTE EXERCISE RESPONSE OF CAFFEINE AND NITRIC OXIDE STIMULATING PRE-WORKOUT SUPPLEMENT AMONG HEALTHY MALE RECREATIONAL ATHLETES

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Title

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

The effect of cocktail or “pre-workout” supplements containing L-arginine nitrate and caffeine are equivocal with anaerobic performance. The purpose of this study was to compare anaerobic performance and blood flow of pre-workout supplement containing L-arginine-nitrate and caffeine-to-caffeine and placebo. In a randomized, double-blind study, 12 resistance-trained males (caffeine users) completed three trials. Biodex concentric-concentric elbow-flexion and extension (5-sets, 10-repetitions). Ultrasound measured brachial blood flow (M-*Vel*, V- r^2 and V-*C*). Statistical analyses revealed a significant difference in total dynamic work PRE-PLA (P<0.0001) and CAF-to-PLA (P<0.0001) but not PRE-to-CAF (P=0.9581). Furthermore, a significant difference V- r^2 PRE-to-CAF (P=0.0391) and PLA-to-CAF (P=0.0070) and M-*Vel* PRE-to-CAF (P=0.0281). Conclusion, PRE did not differ CAF in strength measures other than a difference in M-*Vel*. PRE compared to PLA were not statically different in blood flow. The study illustrated no improvement beyond individual ingredient. This research may be useful for future cocktail supplement and Nitric Oxide research.

Keywords: Cocktail mixtures, L-arginine, NO, Anaerobic exercise, ergogenic-aid

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Aerobic exercise……Exercise that relies on oxygen-dependent energy produced through slow substrate reduction, also referred to as **endurance exercise** (Katch, McArdle, & Katch, 2011, p152)

Anaerobic exercise…….Exercise that relies on the fast chemical reactions to reduce energy substrates without oxygen, usually less than 90 seconds (Katch et al., 2011, p. 152)

Angiotensin II……A powerful stimulant that increases blood pressure by stimulating the release of aldosterone, stimulating sympathetic nerves, and causes vasoconstriction or narrowing of the blood vessel (Fyhrquits, Metsärinne, and Tikkanen, 1995)

Clearance……Removal or withdraw from a substance to potentially return to a state before consumption (Graham, 2001a)

Cocktail……..Combination of individual nutritional supplements or ergogenic compounds mixed within a formula among the field of sports nutrition

Concentric……..Concentric muscle contraction occurs when skeletal muscle shortens resulting from increased tension on the muscle or muscle groups. This contraction is the most common in weight-bearing exercise (Katch et al., 2011, p. 446)

Eccentric……Eccentric muscle contractions occur when skeletal muscles lengthen in response to resisting tension (Katch et al., 2011, p. 447)

Forearm Blood Flow (FBF)……...Is a regional area that blood flow is measured from the superficial portion of the brachial artery (Daniels et al., 1999)

Intoxication……...Consumption of a substance beyond recommended dosage that results in side effects, adverse effects, injury, or death

Isokinetic……...Muscular action that maintains constant speed in space (Katch et al., 2011)

Nutritional supplement……..Agent or procedure added to complete, extend, or reinforce nutrition (**supplement, supplemented**). Aspect: “sports nutritional supplement” a designed, specialized nutritional supplement to support: metabolism, energy, athletic performance and recovery (Buell et al., 2013; Katch et al., 2011)

One repetition maximum (1RM)…...A dynamic exercise test that assesses concentric and eccentric maximum lifting capacity (Katch et al., 2011, p. 448)

Resistance training (RT)……Resistance training is physical activity that improves muscular fitness among exercised muscle groups with external resistance, also referred to as strength training (American College of Sports Medicine, 2013)

VO_2max…….This is the maximum amount of oxygen uptake the can occur during exercise, also referred to as maximal oxygen uptake (Katch et al., 2011, p. 192)

VO_2peak…….Point at which VO_2max levels (peaks) during a maximal exercise test, this value is the highest amount of oxygen uptake (Katch et al., 2011, p. 222), commonly VO_2max and VO_2peak are used interchangeably

Wingate test……...Involves 30 seconds of all-out exercise on either an arm or cycle ergometer, with weight determined by body mass which applies friction to the cycle, and enables the calculation for peak power output and average power output (Katch et al., 2011, p. 208)

CHAPTER 1. INTRODUCTION

Nutritional supplements are an estimated $32 billion-dollar industry annually in the Unites States (Forbes Magazine) (Lariviere, 2013). Industry leaders such as MusclePharm reach near hundreds of millions of dollars in revenue each year, purchased by consumers, many younger, for use in improved results from workouts, often called "pre-workout" supplements. The nutritional supplement industry is poorly regulated particularly in the areas of supplement safety, efficacy, and purity (Buell et al., 2013). The Food and Drug Administration (FDA) is not required to evaluate nutritional supplements before they are made available to consumers (FDA, 2015). In addition, supplements are not removed from the market until the reported risks outweigh the products advertised (or "on the label") purpose (FDA, 2015). Especially among competitive athletes, purity is important. Unknown ingredients could result in disqualification from competition. Unknown purity could also result in unknown side effects, even death (e.g. from ephedra containing supplements in the last decade). In the recent decade, FDA regulations go unchanged. Compounding the problem, most supplements available to recreationally active individuals are mixtures of ingredients rather than pure substances making it difficult to test for safety, efficacy, and purity for single ingredients (Buell et al., 2013).

These multi-ingredient mixtures are often called: energy drinks, dietary supplements, mixed supplements, "pre-workout", *"cocktail"* supplements, and many others. The combination cocktail mixed supplements that contain unknown doses of individual ingredients per dose, making it challenging to compare one product among other pre-workout supplements (Kedia et al., 2014; Smith et al., 2010; Spradley et al., 2012; and Outlaw et al., 2014). Furthermore, the research related to common pre-workout ingredients such as nitric oxide warrants investigation into testing as individual substances to show improved ergogenic potential (Bescós et al., 2012;

Bailey et al., 2010; Camic et al., 2010a; Camic et al., 2010b). Caffeine, another common ingredient in pre-workout supplements, has been an individual ingredient shown to potentially be ergogenic both in combination with cocktail mixtures and alone.

Statement of the problem

This inability to test for safety, purity, and to test for efficacy of the ergogenic potential of individually combined ergogenic substances is under-researched and needed. The combined effects of such mixtures may result in unwanted side effects, injury, or death (Bunsawat et al., 2014; Jacobs et al., 2003).

Purpose of the study

The purpose of this literature review is to identify the need to investigate both combination mixtures as well as individual mixtures. Therefore, investigation into the ergogenic potential of the combination L-arginine nitrate and caffeine in comparison to caffeine alone or placebo will be reinforced.

Hypothesis of the study

The hypothesis of this research is that a single dose of caffeine will not illustrated difference from a combined mixture including a similar dose of caffeine in compared short term effects, anaerobic (ergogenic) performance, or blood flow measures.

CHAPTER 2. LITERATURE REVIEW

Introduction

Nutritional ergogenic aids, also referred to as performance enhancing substances, dietary substances or practices are not a new idea (Katch, McArdle, & Katch, 2011; Mahan, Escott-Stump, & Raymond, 2012). Athletes, whether professional, collegiate, or recreational, commonly use dietary supplements, however, all supplements are not, and should be evaluated for efficacy and safety (Buell et al., 2013). In recent years, all types of ergogenic nutritional aids have increased in availability. Gaining in popularity, ergogenic nutritional mixtures, which are a combination of consumable dietary supplements, have been called *cocktails* (Spradley et al., 2012). These cocktail mixtures combine separate ergogenic substances that may improve performance alone but are generally not tested together. To better understand how cocktail mixes effect ergogenic potential, research is needed comparing specific cocktail mixtures to individual ingredients to identify ergogenic potential and safety beyond individual ingredients alone. The purpose of this literature review is to examine the potential ergogenic effects separately, safety and efficacy of single ingredients caffeine, and L-arginine nitrate alone, to estimate effects of the combined mixture.

Cocktail pre-workout supplement

Cocktail mixtures marketed as pre- and post-workout supplements are often specific to anaerobic training or resistance training (Kedia et al., 2014). A specific research focus has been on pre-workout supplements and investigated by several teams (Kedia et al., 2014; Hoffman et al., 2008; Smith, Fukuda, Kendall, & Stout, 2010; Spradley et al., 2012; Outlaw et al., 2014). It is difficult to show interrelation among studies because none of the listed studies used the same mixture products in research design.

Kedia et al. (2014) used a double-blind randomized trial that consisted of two segments of resistance trained male and female participants (n=40, 26 ± 5.3 years). The measure of resistance training was three workouts per week for more than or equal to 2 years. This study did not use a gender divided participant group. The investigational study used a multi-ingredient (cocktail mixture) pre-workout supplement measuring the safety in segment 1 (Kedia et al., 2014). Kedia et al. (2014) used the first segment to identify an acute ingestion of the cocktail mixture and measured the effects on blood measures including blood pressure (BP), heart rate (HR), and heart rate variability. The HR and BP were measured at baseline and then repeated every 30 minutes post consumption, whereas the heart rate variability was measured pre-ingestion and 180 minutes post by 12-lead ECG, using Friedericia's formula ($QTc=QT/3\sqrt{RR}$). The participants were excluded from both segments if they reported a history of taking dietary supplements such as creatine or betaine (both found in the multi-ingredient supplement) within 30 days of the study (Kedia et al., 2014). The participant inclusion criteria did not restrict, measure, or control caffeine intake during the study. The results of segment 1 reached statistical significance ($p<0.01$, and $p<0.05$) with increases in both Systolic Blood Pressure, SBP (3.4-5.4 mmHg) and Diastolic Blood Pressure, DBP (2.3-42 mmHg), respectively, within supplement participants, with intervention. These results did not reach significance in the intergroup comparison as well the ECG results did not influence intergroup difference ($p<0.11$).

The second segment of the Kedia et al. (2014) study was a 6 week resistance training program 4 days per week. Participants (n=43, 24.3 ± 2.9 years) used a blinded supplement consumed 30 minutes prior to exercise after a 10 hour fast. The investigators did not discuss the difference between participants in segment 1 and 2. The exercise program consisted of 10 to 12 exercises per day at 3 sets by 12-15 repetitions (Kedia et al., 2014). The program measured

muscular performance at week 3 and week 6, with 1RM bench press and calculated muscular endurance (volume and total number reps during 1RM) (Kedia et al., 2014). Lower extremity muscle performance was not assessed in this study. The comparison of statistical analysis was between-group (supplement and placebo) with independent sample t-tests. The results of muscular performance did not reach statistical significance intergroup with the exception of repetitions to failure within supplement group from baseline in week 3 ($p<0.01$). The efficacy of supplement use was assessed by differences in perceived energy both at week 3 ($p=0.006$, set 2 and $=0.024$, set 3) and week 6 ($p=0.034$, set 2 and $=0.040$, set 3) via self-questionnaire. Other measures of efficacy included side effects ranging from headache, upset stomach, and insomnia (Kedia et al., 2014). Results suggest that a multi-ingredient (cocktail) supplement taken before exercise may improve athletic performance during exercise (Kedia et al., 2014).

Hoffman et al. (2008) investigated the acute hormonal effects of a "pre-exercise energy supplement" on resistance trained males ($n=8$, 20.3 ± 1.6 years). Participants were screened for nutritional supplements for study eligibility but were not controlled or screened for habitual caffeine intake. These investigators did, however, effectively list the dose of individual ingredients in the commercially available supplement (Amino Shooters™ by Champion Nutrition Inc.) (Hoffman et al., 2008, pg 878). The ingredients contained in that cocktail mixture are listed in Table 1. The experiment consisted of 5 total sessions (testing, protocol, loading, and two post-testing) in a double-blind crossover design that comparatively examined the hemodynamic (or physical blood make-up), effects pre and post exercise (Hoffman et al., 2008). The exercise entailed 1RM back squat (initial session), followed by familiarization sessions (second and third) of 6 sets by $\leq$10 repetitions of back squat (at 75% 1RM) with 2 minutes between sets (Hoffman et al., 2008).

Table 1

Pre-workout (cocktail mixture) ingredients

Hoffman et al. (2008) (n=8)	serving size 19 g	Spradely et al. (2012) (n=12)	serving size 23 g (1/2 scoop)
Total caffeine content	110 mg	**Total caffeine content**	unknown
Amino Shooters™ ingredients:		Assualt™ ingredients:	
Branch chain amino acids	(3000 mg L-leucine, 1100 mg L-isoleucine, and 1100 mg valine)	Total carbohydrate	9 g
		Vitamin B6	14 mg
		Vitamin B12	85 µg
Essential amino acids	(1100 mg L-lysine, 300 mg L-methionine, 1100 mg L-phenylalanine, 700 mg histidine, 1100 mg L-threonine)	Calcium	247 mg
		Sodium	45 mg
		Potassium	40 mg
Creatine monohydrate	5000 mg	*Assualt proprietary blend*	23000 mg
L-taurine	1500 mg	CarnoSyn® Beta-alanine, citrulline malate, (DMG) Dimethylglycerine, rhodiola rosea root, creatine monohydrate, Con-Cret®, creatine HCl, ****caffeine anhydrous**, BCAAs (L-leucine, L-valine, L-isoleucine), L-glycine, ****L-arginine blend (L-arginine AKG, Di-arginine malate, L-arginine HCl)**, Astragin®, Cinnulin®, L-glutamine, phosphorus, sodium, potassium, and other ingredients	
Glucuronolactone	350 mg		
Caffeine	110 mg		

The familiarization was followed by a 1 week creatine-loading of 20 g/day before the final two crossover testing sessions (similar to familiarization) where the supplement and/or placebo was consumed 10 minutes prior to exercise. The creatine-loading phase was used in addition to the tested supplements prior to exercise test sessions. The investigators then measured acute hormonal effects with blood draws pre-exercise, immediately post exercise, as well as 15 and 30 minutes post-exercise (Hoffman et al., 2008).

Other than a statistically significant increase ($p \leq 0.05$) in total number of repetitions in set 5 the results of this study did not comprehensively compare dose to anaerobic exercise or ergogenic potential (Hoffman et al., 2008). The author did not provide the exact *p*-value but total work volume was higher in supplement groups. The volume of exercise was estimated from blood lactate concentration with an eight percent increase ($p = 0.07$) in supplement compared to placebo group immediately post-exercise. Furthermore, there was a significant increase (no *p*-value provided) in acute growth hormone. The response increased from pre-exercise to both 15 and 30 minutes post-exercise in supplement group whereas placebo increased pre-exercise to 30 minutes post only (Hoffman et al., 2008). The total testosterone concentration increased pre- to post-exercise but did not reach statistical significance (no p-value provided). There were no reports of unwanted side effects. This suggests that the supplement may increase the response of growth hormone and testosterone during exercise but require further investigation.

Smith et al. (2010) identified the effects of a pre-workout supplement on both aerobic and anaerobic performance in a randomized parallel trial that was single blinded. The experimental design incorporated the supplement use into a 3 week training program of high-intensity interval training (HIIT) with moderately trained recreational athletes (n=24, 21.1 ± 1.9 years). The pre-workout supplement Game Time® (Corr-Jenson Laboratories Inc, Aurora, CO) contained a

proprietary blend of whey protein, creatine, citrulline, ginseng, caffeine 100 mg, and additional ingredients (Smith et al., 2010). The participants were screened for prior caffeine and supplement use however these surveys were not discussed. Eighteen total sessions were used throughout the study detailed below (Smith et al., 2010). The trial familiarized the participants on three separate sessions prior to the initiation of baseline testing in sessions four, five, and six. The baseline testing measured VO_2max with exercise treadmill protocol beginning at 10 km/h and 0% grade increasing 2 km/h every two minutes until participant reached 16 km/h and following with 1 km/h increase per minute until 18 km/h was achieved (Smith et al., 2010). The VO_2max was instrumentally measured with a metabolic cart (True One 2400® Metabolic Measurement System, Parvo-Medics Inc., Sandy, UT).

After testing the participants began the HIIT program 3 days per week for 3 weeks, beginning each training session pre-fasted (4 hours) and 30 minutes after ingesting the supplement or placebo prior to exercise (Smith et al., 2010). The HIIT consisted of a treadmill run with a series of 5 sets by 2 minutes in repetitions (with 1 minute of rest) at 90% VO_2max from baseline measure (Smith et al., 2010). The VO_2max (l/min) results reached significance ($p = 0.028$) in comparison to time baseline to post-test in both supplement and placebo groups. The total training volume increased in the supplement group reaching significance ($p = 0.041$) over placebo. This study did not discuss possible side effects of the Game Time® supplement. The consensus of this study suggests that performance related to cardiovascular and VO_2max is improved by the consumption of this supplement (Smith et al., 2010).

Spradley et al. (2012) examined the acute ergogenic potential of pre-workout supplement, Assault™ (MusclePharm, Denver, CO) on muscular endurance, anaerobic capacity, and aerobic capacity. The ingredients are listed in Table 1. This experiment used a randomized double-blind

design with crossover investigation in recreationally trained males (n=12, 28 ± 5 years). For eligibility participants were required to refrain from nutritional supplements for 6 weeks prior to the study and for the duration of the study (Spradley et al., 2012). There was no evidence of dietary screening or habitual caffeine use before the study. However, participants were asked to record a 2-day food diary before the first test session in order to repeat that diet before the following testing session and to report 12 hours fasted for testing (Spradley et al., 2012). The participants completed three different visits, including a baseline familiarization and two following test sessions. This investigation measured ergogenic performance with; muscular reaction measured by martial arts protocol, muscular strength measured by 1RM for both free bench press, seated leg press, muscular endurance measured by 75% 1RM, 1 set ≥5 reps, aerobic and anaerobic capacities (modified treadmill protocols) (Spradley et al., 2012). The supplement (see Table 1, for dose) or placebo was ingested 20 minutes prior to exercise and heart rate and blood pressure was measured pre-ingestion, 20 minutes post, and post-exercise. Meanwhile participants were asked to complete a short 4 question Likert-scale survey on feelings of: energy, alertness, focus, and level of fatigue (Spradley et al., 2012). There was no mention of the number of times participants filled out the questionnaire.

After investigation, the results were skewed towards the supplement group. Participants in the supplement group on average had higher heart rates 102 ± 12 compared to the placebo group 94 ± 15 ($p < 0.05$) (Spradley et al., 2012). In addition, leg press repetitions reached significance ($p < 0.05$) in supplement group 13 ± 6 compared to placebo 11 ± 3. Finally, the questionnaire revealed differences between supplement and placebo (respectively): energy 3.6 ± 0.8, 2.7 ± 0.5; alertness 4.0 ± 0.7, 3.6 ± 0.8; focus 4.1 ± 0.6, 3.6 ± 0.8; and fatigue 2.6 ± 0.8, 3.7 ± 0.7 ($p < 0.05$) (Spradley et al., 2012). This investigation suggests that ergogenic performance

except for aerobic capacity (VO_2max) is improved with consumption of this multi-ingredient cocktail supplement.

Outlaw et al. (2014) used a randomized double-blind, match-pair design to investigate anaerobic ergogenic potential of a pre-workout supplement containing creatine monohydrate, beta-alanine, L-taurine, L-leucine, and caffeine. This study included resistance trained males (n=20, 22.4 ± 9.5 years) that were required to bench 1.2 and leg press 2.5 times individual body weight (Outlaw et al., 2014). There was no inclusion or exclusion criterion for habitual caffeine use other than nutritional supplements. The study used a 9-day food diary, as pre-dietary screening that was analyzed by lab personnel using The Food Processor (ESHA Research, Salem, OR) (Outlaw et al., 2014). Baseline testing consisting of vertical jump, 1RM bench, muscular endurance bench (repetitions to failure at 85% 1RM), 1RM leg press, muscular endurance leg press (repetitions to failure at 85% 1RM) and a Wingate cycle ergometer test (Outlaw et al., 2014). The investigators then matched and randomized participants into supplement or placebo training groups. The pre-workout supplement (or placebo) was consumed 30 minutes prior to resistance exercise.

The training program consisted of four sessions of split body workouts (upper and lower) with 10 exercises per session at 3 sets by 8 repetitions targeting 80% of baseline 1RM (Outlaw et al., 2014). The rest ratio for the training program was 1 to 2 minutes between sets and minutes between exercises respectively. The training program lasted seven days in total at which time participants retested the previous exercise tests (Outlaw et al., 2014). The overall results showed significance within time effect for peak power measured in Watts (Wingate test, p =0.001; η_p^2 = 0.550), bench 1RM (p = 0.001; η_p^2 = 0.448), and leg press 1RM (p = 0.001; η_p^2 = 0.632) (Outlaw et al., 2014). However there were no differences between group comparisons. The data of this

study suggests that a pre-workout supplement has the potential to improve performance during anaerobic exercise.

Table 2

Caffeine supplement ingredients contained in one dose of cocktail mixtures described by Beck et al. (2006); Beck et al. (2008) (3 pills)

Ingredients	Beck et al. (2006) (n=37)	Beck et al. (2008) (n=31)	
Total caffeine content	201 mg	201 mg	(201 mg)
Yerba Mate	500 mg	500 mg	(40 mg caffeine)
Guarana seed extract	422 mg	422 mg	(152 mg caffeine)
Black tea extract	100 mg	100 mg	(9 mg caffeine)
Ginger extract	500 mg	500 mg	
Schisandra chinensis fruit extract	100 mg	100 mg	
Dill weed extract	5 mg	5 mg	
Grape seed extract	1 mg	1 mg	
Vitamin C	120 mg	120 mg	
Niacin	20 mg	40 mg	
Vitamin B6	2 mg	2 mg	
Pantothenic acid	10 mg	10.4 mg	
Vaccininium angustifolium (Wild blueberry extract, Vitablue)	50 mg	100 mg	
Cinnamon	25 mg	25 mg	
Red pepper extract	-	10 mg	
Black pepper extract	-	5 mg	

Several studies used cocktail mixtures not related to pre-workout supplements but focused on caffeine as an ingredient (Beck et al., 2006; Beck, Housh, Malek, Mielke, & Hendrix, 2008; Forbes, Candow, Little, Magnus, & Chilibeck, 2007). Beck et al. (2006) tested a caffeine containing supplement (1 dose = 3 tablets, = 201 mg) compared to placebo (Table 2). This investigation used a randomized, double-blinded parallel design with resistance trained males (n=37, 20.8 ± 2.0 years). The purpose was to measure anaerobic performances markers including Wingate, 1RM on bench press, 1RM on seated leg press, and muscular endurance of both bench

press and seated leg press (Beck et al., 2006). Muscular endurance was measured with 1 set to fatigue at 80% 1RM, 2 minutes post 1RM.

The participants were not allowed to consume nutritional supplements within 6 weeks and throughout the duration of the study (Beck et al., 2006). The participant habitual caffeine use was not controlled or accounted for in this study. Beck et al. (2006) conducted three total visits starting with baseline testing, followed by 2 supplementation visits. Participants performed 2 consecutive Wingate tests one hour after supplement or placebo ingestion. The participants returned for the final visits (≥ 24 hours) after Wingate measures were taken. The final visit concluded testing with both 1RM and muscular endurance for both bench and seated leg press (Beck et al., 2006). The outcome of this investigation illustrated a 2.1% increase in bench 1RM in supplement compared to placebo reaching significance ($p \leq 0.05$) (Beck et al., 2006). Bench muscular endurance increased 5% but did not reach significance ($p = 0.074$) (Beck et al., 2006). The caffeine containing supplement had no effect on the remaining measures compared to placebo. This suggests that the supplement is a potential ergogenic aide and caffeine can hypothetically increase bench 1RM performance.

The later research by Beck et al. (2008) was a follow-up study to the previous study by Beck et al. (2006). This study was a double-blinded, and crossover design that examined the anaerobic and aerobic potential of a caffeine supplement (201 mg) compared to placebo on 1RM bench press, and running treadmill test running to volitional exhaustion at 85% of VO_2peak in untrained males (both resistance and aerobic, n=31, 23.0 ± 2.6 years) (Beck et al., 2008). Caffeine intake was not screened or controlled for throughout the investigation. Beck et al. (2008) used a similar supplement “cocktail mixture” from Beck et al. (2006). Individual ingredients appear in Table 2. Baseline testing included predicted VO_2peak during a treadmill

protocol starting participants at 6.44 km/h, 0% grade increasing 1.61 km/h every two minutes until 14.49 km/h after intensity increased by 2% grade every two minutes concluding at volitional exhaustion (Beck et al., 2008). The VO_2peak was measured by TrueMax metabolic cart (Parvo Medics, Sandy, UT). During testing participants consumed a randomized trial (supplement or placebo) and ingested 45 minutes before exercise. The results showed no significant mean differences between supplement and placebo for 1RM bench or treadmill testing (Beck et al., 2008). This suggests a possible link between ergogenic potential, trained versus untrained individuals, and the effectiveness of caffeine as an ergogenic performance aid on select population.

The caffeine supplement (3 pills) used in Beck et al. (2008) contained red pepper extract, and black pepper extract which was not consistent with the previous study (Beck et al., 2006). The dose of niacin, and pantothenic acid varied within the two Beck studies 40 mg, and 10.4mg respectively (Beck et al., 2008) compared to 20 mg and 10 mg respectively (Beck et al., 2006).

Forbes et al. (2007) investigated the use of a caffeinated energy drink (Red Bull®) and effects on anaerobic power by Wingate cycle test and muscular endurance on physically active male (n=12) and female (n=4) participants. The age and standard deviation of participants both male and female was 24 ± 6 years. Physical activity was self-reported equating to moderate activity 2 to 3 days per week as well participants completed Physical Activity Readiness Questionnaire (PAR-Q) (Forbes et al., 2007). The dietary intake was not screened controlled for throughout the study. Other than the researcher staff requesting participants not to change any dietary patterns (Forbes et al., 2007). However researchers screened participants for habitual caffeine intake through self-reported questionnaire. The questionnaire revealed that some participants (n=8) reported caffeine naïve 0 $mg \cdot d^{-1}$, n=4 <100 $mg \cdot d^{-1}$, and n=3 >200 $mg \cdot d^{-1}$

(Forbes et al., 2007). This investigation used a double-blinded, crossover design and previous listed performance measures. Blood lactate values were also measured but not reported in methods section. The participants attended 4 separate sessions starting with baseline 1RM on bench, followed by a familiarization to exercise protocol. The familiarization and exercise protocol consisted of muscular endurance measured by 3 sets of repeated bench press to fatigue (failure) at 70 to 80% 1RM followed by three repeated Wingate tests (Forbes et al., 2007). The rest ratio was 1 to 2 minutes bench and Wingate respectively with 10 minutes between both measures.

After both baseline testing and familiarization sessions participants returned for 2 more supplemental testing sessions. Participants arrived fasted (3 hours) and refrained from caffeine use for 48 hours (Forbes et al., 2007). The supplement, Redbull®, was compared to a noncaffeinated lemon citrus soft drink (noncaffeinated Mountain Dew®) ingested one hour prior to exercise testing (Forbes et al., 2007). Redbull® is an example of cocktail mixtures containing ingredients other than a pure caffeine dose. Forbes et al. (2007) used the caffeinated beverage Red Bull® is dosages to equate to 2.0 mg/kg of caffeine including: sugar 0.65 mg/kg, taurine 25 mg/kg, Glucuronolactone 15 mg.kg, niacin 0.45 mg/kg, pantothenic acid 0.15 mg/kg, vitamin B6 0.05 mg/kg, riboflavin 0.04 mg/kg, and vitamin B12 0.025 µg/kg. The protocol for testing was the same as the familiarization. There were no differences in caffeine groups (habitual versus naïve) and both groups were assessed together.

The results found Redbull® increased total number of repetitions 34 ± 9, compared to placebo 32 ± 8 reaching significance ($P = 0.031$) (Forbes et al, 2007). There was no difference in Wingate performance peak power, or average power in Redbull® compared to placebo. The fingertip blood lactate (mmol/L) measures showed a time effect within group at baseline (*P*

<0.01), after set 1 (P <0.01), and set 1 to set 2 (P =0.016) (Forbes et al., 2007). No difference was observed after set 3 or 2 minute post measure. Group comparison found that supplementation significantly increase muscular endurance but did not affect Wingate measures (peak and average power) or blood lactate concentration (Forbes et al., 2007).

All the cocktail mixtures contained niacin, pantothenic acid, and vitamin B6 (Beck et al., 2006; Beck et al., 2008; Forbes et al., 2007) in addition to caffeine. The listed studies all examined performance markers of resistance training and capabilities of anaerobic exercise in comparison to placebo. The interrelation between these studies is that there was no isolation of ingredients. This suggests that an increase in 1RM bench (Beck et al., 2006) will not transfer to increased number of repetitions of bench in Forbes et al. (2007) because of ingredients that are unaccounted for.

Ergogenic nutrition supplements released to consumers are not regulated routinely nor tested for safety or effectiveness by the Food and Drug Administration (FDA) (FDA, 2015). This lack of regulation of efficacy and safety is compounded by mixed ingredients of unknown quantities in cocktail supplements such as those investigated by Kedia et al. (2014); Smith et al. (2010); Spradley et al. (2012); and Outlaw et al. (2014). It is increasingly difficulty to investigate claims because companies generally do not disclose individual ingredient dosages. The predominate standard of investigation for claims for performance enhancement or ergogenic potential is to identify individual ingredients. Kedia et al. (2014) examined the safety and efficacy of pre-workout supplementation but noted that comparing individual ingredients of cocktail supplements is difficult in determining combined performance potential. Overall the acute effect of pre-workout supplements for ergogenic potential in anaerobic exercise is not well researched.

In the study by Spradley et al. (2012) investigators measured acute ergogenic potential of a pre-workout supplement (Assault™ by MusclePharm) to placebo but failed to compare it to individual ingredients including branched chain amino acids, caffeine and numerous other potentially performance-enhancing substances (Table 1). Research studies comparing both individual and combined ingredients in a pre-workout supplement could potentially test for a synergistic effect beyond consumption of individual substances. In another study, Hoffman et al. (2008) investigated the acute hormonal effects of a "pre-exercise energy supplement" but did not comprehensively compare the study dose to anaerobic exercise or ergogenic potential. This study did, however, effectively list the dose of each ingredient in the commercially available supplement (Amino Shooters™ by Champion Nutrition Inc.) (Hoffman et al., 2008, pg 878). This as previously noted is not a common occurrence in multi-ingredient "cocktail" supplements.

Finally investigators Smith et al. (2014); and Kedia et al. (2014) researched and focused on exercise training studies. First Smith et al. (2014) experimentally tested 3 weeks of HIIT on a treadmill compared to Kedia et al. (2014) using 6 weeks of muscular strength training respectively using multiple doses of pre-workout supplements. Furthermore, Outlaw et al. (2014) used a four-day spilt training program for the duration of an eight-day study. This study did not account for adequate time after eight days of supplementation for participant recovery. Likewise with longer investigations, training studies mask the acute ergogenic potential of these pre-workout supplements due to neuromuscular adaptation, recovery-time, and muscle hypertrophy (Katch et al., 2011). After reviewing each investigation, the results are applicable only to the specific cocktail mixture investigated as specific dosage, individual ingredients nor their individual affects are not known (Kedia et al., 2014; Smith et al., 2010; Spradley et al., 2012; and Outlaw et al., 2014). The report results of the acute effects for ergogenic potential are limited but

warrant further investigating. Also, cocktail mixtures may result in unwanted side effects as reported by Kedia et al. (2014) and Mahan et al. (2012). This is an important consideration involved with choosing the correct supplement.

Iron Pump™ is pre-workout supplement created by MusclePharm (Denver, Colorado). This supplement is marketed as having the ergogenic potential to affect vasodilation and is intended to increase blood flow to muscles during exercise (MusclePharm, 2013). This ergogenic supplement functions with the nutritional compound L-arginine nitrate, which is a salt combination of l-arginine and nitric acid and a precursor to nitric oxide (MusclePharm, 2013). The combined ingredients of this pre-workout supplement are similar to *Assault* ™ (MusclePharm Corp, 2013) investigated by Spradley et al. (2012) because both contain undefined energy blends produced by MusclePharm and both cocktail mixtures contain unknown doses of L-arginine. The breakdown of *Iron Pump*™ includes a combination of multiple ergogenic ingredients and two primary ingredients that may affect muscle activity and blood flow: L-arginine nitrate and caffeine as an energy blend including anhydrous caffeine (Spradley et al., 2012; MusclePharm, 2013).

Although several researchers have attempted to pinpoint effects of specific ingredients within cocktail ergogenic mixtures, these studies have limited ability to generalize the ergogenic potential effects of individual ingredients. This is because researchers do not know the given amount (dosage) or they do not contain the same specific ingredients in each supplement. In comparison to the apparent lack of research regarding a similar supplement (to *Iron Pump*™) it may be more important to compare the individual ingredients of *Iron Pump*™ to ergogenic effects of those individual ingredients in scholarly work, in order to arrive at a possible ergogenic potential. Therefore, the next several sections break down individual *Iron Pump*™ ingredients

including L-arginine nitrate and caffeine, specifically related to ergogenic potential, safety and efficacy of anaerobic training (Astorino, & Roberson, 2010a) and blood flow. MusclePharm (2013) claims:

"Iron Pump™ utilizes "Super Nitric Oxide" ION-3 Nitrate Technology to open up blood pathways into the muscles, improving the effectiveness of the ordinary molecule. As the world's first molecularly-modified arginine, L-Arginine Nitrate is a fusion of l-arginine and nitric acid that increases blood flow to enhance distribution of nutrients."

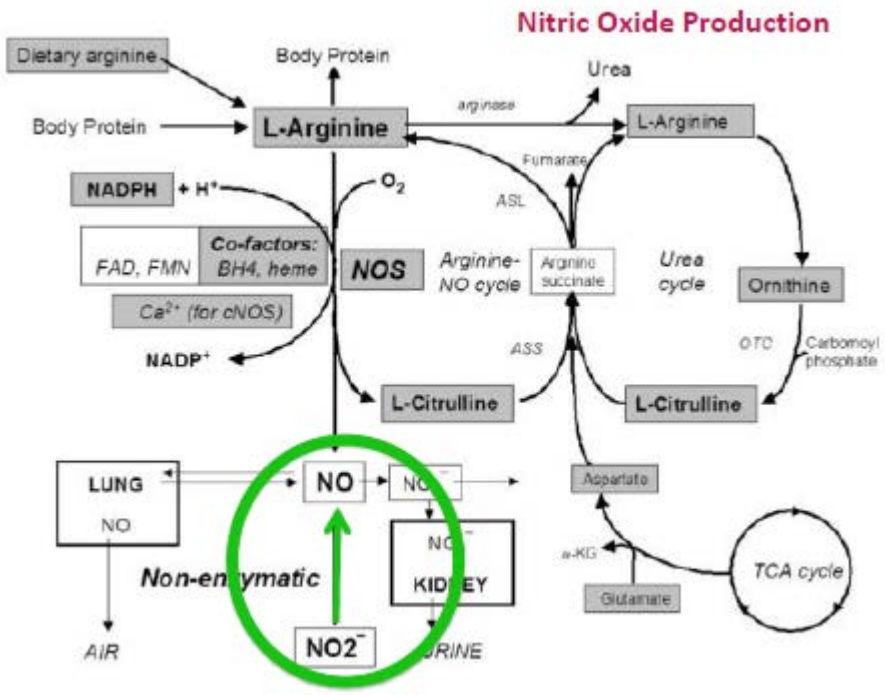


Figure 1. A schematic diagram of the physiologic disposition of nitrate, nitrite, and nitric oxide from exogenous (dietary) and endogenous sources (Ivy, 2015).

Nitric oxide, nitrate, nitrite, and L-arginine

Iron Pump™ is promoted as an L-arginine nitrate supplement (MusclePharm, 2013). L-arginine is a precursor to nitric oxide synthases, whereas, nitrate or nitrite are more immediate precursors to nitric oxide (Hord, Tang, & Bryan, 2009). The nitric oxide released by cells after synthesis or absorption of nitrate or nitrite, has a vasodilating effect that is localized in blood

vessels (Mahan et al., 2012). There are a number of exogenic and endogenic pathways for nitric oxide as illustrated in Figure 1. Search terms for citations found using supplements or exercise in association with *L-arginine, nitric oxide, nitrate, nitrite, caffeine, and caffeinated.*

Further, the USDA Nutrient Database, the Food and Agriculture Organization (FAO), and World Health Organization (WHO) do not report any nitric oxide, nitrite, or nitrate (USDA, 2015; FAO, 2015; WHO, 2015). There is more information available, about caffeine as an individual ingredient than L-arginine in research (Bescós, Sureda, Tur, & Pons, 2012). This compares to database search results including *PubMed, Google Scholar, and ProQuest,* caffeine as an individual supplement (≈ 94,000 hits) than L-arginine alone (≈ 33,000 hits).

L-arginine is an essential amino acid that works to synthesize nitric oxide in cells (Hord et al., 2009; Mahan et al., 2012). Good dietary sources of nitric oxide include beets, beet root juice, kale, arugula, spinach, and Swiss chard. Therefore the supplement is intended to mimic this natural process illustrated in Figure 1.

The supplementation of L-arginine, or nitric oxide, including cocktail mixtures has ergogenic potential to enhance endurance exercise through cardiorespiratory adaptation and increased tolerance to exercise (Bescós et al., 2012; Bailey et al., 2010; Camic et al., 2010a; Camic et al., 2010b). In a systematic review, Bescós et al. (2012) found the majority of research on L-arginine or nitric oxide to be based on endurance ergogenic potential (e.g. aerobic exercise to exhaustion) rather than resistance training (e.g. measured muscular strength, muscular endurance, and anaerobic capacity ~Wingate test). This warrants further investigations of nitric oxide and L-arginine related to resistance training. Another problem with L-arginine nitrate is that the ergogenic potential of the individual substance has yet to be established and have primarily shown results in cocktail mixtures (Bescós et al., 2012; Bailey et al., 2010; Camic et

al., 2010a; Camic et al., 2010b). The article by Bescós et al. (2012) identified the lack of research involving nitric oxide in isolation. Therefore, further research analyzing these compounds in a mixture and as individual ingredients is important to better understand the effects of combining cocktail supplements. However a single “dose” of beet-root juice costs approximately ≈ $15.00 (USD) (Swanson Health Products, 2015).

Furthermore *Iron Pump*™ does not disclose the amount of L-arginine nitrate nor the caffeine content of its energy blend per dose (1 Scoop) (MusclePharm, 2013). While the manufacturer claims that L-arginine nitrate is responsible for vasodilation, caffeine may also play a crucial role yet to be determined in the cocktail mixture (Bescós et al., 2012; Kedia et al., 2014). For simplicity L-arginine, nitrite, nitrate and nitric oxide will be referred to as nitric oxide (NO).

Caffeine use as ergogenic aid

For the purpose of this study the aerobic effects of caffeine are positioned by Rosenbloom, & Coleman (2012):

"The strongest evidence is that caffeine can enhance endurance performance.”

This increased performance is likely related to the stimulation of the central nervous system and decreased rate of perceived effort (Rosenbloom et al., 2012). This position stand was established in accordance with American College of Sports Medicine ACSM (2009) related to sufficient evidence. However few studies have investigated the acute effect of caffeine with anaerobic exercises (Astorino et al., 2010a).

Strategic intake of caffeine has been documented since the early 1900s and has been rigorously studied for its ergogenic effects during exercise (Astorino et al., 2010a; Jacobson, &

Kulling, 1989). Although caffeine is naturally found in a variety of foods and drinks, it is often supplemented. The habitual use of caffeine as an ergogenic aid is more common than all others (Jacobson et al., 1989; Graham, 2001b), which could be attributed to its wide availability. The caffeine complex or 1, 3, 7-trimethylxanthine is readily absorbed in the small intestine within 45 minutes (range, 15 to 120 minutes) after consumption (Higgins, & Babu, 2013; Magkos, & Kavouras, 2005). After ingestion caffeine has a reported half-life of up to 3 to 4 hours which can vary from 2 to 12 hours, which provides prolonged circulation of caffeine until excreted or otherwise, used (Graham, 2001a; Higgins et al., 2013; Magkos et al., 2005).

Caffeine has ergogenic potential both as an individual supplement and as a combined ingredient in a cocktail mixture, as explained previously (Beck et al., 2006; Beck et al,, 2008; Forbes et al., 2007; Kedia et al., 2014; Hoffman et al., 2008; Smith et al., 2010; Spradley et al., 2012; Outlaw et al., 2014). The effect that caffeine has on enhancing aerobic performance in endurance exercises is well researched (Bell & McLellan, 2003; Davis, & Green, 2009). Bell et al. (2003) examined the effect of a repeated dose of caffeine during repeated maximal endurance exercise in recreational male cyclists (n=9, 33 ± 7 years). The study used a double-blinded, randomized design to examine four treatment protocols measuring VO_2max, heart rate, blood lipids (free fatty acids), blood glucose, and blood caffeine concentration. All participants habitually used caffeine ($\geq$ 300 mg·d^{-1}) measured by a self-reported questionnaire.

This study consisted of 7 total visits, starting with VO_2max measured on an electrically brake cycle ergometer followed by two familiarization trials (no treatment). The two-day food diaries were collected before the familiarization trial in order to replicate that diet for all subsequent trials (Bell et al., 2003). The repeated exercise consisted of two sets of cycle ergometer testing per day separated by 5 hours (AM, PM) one day per week was examined

compared to placebo. Participants refrained from caffeine for 12 hours prior to each trial and ingested treatment (1, 2, 3, or 4) 1 hour prior to exercise and consumed a cereal bar 15 minutes post caffeine ingestion (Bell et al., 2003). There type of cereal bar including ingredients and calorie content was not discussed. The treatment protocols were as follows: treatment 1 =5 mg/kg caffeine AM and 2.5 mg/kg PM, treatment 2 = placebo AM/PM, treatment 3 =5mg/kg AM and placebo PM, treatment 4 =5 mg/kg AM/PM (Bell et al., 2003). The cycle ergometer test used two phases: phase one was a 5 minute ride at 50% VO_2max, the second phase was time to exhaustion (≈15 minutes) at 80% VO_2max (Bell et al., 2003).

The groups treated with caffeine significantly ($P < 0.05$) increased time to exhaustion 31 ± 18% compared to placebo (Bell et al., 2003). However treatment 1 and 3 did not show strong efficacy for improved performance with a second dose of caffeine in the PM trial. The caffeine supplement also decreased rate of perceived exertion (RPE, 14.7 ± 2.2) in 80% time to exhaustion ride compared to placebo (15.6 ± 2.3) (Bell et al., 2003). Furthermore group differences of lactate concentrations were significantly higher ($P < 0.05$) with caffeine ingestion (6.87 ± 1.59) compared to placebo (6.30 ± 1.60). Caffeine did not have any effect on free fatty acids but increased glucose concentrations. This observation is expected during the physiologic stress of exercise on the body. This investigation suggests that there is a strong correlation between caffeine intake and RPE during aerobic performance (Bell et al., 2003). Therefore this study illustrates caffeine has an ergogenic effect of aerobic performance.

Caffeine use in study populations

Published caffeine related research studies include various doses of caffeine investigating as low as 2 mg/kg to as high as 9 mg/kg (Graham, 2001a). The National Collegiate Athletic Association (NCAA) restricts the use of caffeine at doses ≥ 9 mg/kg, because doses this high

have the possibility of a positive doping test at a urine concentration > 15 $\mu g \cdot ml^{-1}$ (ACSM, 2009). Many studies have tested only one dose of caffeine relating to anaerobic performance measures, while some used various levels of doses within anaerobic testing (Astorino, Terzi, Roberson, & Burnett, 2010b; Beaven et al., 2008; Jacobson & Thurman-Lacey, 1992a; Jacobson & Edwards, 1991).

Astorino et al. (2010b) examined caffeine intake and the isokinetic potential of exercise in recreational active males (n=15, 26.4 ± 3.9 years). This investigation used a single-blinded crossover design with counterbalance. Astorino et al. (2010b) counterbalance ensured that no participants knew the order of treatment as well all supplements were prepared by the same co-investigator. Participants were screened for caffeine intake and required >100 $mg \cdot d^{-1}$ of habitual use (Astorino et al., 2010b). There was no maximum caffeine intake or methods of measurement for habitual caffeine use. Further, participants were required to be nonsmokers and recreationally active for four hours per week. Astorino et al. (2010b) investigated two separate dosage of anhydrous caffeine (2 and 5 mg/kg) compared to placebo (lemon-flavored noncaloric beverage, Crystal Light, Northfield, IL). Isokinetic strength potential was measured using knee extension and flexion of the dominant leg in a seated dynamometer (Biodex System 4; Biodex, Shirley, NY) (Astorino et al., 2010b). The Biodex System 4 was used to measure peak and average torque, power, total work, and work fatigue. Astorino et al. (2010b) revealed through pilot testing that the coefficient variance for extension; peak torque (5.3%), total work (7.8%) and peak flexion torque (6.5%).

A total of four trials were conducted first beginning with a familiarization trial 1 set of 40 repetitions on the Biodex. Additionally researchers recorded participant individualized seat measurements including seat angle, chair location, lever arm length, and isokinetic attachment

during familiarization for subsequent trials (Astorino et al., 2010b). Participants were required to refrain from caffeine for 48 hours prior to each trial. During supplementation, the same assistant mixed one of the three total samples for each participant and likewise that supplement was ingested 1 hour prior to exercise (Astorino et al., 2010b). The exercise protocol incorporated 2 maximal sets at 40 repetitions knee flexion/extension (1 flexion + 1 extension = 1 repetition) (Astorino et al., 2010b). The result revealed a significant decrease between set 1 and 2 for peak torque extension and flexion ($P < 0.05$) with intergroup comparison (3x2, ANOVA, repeated measure) (Astorino et al., 2010b). The decline between set 1 and 2 was consistent for all measures including average torque, total work, and power output ($P < 0.05$). However peak knee flexion reached significance in set 1 ($P < 0.05$, $F(2,28) = 4.24$) in 5 mg/kg supplement compared to placebo (Astorino et al., 2010b). The same result effect ($P < 0.05$) was found for total work in both knee extension, 5 mg/kg compared to placebo and 2 mg/kg in set 1; and knee flexion, 5 mg/kg compared to placebo only in set 1 (Astorino et al., 2010b).

Beaven et al. (2008) investigated the acute effect of caffeine related to hormonal response during exercise in professional male rugby players (n=24, 22.3 ± 3.0 years). The study was double-blinded and consisted of 4 randomized trials (Beaven et al., 2008). Participants completed a 24-hour food recall to assess caffeine intake and were asked to refrain from ingesting dietary caffeine before trials, no washout period. This investigation used four definitive but separate doses of caffeine 200 mg, 400 mg, 800 mg, and 0 mg (placebo) ingested 1 hour prior to exercise (Beaven et al., 2008). The resistance training portion of this study was based around a rugby practice including back squat, deadlift, lunge, and sprinting. This study did not directly propose to measure ergogenic potential of exercise other than focusing on effects of resistance exercise and hormonal concentrations. These concentrations were measured with

salivary samples (2ml) collected at dosing, immediately before exercise, every 15 minutes during exercise, and at 15- and 30 minutes post-exercise (Beaven et al., 2008). The salivary samples could have been contaminated if participants consumed hot beverages or brushed their teeth within 2 hours of each trial (Beaven et al., 2008). Although athletic performance was not directly measured there were improvements within caffeine dose. The larger dose of caffeine (800 mg) showed a relationship with decreasing sprint times during practice (1.7%; 90% confidence limits ± 2.5) (Beaven et al., 2008). Additionally, the salivary samples revealed an increase in testosterone concentration (15% ± 19%) with ≥400 mg of caffeine (Beaven et al., 2008). Similarly salivary cortisol increased with doses of caffeine ≥400 mg with 800 mg 51% increase (±41%) dose-response. This study suggests a dose-response relationship between caffeine supplementation and both cortisol and testosterone concentration during exercise.

An early experiment by Jacobson and Thurman-Lacey, (1992a) included female participants (n=40, 21 ± 1.7 years) the effect of two doses of caffeine on manipulative skills. Fitness level of the participants was not discussed other than using a college student sample. This sample included 20 habitual caffeine (caffeine familiar) users identified by caffeine intake > 750 $mg \cdot d^{-1}$, and 20 naïve or caffeine intake < 90 $mg \cdot d^{-1}$. This study used a double-blinded crossover design with a 5-day caffeine questionnaire to recall caffeine use in determining habitual versus naïve caffeine users. The purpose of this investigation was to examine the physiological effects on the body measured by steadiness, dexterity, and tracing ability (Jacobson et al., 1992a). Prior to the investigation caffeine naïve subjects abstained from caffeine for 4 days whereas habitual caffeine users maintained normal caffeine intake (Jacobson et al., 1992a). The doses of caffeine used within this study 2.5 and 5 mg/kg compared to placebo were intakes were randomized and ingested 1 hour prior to testing. The results show significant differences (p <0.05) in

physiological effects of caffeine on dexterity and hand steadiness within naïve caffeine users but not within caffeine familiar (habitual caffeine users) group (Jacobson et al., 1992a). Specifically, results were altered between the 5 mg/kg and both 2.5 mg.kg or placebo within sample comparison. No significant differences existed between dose and habitual caffeine users because confounding factors such as high caffeine intake (> 750 $mg \cdot d^{-1}$) or the lack of constraints for participants to refrain from caffeine prior to testing.

Jacobson & Edwards, (1991) examined the effect of two doses of caffeine on peak torque and muscular endurance in male and female participants (n=20, n=16 respectively) (n=36, 20.5 years mean no SD). All participants reported a habitual caffeine intake >200 $mg \cdot d^{-1}$ and screened (questionnaire) for excess caffeine use (Jacobson et al., 1991). This study used a double-blinded randomized trial with three treatments: 600 mg, 300 mg, and placebo. The participants refrained from caffeine for 24 hours and ingested supplement 1 hour prior to testing (Jacobson et al., 1991). The exercise protocol included peak torque and muscular endurance for both knee flexion and knee extension. Furthermore three isokinetic velocities were measured and recorded using a Cybex II Isokinetic Dynamometer at 75 º/s (3 reps, 5 min rest), 180 º/s (3 reps, 5 min rest), and 300º/s (15 reps, 10 min rest) (Jacobson et al., 1991). The results of the habitual caffeine use questionnaire revealed ≈89.4 and 109.2 $mg \cdot wk^{-1}$ (Jacobson et al., 1991). There was an increase in combined torque and velocity within both 600 mg and 300 mg treatments however the response did not reach significance (p >0.05) (Jacobson et al., 1991). This suggests that caffeine does not affect ergogenic potential in relation to maximal strength.

The studies that used multiple doses of caffeine found a mixture of results, depending upon the dose. While Jacobson et al. (1992a) found a statistical significance of motor performance measured by hand steadiness and tracing “drawing” tasks among caffeine naïve

subjects, no significance was discovered within habitual caffeine users. Although ergogenic potential was not examined in this investigation this research identifies possible differences in response to caffeine in habitual and naïve participants. Both Bell et al. (2003) and Jacobson et al. (1992a) used similar dosing procedures of a placebo, 2.5 mg/kg, and 5 mg/kg of caffeine. It was discovered that a repeat dose of caffeine 5 mg/kg in the morning and 2.5 mg/kg, 5 hours later did not reveal significance compared to a single dose of caffeine (5 mg/kg) and placebo (Bell et al., 2003). However the dose of caffeine significantly increased time to exhaustion in both caffeine trials versus placebo trial, therefore it is suggested that one dose of caffeine is sufficient to maintain ergogenic potential. Although Bell et al. (2003) was an aerobic investigation the dose relationship and caffeine treatment directly related to Jacobson et al. (1992a). Therefore Bell et al. (2003) is excluded from further discussion beyond this point.

In a similar design Astorino et al. (2010b) examined two separate doses of caffeine intake and effect on muscular endurance measured with Biodex System 4. The significance of this study was that it measured unilateral isokinetic knee extension and flexion in the dominant leg (Astorino et al., 2010b). The dominant leg is determined by reaction and strength difference which is common among athletes. This study was unique in that supplementation was non-controlled, referring to no visual consumption of caffeine supplement because supplements were provided to participants during familiarization trial (e.g. three bottled, sealed supplements). The participants were instructed to consume one supplement 1 hour before testing and return the empty container to researchers (Astorino et al., 2010b). The results of this study did not show efficacy (did not reach significance) for caffeine use < 5 mg/kg to be ergogenic potential. This suggests that a dose ≈ 2 mg/kg is not ergogenic in habitual caffeine users during isokinetic knee flexion and extension.

Beaven et al. (2008) used set doses of caffeine rather than weight measured doses (mg/kg). This investigation revealed a possible beneficial effect related to an increase in testosterone response to exercise with doses ≥ 400 mg of caffeine (Beaven et al., 2008). The results for the most part were inconclusive for acute hormonal affect after ingesting caffeine. These investigations did not find a strong efficacy for examining more than a single dose of caffeine per activity or period of exercise for ergogenic potential other than possible effects between habitual and naïve caffeine users. In addition, this premise illustrates a possible reaction to caffeine in trained versus untrained participants. Overall research suggests that multiple dose studies are unnecessary as there is not a minimum recommended dose of caffeine for increased ergogenic potential. Therefore investigations should focus on single doses in relation to ergogenic potential.

The previous research used of multiple doses caffeine in their investigations however there was no strong efficacy found within that design. The following investigations are focused on single doses of caffeine and the dose-response relationship of anaerobic exercise (Anselme, Collomp, Mercier, Ahmaidi, & Prefaut, 1992; Astorino, Firth, &, Rohnmann, 2008; Beaven et al., 2008; Beck et al., 2006; Beck et al., 2008; Forbes et al., 2007; Hudson, Green, Bishop, & Richardson, 2008; Jacobs, Pasternak, & Bell, 2003; Kalmar et al., 1999; Timmins, & Saunders, 2014). The minimum effective dose of caffeine has not been established for ergogenic potential within resistance training, but research shows improvements in ergogenic potential of all anaerobic athletic performance (resistance training, Wingate, sprint) with doses from 2.5 mg/kg to 7 mg/kg (Astorino et al., 2010a).

Anselme et al. (1992) investigated caffeine supplementation in association with maximal anaerobic potential in recreationally active males (n=10) and females (n=4). Participants (n=14,

26 ± 1.72 years) were required to accumulate ≈ 3.5 hour per week of physical activity related to recreational sports. This investigation was double-blinded and randomized comparing 250 mg caffeine to placebo ingested 30 minutes prior to exercise (Anselme et al., 1992). The habitual use of caffeine was not measured or controlled throughout the study. Researchers measured blood lactate with intravenous samples during peak cycle ergometer sprints (Anselme et al., 1992). Blood samples were collected during rest/recovery of each load. The sprint protocol consisted of a maximum duration of 6 seconds starting with 2 kilograms of weight load on the cycle. After each set participants would recover for 5 minutes before the next sprint increasing by 2 kg, 1 kg, and 0.5 kg until maximum velocity was reached (Anselme et al., 1992). The peak load was the same for both treatments but the pedaling frequency of the caffeine group significantly increased (P <0.001) compared to placebo Anselme et al., 1992). Moreover, blood lactate reached significance in the peak load (8 kg) in caffeine group (P <0.01) and 5 minutes post recovery (P <0.04) to placebo comparison Anselme et al., 1992). This investigation suggests a positive relationship between peak anaerobic capacity and caffeine supplementation including increased levels of blood lactate.

Astorino et al., 2008 used a double-blinded crossover method to investigate the acute effect of caffeine on anaerobic exercise. Researchers recruited resistance trained males (n=21, 23.4 ±3.6 years) screened for habitual caffeine use. The method of screen for caffeine intake was not discussed however 4 participants reported being naïve users of caffeine (≈ 0 $mg \cdot d^{-1}$). Participants were required to refrain from caffeine for 48 hours pre-trial. The treatment 6 mg/kg of caffeine or placebo was ingested 1 hour pre-exercise (Astorino et al., 2008). During each trial researchers collected heart rate (HR) and blood pressure (BP) and measured anaerobic capacity with a 1RM test and repetitions to failure (fatigue) for both standard bench press and seated leg

press Astorino et al., 2008). The exercise protocol incorporated a 12-15 repetition warm prior to 1RM determined within 3-6 sets and maximum weight lifted one complete rep (Astorino et al., 2008). The repetitions to failure or muscular endurance was the total number of repetitions (to failure) at 60% 1RM. Astorino et al. (2008) reported side effects of caffeine supplementation similar to Kedia et al. (2014) with the addition of tremors, higher energy, and elevated heart rate. The results showed increased in weight lifted for both bench press and leg press but failed to reach significance (P >0.05). For example, twelve participants increased bench press (≥3 kg) with caffeine but five participants increased bench (≥3 kg) with placebo (Astorino et al., 2008). Likewise both bench and leg press muscular endurance increased total number of repetitions and total load but did not reach significance (P >0.05) (Table 3). Overall both HR and BP significantly increased with caffeine consumption (P <0.05) in naïve participants during warm-up. The dose of caffeine and results from this study suggest a response-relationship of increased side effects in caffeine naïve participants.

Hudson et al. (2008) investigated the independent effects of caffeine and aspirin in combination with light intensity resistance trained (RT) in resistance trained males (n=15, 22 ± 1.3 years, >8 weeks of training experience). All participants maintained RT 2-4 days per week and caffeine intake throughout the study, except within 24 hours of each trial (Hudson et al., 2008).

Table 3

Astorino et al. (2008) effect of caffeine on anaerobic performance

Parameter	Caffeine	Placebo
1-RM bench press (kg)	116.4 ± 23.6	114.9 ± 22.8
Repetitions at 60% 1-RM (kg)	19.9 ± 4.3	18.4 ± 4.0
Weight at 60% 1-RM (kg)	69.9 ± 14.3	68.9 ± 13.3
Total weight lifted (kg)	1369.7 ± 383.1	1226.2 ± 357.3
1-RM leg press (kg)	410.6 ± 92.4	394.8 ± 95.4
Repetitions at 60% 1-RM (reps)	23.9 ± 13.0	22.5 ± 11.0
Weight at 60% 1-RM (kg)	247.9 ± 57.5	238.6 ± 55.5
Total weight lifted (kg)	5945.9 ± 3275.6	5358.0 ± 2148.5

This study used a double-blinded within subject design to measure the associated effects on muscular endurance, HR, RPE, and pain perception index (PPI). The participants reported habitual caffeine use ≈100-400 $mg \cdot d^{-1}$ the measurement used was not reported. The exercise protocol consisted of 3 treatment trials testing seated leg extension and seated arm curl (4 sets for 12 repetitions) (Hudson et al., 2008). The twelve repetition sets were participant estimated repetition maximums counter-balanced for reps/set <11 or >13 was adjusted with increased or decreased weight (Hudson et al., 2008). The rest ratio was 3minute between sets and 5 minutes between exercises. The participants ingested caffeine (6-6.4 mg/kg), aspirin (10-10.4 mg/kg) or placebo 1 hour pre-exercise. The non-exercise measures (HR, PPI, and RPE) were measured at rest, pre-set, immediately post-set (within 10s) and 5 minutes post exercise. In addition

researchers used a questionnaire to measure symptoms related to supplement intake and possible side effects post-exercise (Hudson et al., 2008).

The results of this study revealed a significant (p <0.05) increase in leg extension total repetitions within the caffeine group compared to across group samples. Additionally, a dose of caffeine significantly (p <0.05) increased HR in leg extension greater than aspirin and placebo (Hudson et al., 2008). However aspirin significantly (p <0.05) increased the RPE over caffeine supplementation. Inversely caffeine did not result in significant (p =0.051) increase in arm curl repetitions but significantly (p <0.05) increased HR compared to aspirin and placebo (Hudson et al., 2008). Furthermore post-exercise questionnaire resulted in significant (p <0.05) increased reports of side effects with caffeine ingestion only (Hudson et al., 2008). The side effects reported were similar to those previously reported (Astorino et al., 2010a; Kedia et al., 2014) and included restlessness, tremors, and stomach distress (Hudson et al., 2014). Overall the results of this study suggest a dose response reaction to caffeine consumption and HR variability. Further a dose of aspirin has shown a greater anti-algesia effect than caffeine.

Jacobs et al. (2003) investigated the independent and combined effect of caffeine ephedrine on muscular endurance in recreational active males (n=13, 18-34 years of age). This study used a double blinded, repeated measures. The researchers used the *a priori* analysis in order to estimate the number of participants required with a statistical power of 0.8 and expectation that treatment would cause 30% difference in repetitions performed during one set of exercise (Jacobs et al., 2003). The dietary intake of caffeine was not controlled nor measured except that participants did not avoid caffeine. This study required participants to attend 9 sessions that began with an initial visit to measure 1RM for leg press and bench press was measured followed by 2 succeeding familiarization trials to adapt participants to fasting but

euhydrated state. Participants were given a standardized breakfast with choice of white toast, muffin, or bagel and the choice of apple or orange juice all subsequent trials used the same meal. Participants' refrained from caffeine, alcohol, and medication 48 hours pre-exercise sessions (4-8) all participant trials were randomized (Jacobs et al., 2003).

Trials tested one of four treatments including caffeine (4 mg/kg), ephedrine (0.8 mg/kg), both caffeine and ephedrine combined or placebo, each treatment was ingested 90 minutes prior to exercise. The exercise protocol consisted of a warm up of 10 reps at ≈30%, and 3 supersets, supine/seated leg press at 80% 1RM to failure, followed by bench press at 70% 1RM to failure (Jacobs et al., 2003). The superset was defined as one leg set to one bench set to failure without rest (Jacobs et al., 2003). Each superset was then followed by 2 minutes of rest. The exercise measures were recorded as total work calculated from total number of repetitions and total weight lifted. The non-exercise measurements included BP, HR, blood hemodynamics specifically concentrations of caffeine and ephedrine analyzed by mass spectrometry (GC-MS) (Jacobs et al., 2003). The data was analyzed by a repeated factorial measure (2 X 2 X 3) ANOVA determining effects of *caffeine (present or absent) an ephedrine (present or absent) and set (1, 2, or 3)* (Jacobs et al., 2003).

The consumption of caffeine increased the total number of repetitions (13.6 ± 6.5) but did not reach significance (P <0.05) compared to placebo (12.5 ± 5.0, set 1) (Jacobs et al., 2003). The combination of caffeine and ephedrine significantly (P<0.05) increased the number of repetitions for leg press (18.5± 8.4) and bench press (14.3 ± 3.1) compared to caffeine and placebo (set 1) (Jacobs et al., 2003). However this study revealed a hypertensive reaction to the combination treatment (caffeine and ephedrine) resulting in participants (n=2) BP to increase to 204/ 90 and 214/112 pre-exercise 90 minutes post consumption (Jacobs et al., 2003). This

relationship demonstrates evidence of a synergistic effect of nutritional supplements. There was no statistical effect after set 1 in any treatment. The results reflecting ephedrine are not considered relevant to caffeine as ephedrine is a banned substance FDA (FDA, 2015).

Kalmar et al. (1999) investigated the effects of caffeine on neuromuscular function in healthy males (n=11, 22.3 ± 2.4 years). This study used a double blind repeated measure with a caffeine dose of 6 mg/kg compared to placebo (Kalmar et al., 1999). The activity level of participants was not discussed nor was the inclusion criteria other than habitual caffeine use. Participants self-reported caffeine intake ≤ 200 mg·wk^{-1}; in addition, they were required to abstain from caffeine for seven days before and throughout the study. The researchers did not report the measurement tool used to assess caffeine intake. The blinding procedures used a coded envelopes system that was identified by the participant's initials and the day the dose was used (Kalmar et al., 1999). Each trial consisted of a randomized treatment and each treatment was separated by three days. Caffeine was administered and ingested one hour pre-exercise. The exercise protocol consisted of four parts including 1) tibialis nerve stimulation of the left soleus 10 times lasting 1-ms, 2) maximal voluntary contraction (MVC) of the right knee extensors 4 repetitions, 3) 6 submaximal contractions, and 4) 50% MVC held to failure (Kalmar et al., 1999). The results found MVC (Part 2) reached significance ($P < 0.01$) with an increase of 3.5 ± 1.01% in caffeine compared to placebo (Kalmar et al., 1999). There was no significant effect in reflex of the soleus as well as submaximal contractions with caffeine consumption. Caffeine, however resulted in a significant increase ($P < 0.05$) the time to failure at 50% MVC (Kalmar et al., 1999). This suggests that caffeine may increase maximal contraction and beneficially effect sustained muscle activation.

Timmins et al. (2014) examines the effect of caffeine on MVC in resistance trained males (n=16, 21.1 ±0.8 years). This study used a single blind cross over randomized trial with a 6 mg/kg caffeine dose compared to placebo with both upper and lower segments (Timmins et al., 2014). Participants completed a habitual caffeine use questionnaire which identifying participants ≤ 300 $mg \cdot d^{-1}$ (Timmins et al., 2014). Participants were excluded if they had <1year RT experience or if they used nicotine or any other tobacco product. The researchers first of three sessions included a participant familiarization to the exercise protocol.

The exercise protocol measured the isokinetic peek torque of knee extensors, ankle plantar flexors, elbow flexors and wrist flexors in the subsequent trials (Timmins et al., 2014). Each exercise was measured at an angular velocity of 60 º/s with 3 repeated 1RM. Participants were asked to refrain from caffeine for 24 hours pre-exercise with a single week separation between treatment (Timmins et al., 2014). Thirty minutes after ingesting the respective treatment participants started MVC using the isokinetic dynamometer Biodex System 3 Pro (Biodex Medical Systems, Shirley, NY).

The caffeine frequency questionnaire revealed participants consumed ~95.4± 80.0 $mg \cdot d^{-1}$ (Timmins et al., 2014). Further results revealed a significant difference ($p<0.001$) in peak isokinetic torque between muscle groups (upper/lower) (Timmins et al., 2014). There was also a significant increase ($p =0.011$) in peak torque from placebo to caffeine. The total effect of caffeine increased knee extension 13.7% (237.0 ± 69.5) compared to placebo (208.4 ± 38.3) (Timmins et al., 2014). The results found within this research support caffeine supplementation for ergogenic potential on peak isokinetic torque.

The analysis of select caffeine related anaerobic studies that used a single dose can be divided into two groups; 1) caffeine ingestion ≥ 4 mg/kg, and 2) < 4 mg/kg. The first group

(Astorino et al., 2008; Hudson et al., 2008; Jacobs et al., 2003; Kalmar et al., 1999; Timmins et al., 2014) are studies that used caffeine ≥ 4 mg/kg (or equal about or greater than 300 mg) of caffeine ingested and the second group (Anselme et al., 1992; Beaven et al., 2008; Beck et al., 2006; Beck et al., 2008; Forbes et al., 2007) researched dosage < 4 mg/kg (or less than about 300 mg) of caffeine ingested.

In addition, most studies used a randomized, double-blind controlled design and included a 24-hour period (minimum period) non-controlled to withdraw caffeine from dietary consumption (Astorino et al., 2010a). However a review by Graham (2001a) found that exclusion periods from caffeine whether there was no withdrawal, 24 hours, or 4 days shows no difference in exercise performance. This suggests that depending of the level of habitual caffeine intake, withdrawal periods may not differ beyond 12 to 48 hours. Conversely this withdrawal period when greater than 48 hours may increase participants' sensitivity to caffeine, causing intoxication when consuming high doses (Graham 2001a). Much of the research recommends a 12 to 48-hour abstinence period to remove caffeine and re-sensitize the body (Astorino et al., 2010a; Astorino et al., 2008; Astorino et al., 2010b; Forbes et al., 2007; Hudson et al., 2008; Jacobs et al., 2003). One research team had participants "rinse" from caffeine-containing beverages for 7 days and throughout the study (Kalmar et al., 1999). "Cleansing is the withdrawal, or re-sensitizing are periods of time without caffeine. These are common in most research where the use of habitual caffeine users versus non-caffeine users (naïve) has presented another possible shape for research design.

Another experiment by Tarnopolsky and Cupido (2000) supported the use of habitual (n=6, 29 ± 7 years) and naïve (n=6, 26 ± 4 years) caffeine users within physically active males (n=12, ≥3 workout day·wk^{-1}). This study was double-blinded, crossover, and counter-balanced

design. The caffeine intake >500 mg·day^{-1} (actual ≈771 ± 295, mg) was deemed as habitual use (Tarnopolsky et al., 2000). Whereas naïve or noncaffeine users intake <50 mg·day^{-1} (actual ≈14 ± 17, mg) (Tarnopolsky et al., 2000). The participants completed self-reported food frequency questionnaires (FFQ) to estimate caffeine, and caloric intake with a computer program (Nutritionist IV, Silverton, OR). The experimental protocol used randomized (right or left) leg muscle stimulation to fatigue (failure) at one of two electrical hertz (Hz) (20Hz twice, and 40Hz) which measured the peak twitch torque and tetanic torque of the dorsiflexor muscle (Tarnopolsky et al., 2000). This protocol was then measured with one of two randomized treatments: 1) caffeine dose (6 mg/kg) or 2) placebo (Tarnopolsky et al., 2000). Participants refrained from caffeine for 12 hours prior to each investigation and began exercise 90 minutes after ingestion. The results found within this study was that there were no effects of interactions between habitual and naïve caffeine users (Tarnopolosky et al., 2000). The related caffeine groups were assessed together in an across group comparison. There was a significant increase (P <0.005) in tetanic torque with caffeine supplementation and 20Hz stimulant compared to placebo (Tarnopolsky et al., 2000). However the 40Hz stimulant did not reveal any significant difference between groups. This study suggests that caffeine may directly and ergogenic effect of muscle.

Similarly Forbes et al. (2007) and Astorino et al. (2008) classified users as caffeine naïve with intake <100 mg·day^{-1} and ≈ 0 mg·day^{-1} respectively which is consistent with the measure used by Tarnopolsky et al. (2000). A concurrent finding within Tarnopolsky et al. (2000), Forbes et al. (2007) and Astorino et al. (2008) was that there were no differences in measured torque, Wingate/muscular endurance, or 1RM/muscular endurance respectively between habitual and naïve users. The only difference was that naïve users experienced more pronounced symptoms

such as: *tremors, insomnia, greater energy, elevated heart rate, headache, stomach distress (upset), and restlessness* associated with caffeine intake (Astorino et al., 2008; Hudson et al., 2014; Kedia et al., 2014).

In another perspective Jacobs et al. (2003) did not control or make an effort to estimate caffeine intake in participants prior to participation and asked participants to not consume coffee 48 hours prior to testing. This was despite caffeine content of other substances. Most studies identified participants either as habitual users or nonhabitual (naïve) users by measuring caffeine intake with the exception of previously listed research examples. Furthermore those studies that identified habitual caffeine intake also used withdrawal periods for caffeine ranging from 0 to 4 days. Overall the listed studies have not effectively or concisely determined the ergogenic effect of caffeine. The major impact of including nonhabitual (naïve) users is that the side effects are more apparent than those that regularly use caffeine (habitual users).

Furthermore gender which has lead research direction annotated by Graham (2001b) revealed no difference in caffeine clearance after 90 minutes of exercise related to gender in studies that used females that were not on oral contraceptives. On the contrary evidence suggests that female participants using daily contraceptives have a decreased clearance (removal from the body) of caffeine (Abernethy & Todd. 1985) and that the luteal phase (latter stage) of the menstrual cycle also impairs caffeine degradation (breakdown and clearance) (Lane, Steege, Rupp, & Kuhn, 1992). This evidence was cited as exclusion criteria for participants in Kalmar and Cafarelli (1999); Astorino et al. 2008; Hudson et al. 2008; whereas some studies did not account for this information and investigated both male and female participants (Forbes et al., 2007). Others justified the exclusion of female participants by other means such as unknown effects on a potential fetus with a combination of caffeine and ephedrine (Jacobs et al., 2003).

Research by Jacobson et al. (1992a) included a total female cohort, disregarding research related to females using oral contraceptives. This study did not investigate ergogenic potential but found differences within naïve compared to habitual caffeine users. Another study, Kamimori, Jubert, Otterstetter, Santaromana, and Eddington (1999) found evidence to refute decreased clearance related to the luteal phase of the menstrual cycle as suggested earlier (Lane et al., 1992). Gender differences are not fully understood and may not affect the outcome of research.

Caffeine side effects

Bunsawat, White, Kappus, and Baynard (2014) investigated pre-exercise caffeine consumption and autonomic recovery related side effects in healthy participants (n=18, 26 ± 1 years, male n=10, female n=8). This study used a double-blinded crossover design measuring HR, heart rate variability HRV, heart rate recovery HRR, and VO_2max with a maximal treadmill test (Bunsawat et al., 2014). Heart rate variability was collected with a continuous 5 minute ECG at a sample rate of 1000Hz (Biopac Systems, Santa Barbara, CA) (Bunsawat et al., 2014). This measure detected the rate and variance at which a heart muscle contraction occurred. There were two randomized treatments of caffeine (400 mg) and placebo ingested 45 minute pre-exercise. Participants who reported ≤285 $mg \cdot d^{-1}$ and were required to refrain from caffeine for 12 hours prior to exercise. The results revealed that caffeine significantly increased (p <0.05) HR max (192 ± 2) compared to placebo (190 ± 2) (Bunsawat et al., 2014). Caffeine, as expected significantly increased (p <0.05) time to exhaustion (13.3 ± 0.7) compared to placebo (12.8 ± 0.7) (Bunsawat et al., 2014). The HRV increased in both treatment groups (p <0.05) but caffeine ingestion resulted in less variance post-exercise. Overall this study suggests that caffeine effects BP with delayed recovery post-exercise. The researchers' related effect on autonomic recovery

to detrimental cardiovascular results including sudden death (Bunsawat et al., 2014). This is increasingly important to investigate as the use of caffeine during exercise is a growing trend.

The side effects of excess caffeine consumption or intoxication have been discussed within ACSM (2009), Astorino et al. (2008), Bunsawat et al. (2014), Hudson et al. (2014), Kedia et al. (2014) and Tarnopolsky et al. (2000). These side effects include: anxiety, nervousness, rapid heartbeat, insomnia, jitteriness, gastrointestinal distress.

Caffeine and tobacco use

Caffeine has several interactions with chemicals that affect clearance either increasing or decreasing half-life. Parsons and Neims (1978) reported that tobacco or specifically nicotine, was found to increase the clearance of caffeine, decreasing half-life. A follow-up investigation by Benowitz, Peng, and Jacob (2003) reached significance (P <.001) for oral clearance or decreased half-life in smokers compared to nonsmokers. This research suggested that smoking does not increase urinary secretion of caffeine related to unaffected urine concentration of caffeine in smoking participants. This implication is important to consider in regard to controls within research design.

Caffeine and blood flow

Caffeine, as a supplement can have a positive effect on performance of anaerobic activities; caffeine can affect blood flow, increase blood pressure, and increases heart rate (Daniels, Mole, & Shaffrath, 1998; Shechter et al., 2011; Bell et al., 2003; Bunsawat et al., 2014). In a study by Daniels et al. (1998) the research team investigated the effects of caffeine on blood measures during rest and during exercise in trained cyclists (n=10, 30 ± 0.3). The participants included males (n=3) and females (n=7) that were nonhabitual caffeine users (<100 $mg \cdot d^{-1}$) (Daniels et al., 1999). The measures used in this study were, heart rate, blood pressure,

calculated mean atrial pressure (MAP), forearm blood flow (FBF), forearm vascular conductance (FVC) and angiotensin II (vasoconstrictor) (Daniels et al., 1998). FBF was measured using a strain gauge via venous occlusion plethysmography (Daniels et al., 1999). Participants were required to abstain from caffeine 4 days prior to testing and arrived 12 hours fasted (between 6AM and 10AM) (Daniels et al., 1999). There was no discussion on time of arrival. This could possibly ease the strain of being fasted during exercise. The exercise protocol consisted a 55 minute cycle ergometer trial at 65% VO_2max. The trials were randomized into two treatment groups, caffeine (6 mg/kg) or placebo both ingested 45 minutes pre-exercise. There were no statistical differences between males and females therefore they were assessed together. The results revealed a significant increase (P <0.05) in MAP 40 minutes post-caffeine consumption, likewise both systolic and diastolic BP increased 20 and 40 minutes respectively compared to placebo (Daniels et al., 1999). In addition, caffeine significantly increased (P <0.05) FBF and FVC after ingestion during exercise only compared to placebo (Daniels et al., 1999). Therefore this study suggests caffeine may positively affect angiotensin II, increasing vasoconstriction and blood flow during exercise.

Shechter et al. (2011) examined the acute effect of caffeine on brachial flow-mediated dilation (FMD). This study used a double-blinded, crossover design in participants with and without coronary artery disease (CAD) (n=80, $53 \pm 6_w$, $53 \pm 6_{wo}$). Participants arrived fasted for at least 12 hours and consume a randomized treatment consisting of caffeine 200 mg or placebo (Shechter et al., 2011). The FMD was measured in the brachial artery using a 15- to 6- MHz ultrasound system (HP SONOS 7500 cv System, Agilent Technologies, Inc., Andover, MA) one hour after ingestion. This measure used a pressure occlusion cuff to reduce blood flow prior to measures with ultrasound Doppler and blood vessel diameter after deflation (Shechter et al.,

2011). The results revealed that caffeine significantly increased (p <0.001) FMD in the brachial artery in participants without CAD compared to placebo (Shechter et al., 2011). The results differ from exercise trials in that, exercise studies do not include participants with a chronic disease. Therefore comparisons and conclusions are difficult to compare. However the results of this study are important supporting the possible effect that caffeine may increase FMD in the brachial artery. Overall blood flow is not well researched in anaerobic research with the exception of heart rate, blood pressure, and ECG.

Summary

The effect of cocktail supplements or "pre-workout" supplements containing L-arginine nitrate and caffeine are not fully understood related to anaerobic performance. There is an apparent absence for multi-ingredient supplement regulation. Furthermore, there is a lack of comparative research comparing individual ingredients to multi-ingredients. There is no definitive dose for caffeine or nitric oxide to produce ergogenic effect.

Research questions

1. What are the short term effects of using pre-workout supplements within a healthy adult male population?
2. Does a single dose of caffeine improve the anaerobic performance compared to a cocktail pre-workout supplement?
3. Does a cocktail pre-workout supplement improve blood flow measures compared a single dose of caffeine?

CHAPTER 3. METHODS

Experimental approach to the problem

The purpose of the study was to examine the acute ergogenic effects of a single ingredient caffeine, compared to combined mixture: arginine nitrate, caffeine and other, and placebo. This study used a randomized, double-blind, placebo controlled, and crossover design.

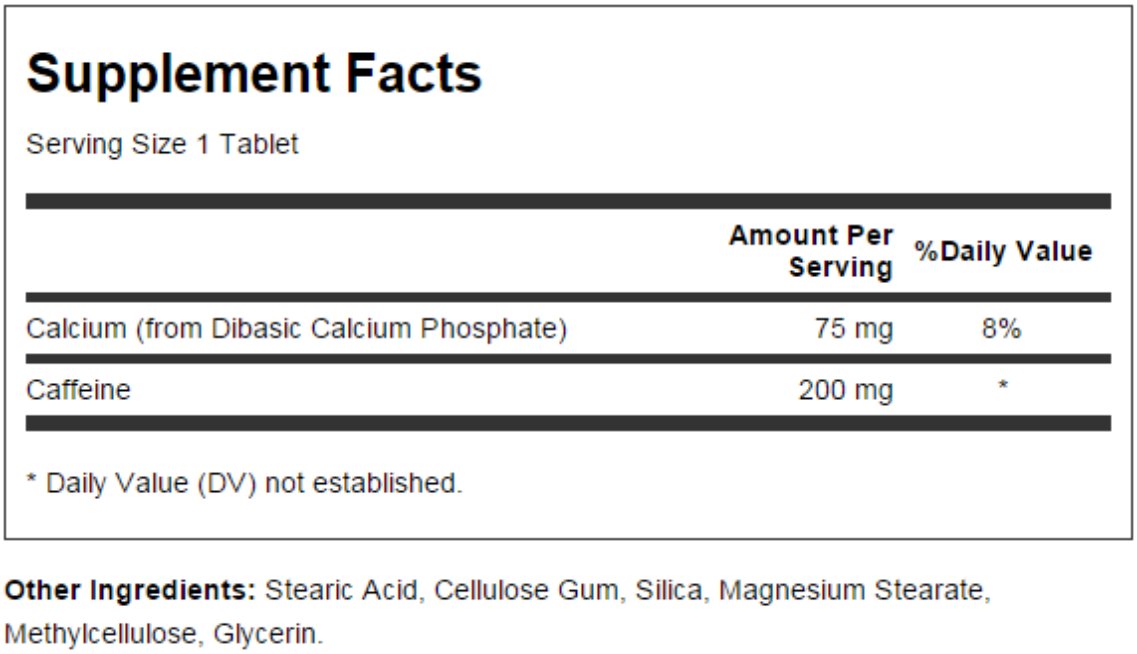
Participants and recruitment

Fifteen recreationally active males (age 18-35 yrs) were recruited for this study. Recruitment flyers and North Dakota State University listservs were used to recruit participants (Appendix D, Appendix E). After preliminary email screening participants were scheduled for a familiarization trial. After receiving study explanation and time to ask questions, participants were asked to sign written informed consent. The informed consent and protocol was approved by the North Dakota State University Internal Review Board (Appendix B, Appendix A). For the first visit the participants were screened using the PAR-Q risk stratification form (ACSM, 2000). The participants were required to be screened for excess or absent caffeine use to classify as habitual or nonhabitual users (Appendix C). In addition to the completion of the informed consent, PAR-Q, and caffeine food frequency questionnaire (CFFQ), participants were required to complete a Health History Questionnaire (HHQ). The inclusion criteria included: 1) caffeine consumption ≥100 mg or ≤ 500 mg of caffeine per day (Graham, 2001a), as estimated by a study team created CFFQ; 2) no current chronic use of another supplement within 30 days as described by Kedia et al. (2014); 3) any chronic disease including but not limited to: affecting cardiovascular, nervous system, and digestive system or other health problems identified by HHQ 4) non-smoking males including all nicotine products. Females were not recruited due to the menstrual cycles and oral contraceptives degradation effect on caffeine as described by

Timmins et al. (2014). All participants were required to be self-reported resistance trained with at least 6 months experience and maintain at least 3 hours of overall exercise per week.

Supplements and placebo

The pre-workout supplement (PRE) was commercially available *Iron Pump*™ (fruit punch flavored, MusclePharm, Denver, Colorado). The dose used for PRE was determined to be one scoop, i.e. 6 g, the amount recommend for pre-workout by the manufacturer. The caffeine supplement (CAF) used for the study was a commercially available caffeine pill ground, using a mortar and pestle in 350 mg doses. (PROLAB Nutrition, Chatsworth, CA).



Supplement Facts

Serving Size 1 Tablet

	Amount Per Serving	%Daily Value
Calcium (from Dibasic Calcium Phosphate)	75 mg	8%
Caffeine	200 mg	*

* Daily Value (DV) not established.

Other Ingredients: Stearic Acid, Cellulose Gum, Silica, Magnesium Stearate, Methylcellulose, Glycerin.

Figure 2. Prolab caffeine pill ingredient list (Caffeine, PROLAB Nutrition, Chatsworth, CA). One individual pill contains 200 mg caffeine

Pills were cut in half using a prescription tablet cutter (EZY Dose Safety Shield Tablet Cutter, Apothecary Products, Inc. ®, Burnsville, MN). Those doses were then mixed with fruit punch flavored, 5 calorie sweetened drink (Crystal Light, Northfield, IL). The dosage was determined after extracting the caffeine from 600 mg of PRE, in a pure chloroform solution for

24 hours. The assumption was that all the caffeine dissolved in the chloroform solution. Then through Nuclear Magnetic Resonance (NMR) spectroscopy, the chloroform solution extract showed that the 600 mg of powder contains 35 mg of pure caffeine. The serving size of PRE is 6 g therefore the approximate dose of caffeine was 350 mg. The placebo dose used was a corn starch mix with a fruit punch flavored, 5 calorie sweetened drink (Crystal Light, Northfield, IL). Both PLA and CAF supplements were an iso-caloric match similar in taste, color and texture to the PRE supplement.

All the supplements were placed into opaque vials which were independently double-blinded into envelopes as described by Kalmar et al. (1999). During each trial the participants were randomly assigned one of the following: (PRE), (CAF), or (PLA). The subsequent trials (T2 and T3) maintained random assignment through double-blinding procedure discussed later. The blinder was otherwise not involved with the study and each subject had their own manila envelope, handled by a research assistant, containing the double-blind, coded vials. The research assistant on the day of each trial selected one envelope containing a vial for participant consumption. The vial was then mixed in 12 oz of cold bottled water in an opaque blender bottle and mixed utilizing a blender ball (Blender Bottle, Lehi, UT). One assistant was the primary observer in mixing and ensuring full consumption of each supplement and was excluded from subsequent data collection.

Procedures

A protocol-trained undergraduate/graduate student research team led participants through the procedure using a protocol spreadsheet and checklist (Appendix F, Appendix G). Participants were asked to attend four visits to the Muscle, Metabolism, and Ergogenics Lab (MME Lab) (NDSU, Fargo, ND). Participants were further instructed on a familiarization procedure with the

Biodex System 4 dynamometer (Biodex Medical Systems, Shirley, NY) as described by Astorino et al. (2010b). Furthermore the participants' individualized Biodex seat measurements were recorded (Astorino et al., 2010b). The use of Biodex is both reliable and valid (Timmins et al., 2014). Participants received a written instruction handout at the end of the FAM to prepare for study procedures (Appendix H). This handout included restrictions, times, and verbal instruction on appropriate actions and attire during trials. The three remaining visits were testing trials (T1, T2, and T3) conducted at the same time as schedules allowed at least 48 hours between trials for the duration of the study. Participants were provided a handout with instructions for T1, T2, and T3 (Appendix I).

Table 4

Completed measurements for each trial (T1, T2, T3)

	Measurements	Baseline	30-Post Supp	Post-Ex	60-Post Supp
T1	M-*Vel*	√	√	√	√
	V- r^2	√	√	√	√
	V-*C*	√	√	√	√
T2	BP-sys	√	√	√	√
	BP-dia	√	√	√	√
	LAC	√	√	√	√
T3	NO sal	√			√
	POMs	√	√		√

Visit 1 was comprised of a familiarization trial (FAM) utilized to measure preliminary data as well as give participants the ability to experience the exercise protocol prior to testing. Upon arrival participants had weight measured using a digital scale to the nearest 0.1 kg (Denver

Instruments DA-150, Denver, CO) and height to the nearest 0.5 cm using a stadiometer (Seca 703 scale, Chino, CA), with jackets and shoes removed. The Biodex was preformatted (protocol preset measures) prior to participant preference. These measures were recorded to set each subsequent trial. The participants were then tested with an exercise protocol for maximum strength peak torque at 0 and 60 degrees to calculate adjusted exercise concentric towards and concentric away loads. Eccentric mechanisms were not used with the Biodex due to irregular movement patterns caused by the equipment. During FAM the pre-trial load measurement was adjustment to 40 percent maximum strength both directions to prevent error during testing. The smallest measures of the four numbers (with respect to one away and one towards) was used for testing trials to ensure participants were able to complete the entire trial. Strength numbers were further rounded to the nearest coordinated number in the Biodex selection. Participants were instructed to fast overnight, and instructed to arrive for testing in a euhydrated state at the scheduled time no more than 7 days from FAM.

For Visit 2, first testing trial (T1) and all succeeding visits 3-4 (respectively T2 and T3) participants arrived at the MME Lab during their assigned time. Participants self-reported no deviations from instruction sheet. Upon arrival participants were prepped for a 12-lead electrocardiogram (EKG), electrodes (3M multipurpose) before positioning the Biodex for participant specification. The participants remained seated for the duration of the trial with the exception of transferring to and from the Biodex. Participants were asked to relax for a minimum of five minutes to prior to recording baseline measures. Each trial used repeated standardized measures at baseline, 30 minutes post supplementation (30-Post Supp), immediately (within <1 minute) post-exercise (Post-Ex), and 60 minutes post supplementation (60-Post Supp). After baseline measures, participants consumed one dose of blinded supplement (PRE, CAF, or PLA)

and remained stationary for 30 minutes. During this time participants were allowed to work on homework, use cellular devices, laptops, or tablets as long as these tasks were not over stimulating. Immediate post-exercise was timed for all ultrasound measures using an ACCUSPILT stop watch (ACCUSPLIT, Pleasanton, California) and repeated in the same time frame of all subsequent trials. The measures for T1, T2, and T3 are depicted in Table 10.

Heart rate

The EKG measured heart rate and heart rate variability assessed by a five minute protocol both pre-consumption and 60 minute-post consumption as well as both pre- and immediately post-exercise. Heart rate was collected during exercise for all sets (1-5) each trial and was recorded as minimum and maximum with focus on peak heart rate. Heart rate was further monitored for abnormalities via EKG connection. Abnormalities were defined as 85 percent maximum heart rate calculate from Max HR = 220 – age during rest or exercise. In case of emergency the lab was equipped with a telephone, research assistants were familiar or trained in CPR, and all assistants were able to locate the AED.

Ultrasound measures

All ultrasound measures were collected from the right arm using a Pulsed Wave Doppler on a Phillips HD11XE ultrasound system (Phillips, Eindhoven, Netherlands). The ultrasound system was equipped with a Phillips L12-5 mm Broadband Linear Array probe from 12-5Mhz for the measures. To ensure accuracy in all trials the ultrasound probe was traced in permanent ink during T1 baseline and each participant received a permanent marker to darken marks between trials. The ultrasound measures included mean blood flow velocity m/s^2 (M-*Vel*), cross-sectional diameter of the right brachial artery cm^2 (V- r^2), and vessel circumference cm^2 (V-*C*).

Blood pressure

Resting blood pressure (BP) was recorded using an American Diagnostic Corporation sphygmometer and stethoscope (American Diagnostic Corp, Hauppauge, NY). The BP measurement obtained at baseline was tracked using EKG input. BP measures were recorded as systolic and diastolic in mmHg.

Blood lactate

Blood lactate (LAC) was measured with a Nova Biomedical portable analyzer (Nova Biomedical, Waltham, Massachusetts) pre- and post-exercise, with the left hand and finger stick of the participant utilizing a BD 30 gauge safety lancet (BD, Franklin Lakes, NJ) in mm/dL.

Salivary nitric oxide

Salivary NO concentration test was administered pre-ingestion (Table 10) measured by (N-O Indicator Strips, HumanN, Greensboro, GA). Saliva was collected using a 15 ml disposable cup. Utilizing a 5 ml pipet with disposable tip each strip was fully saturated with at least enough saliva to repeat measures once. Results were measured as depleted, low, and optimal according to information supplied by the manufacturer.

Modified profile of mood states

The participant then completed the Modified Profile of Mood States (POMs) (Appendix J) (Mackenzie, 2001). POMs analysis collects the following cumulative mood states: Anger (0-48), Confusion (0-28), Depression (0-60), Fatigue (0-28), Tension (0-36), and Vigor (0-32). These moods states are totaled into an accumulative POMs score (-32 to 200). According to author Mackenzie (2001) vigor was associated with preparedness prior to competition among a sample of recreational athletes average (Vigor = 17.78). Behavioral analysis is a test designed to

measure potential vigor and the ability for athletes to perform at international, club, and recreational levels (Mackenzie, 2001).

Exercise protocol

Utilizing the Biodex System 4, participants were measured during visit 1 for maximal strength peak torque at 0 degrees and 60 degrees for both peak elbow flexion and extension. The work load was adjusted in accordance with 40 percent of max for concentric-concentric flexion-extension. The adjusted work load was tested for 5 sets of 10 repetitions with a 60 second rest period between sets. The primary measure recorded was dynamic work. Dynamic work (Joules, J) is defined as the total work (J) for flexion and extension actions summed after each set. All participants received constructive coaching to encourage full effort throughout the exercise trials. Restraint straps were used to maintain physical force and limit body movements in determining muscle torque and total work of muscle contraction. Each subsequent trial consisted of the same exercise protocol listed, 5 sets by 10 repetitions for all treatments.

Statistical analysis

Frequencies and means were estimated for age, height, and weight. Repeated measures ANOVA was used to estimate time compared to supplement. This method was related to participants measured multiple times equating to possible correlation between measures. The variance/co-variance was accessed using compound symmetry. All statistics were performed using SAS Institute Inc. 9.3, 2011 (Cary, NC).

CHAPTER 4. ACUTE EXERCISE RESPONSE OF CAFFEINE AND NITRIC OXIDE STIMULATING PRE-WORKOUT SUPPLEMENT AMONG HEALTHY MALE RECREATIONAL ATHLETES

Abstract

The effect of cocktail supplements or "pre-workout" supplements containing L-arginine nitrate and caffeine are equivocal, especially with regard to anaerobic performance. This is related to poor regulation and the rarity of compared individual ingredients. The purpose of this study was to compare anaerobic performance and blood flow of a pre-workout supplement containing L-arginine nitrate and caffeine to caffeine and placebo. In a randomized, double-blind crossover design, 12 resistance-trained males (caffeine users) completed three trials. Biodex concentric-concentric elbow flexion and extension (5 sets of 10 repetitions each) measured dynamic work. Ultrasound measured brachial artery blood flow assessed M-*Vel*, V- r^2 and V-*C*. Statistical analyses revealed a significant difference in total dynamic work PRE to PLA ($P<0.0001$) and CAF to PLA ($P<0.0001$) but not PRE to CAF ($P=0.9581$). Furthermore, a significant difference V- r^2 PRE to CAF ($P=0.0391$) and PLA to CAF ($P=0.0070$) and M-*Vel* PRE to CAF ($P=0.0281$). In conclusion, the PRE did not differ CAF in strength measures other than a difference in M-*Vel*. PRE did not greatly improve blood flow as CAF suggests vasoconstriction. PRE compared to PLA were not statically different in blood flow. Therefore the use of a cocktail supplement illustrated no difference beyond individual ingredient. This research may be useful for future cocktail supplement and Nitric Oxide research.

Keywords: Cocktail mixtures, L-arginine, NO, Anaerobic exercise, ergogenic-aid

Introduction

Nutritional ergogenic aids, also referred to as performance enhancing substances, dietary substances or practices are not a new idea (Katch, McArdle, & Katch, 2011; Mahan, Escott-Stump, & Raymond, 2012). Athletes, whether professional, collegiate, or recreational, commonly use dietary supplements, however, all supplements are not, and should be evaluated for efficacy and safety (Buell et al., 2013). In recent years, all types of ergogenic nutritional aids have increased in availability. Gaining in popularity, ergogenic nutritional mixtures, which are a combination of consumable dietary supplements, have been called *cocktails* (Spradley et al., 2012). These cocktail mixtures combine separate ergogenic substances that may improve performance alone but are generally not tested together. To better understand how cocktail mixes effect ergogenic potential, research is needed comparing specific cocktail mixtures to individual ingredients to identify ergogenic potential and safety beyond individual ingredients alone. The purpose of this literature review is to examine the separate potential ergogenic effects, safety and efficacy of single ingredients caffeine, and L-arginine nitrate alone, to estimate effects of the combined mixture.

Cocktail pre-workout supplement

Cocktail mixtures marketed as pre- and post-workout supplements are often specific to anaerobic training or resistance training (Kedia et al., 2014). A specific research focus has been on pre-workout supplements and investigated by a number of teams (Kedia et al., 2014; Hoffman et al., 2008; Smith, Fukuda, Kendall, & Stout, 2010; Spradley et al., 2012; Outlaw et al., 2014). It is difficult to show interrelation among studies because none of the listed studies used the same mixture products in research design.

"Iron Pump™ utilizes "Super Nitric Oxide" ION-3 Nitrate Technology to open up blood pathways into the muscles, improving the effectiveness of the ordinary molecule. As the world's first molecularly-modified arginine, L-Arginine Nitrate is a fusion of l-arginine and nitric acid that increases blood flow to enhance distribution of nutrients."

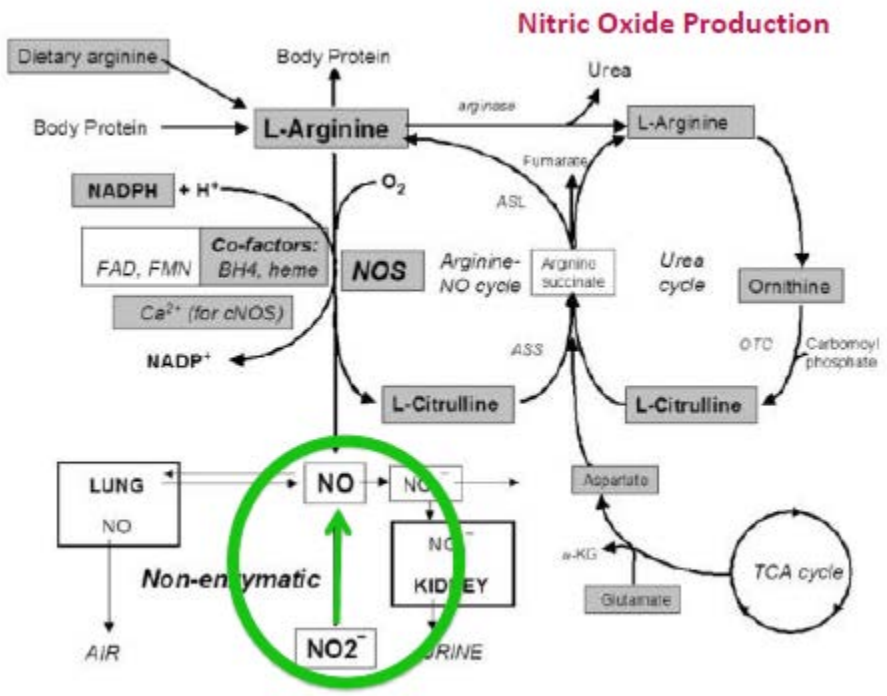


Figure 1. A schematic diagram of the physiologic disposition of nitrate, nitrite, and nitric oxide from exogenous (dietary) and endogenous sources (Ivy, 2015).

Nitric oxide, nitrate, nitrite, and L-arginine

Iron Pump™ is promoted as an L-arginine nitrate supplement (MusclePharm, 2013). L-arginine is a precursor to nitric oxide synthases, whereas, nitrate or nitrite are more immediate precursors to nitric oxide (Hord, Tang, & Bryan, 2009). The nitric oxide released by cells after synthesis or absorption of nitrate or nitrite, has a vasodilating effect that is localized in blood vessels (Mahan et al., 2012). There are a number of exogenic and endogenic pathways for nitric oxide as illustrated in Figure 1. Search terms for citations found using supplements or exercise in association with *L-arginine, nitric oxide, nitrate, nitrite, caffeine, and caffeinated.*

Further, the USDA Nutrient Database, the Food and Agriculture Organization (FAO), and World Health Organization (WHO) do not report any nitric oxide, nitrite, or nitrate (USDA, 2015; FAO, 2015; WHO, 2015). There is more information available, about caffeine as an individual ingredient than L-arginine in research (Bescós, Sureda, Tur, & Pons, 2012). This compares to database search results including *PubMed, Google Scholar, and ProQuest,* caffeine as an individual supplement (≈ 94,000 hits) than L-arginine alone (≈ 33,000 hits).

L-arginine is an essential amino acid that works to synthesize nitric oxide in cells (Hord et al., 2009; Mahan et al., 2012). Good dietary sources of nitric oxide include beets, beet root juice, kale, arugula, spinach, and swiss chard. Therefore the supplement is intended to mimic this natural process illustrated in Figure 1.

The supplementation of L-arginine, or nitric oxide stimulation, included in cocktail mixtures have the ergogenic potential to enhance endurance exercise through cardiorespiratory adaptation and increased tolerance to exercise (Bescós et al., 2012; Bailey et al., 2010; Camic et al., 2010a; Camic et al., 2010b). In a systematic review, Bescós et al. (2012) found the majority of research on L-arginine or nitric oxide to be based on endurance ergogenic potential (e.g. aerobic exercise to exhaustion) rather than resistance training (e.g. measured muscular strength, muscular endurance, and anaerobic capacity ~Wingate test). These results warrant further investigation of nitric oxide and L-arginine related to resistance training. Another problem with L-arginine nitrate is that the ergogenic potential of the individual substance has yet to be established and have primarily shown results in cocktail mixtures (Bescós et al., 2012; Bailey et al., 2010; Camic et al., 2010a; Camic et al., 2010b). The article by Bescós et al. (2012) identified the lack of research involving nitric oxide in isolation. Therefore, further research analyzing these compounds in a mixture and as individual ingredients is important to better understand the

effects of combining cocktail supplements. However a single “dose” of beet-root juice costs approximately ≈ $15.00 (USD) (Swanson Health Products, 2015).

Statement of the problem

This inability to test for safety, purity, and to test for efficacy of the ergogenic potential of individually combined ergogenic substances is under-researched and needed. The combined effects of such mixtures may result in unwanted side effects, injury, or death (Bunsawat et al., 2014; Jacobs et al., 2003).

Purpose of the study

The purpose of this literature review is to identify the need to investigate both combination mixtures as well as individual mixtures. Therefore investigation into the ergogenic potential of the combination L-arginine nitrate and caffeine in comparison to caffeine alone or placebo will be reinforced.

Hypothesis of the study

The hypothesis of this research is that a single dose of caffeine will not illustrate difference from a combined mixture including a similar dose of caffeine in compared short term effects, anaerobic (ergogenic) performance, or blood flow measures.

Methods

Experimental approach to the problem

The purpose of the study was to examine the acute ergogenic effects of a single ingredient caffeine, compared to combined mixture: arginine nitrate, caffeine and other, and placebo. This study used a randomized, double-blind, placebo controlled, and crossover design.

Subjects

Fifteen recreationally active males (age 18-35 yrs) were recruited for this study. Recruitment flyers and North Dakota State University listservs were used to recruit participants (Appendix F, Appendix G). After preliminary email screening participants were scheduled for a familiarization trial. After receiving study explanation and time to ask questions, participants were asked to sign written informed consent. The informed consent and protocol was approved by the North Dakota State University Internal Review Board (Appendix B, Appendix A). For the first visit the participants were screened using the PAR-Q risk stratification form (Appendix C) (ACSM, 2000). The participants were required to be screened for excess or absent caffeine use to classify as habitual or nonhabitual users (Appendix D). In addition to the completion of the informed consent, PAR-Q, and caffeine food frequency questionnaire (CFFQ), participants were required to complete a Health History Questionnaire (HHQ) (Appendix E). The inclusion criteria included: 1) caffeine consumption ≥100 mg or ≤ 500 mg of caffeine per day (Graham, 2001a), as estimated by a study team created CFFQ; 2) no current chronic use of another supplement within 30 days as described by Kedia et al. (2014) 3) any chronic disease including but not limited to; affecting cardiovascular, nervous system, and digestive system or other health problems identified by HHQ 4) non-smoking males including all nicotine products. Females were not recruited due to the menstrual cycles and oral contraceptives degradation effect on caffeine as described by Timmins et al. (2014). All participants were required to be self-reported resistance trained with at least 6 months experience and maintain at least 3 hours of overall exercise per week.

Procedures

A protocol-trained undergraduate/graduate student research team led participants through the procedure using a protocol spreadsheet and checklist (Appendix H, Appendix I). Participants were asked to attend four visits to the Muscle, Metabolism, and Ergogenics Lab (MME Lab) (NDSU, Fargo, ND). Participants were further instructed on a familiarization procedure with the Biodex System 4 dynamometer (Biodex Medical Systems, Shirley, NY) as described by Astorino et al. (2010b). Furthermore the participants' individualized Biodex seat measurements were recorded (Astorino et al., 2010b). The use of Biodex is both reliable and valid (Timmins et al., 2014). Participants received a written instruction handout at the end of the FAM to prepare for study procedures (Appendix J). This handout included restrictions, times, and verbal instruction on appropriate actions and attire during trials. The three remaining visits were testing trials (T1, T2, and T3) conducted at the same time as schedules allowed at least 48 hours between trials for the duration of the study. Participants were provided a handout with instructions for T1, T2, and T3 (Appendix J).

Table 4

Completed measurements for each trial (T1, T2, T3)

	Measurements	Baseline	30-Post Supp	Post-Ex	60-Post Supp
T1	M-*Vel*	√	√	√	√
	V- r^2	√	√	√	√
	V-*C*	√	√	√	√
T2	BP-sys	√	√	√	√
	BP-dia	√	√	√	√
	LAC	√	√	√	√
T3	NO sal	√			√
	POMs	√	√		√

Visit 1 was comprised of a familiarization trial (FAM) utilized to measure preliminary data as well as give participants the ability to experience the exercise protocol prior to testing. Upon arrival participants had weight measured using a digital scale to the nearest 0.1 kg (Denver Instruments DA-150, Denver, CO) and height to the nearest 0.5 cm using a stadiometer (Seca 703 scale, Chino, CA), with jackets and shoes removed. The Biodex was preformatted (protocol preset measures) prior to participant preference. These measures were recorded to set each subsequent trial. The participants were then tested with an exercise protocol for maximum strength peak torque at 0 and 60 degrees to calculate adjusted exercise concentric towards and concentric away loads. Eccentric mechanisms were not used with the Biodex due to irregular movement patterns caused by the equipment. During FAM the pre-trial load measurement was adjustment to 40 percent maximum strength both directions to prevent error during testing. The smallest measures of the four numbers (with respect to one away and one towards) was used for

testing trials to ensure participants were able to complete the entire trial. Strength numbers were further rounded to the nearest coordinated number in the Biodex selection. Participants were instructed to fast overnight, and instructed to arrive for testing in a euhydrated state at the scheduled time no more than 7 days from FAM.

For Visit 2, first testing trial (T1) and all succeeding visits 3-4 (respectively T2 and T3) participants arrived at the MME Lab during their assigned time. Participants self-reported no deviations from instruction sheet. Upon arrival participants were prepped for a 12-lead electrocardiogram (EKG), electrodes (3M multipurpose) before positioning the Biodex for participant specification. The participants remained seated for the duration of the trial with the exception of transferring to and from the Biodex. Participants were asked to relax for a minimum of five minutes to prior to recording baseline measures. Each trial used repeated standardized measures at baseline, 30 minutes post supplementation (30-Post Supp), immediately (within <1 minute) post-exercise (Post-Ex), and 60 minutes post supplementation (60-Post Supp). After baseline measures, participants consumed one dose of blinded supplement (PRE, CAF, or PLA) and remained stationary for 30 minutes. During this time participants were allowed to work on homework, use cellular devices, laptops, or tablets as long as these tasks were not over stimulating. Immediate post-exercise was timed for all ultrasound measures using an ACCUSPILT stop watch (ACCUSPLIT, Pleasanton, California) and repeated in the same time frame of all subsequent trials. The measures for T1, T2, and T3 are depicted in Table 10.

Heart rate

The EKG measured heart rate and heart rate variability assessed by a five minute protocol both pre-consumption and 60 minute-post consumption as well as both pre- and immediately post-exercise. Heart rate was collected during exercise for all sets (1-5) each trial

and was recorded as minimum and maximum with focus on peak heart rate. Heart rate was further monitored for abnormalities via EKG connection. Abnormalities were defined as 85 percent maximum heart rate calculate from Max HR = 220 – age during rest or exercise. In case of emergency the lab was equipped with a telephone, research assistants were familiar or trained in CPR, and all assistants were able to locate the AED.

Ultrasound measures

All ultrasound measures were collected from the right arm using a Pulsed Wave Doppler on a Phillips HD11XE ultrasound system (Phillips, Eindhoven, Netherlands). The ultrasound system was equipped with a Phillips L12-5 mm Broadband Linear Array probe from 12-5Mhz for the measures. To ensure accuracy in all trials the ultrasound probe was traced in permanent ink during T1 baseline and each participant received a permanent marker to darken marks between trials. The ultrasound measures included mean blood flow velocity m/s^2 (M-*Vel*), cross-sectional diameter of the right brachial artery cm^2 (V- r^2), and vessel circumference cm^2 (V-*C*).

Blood pressure

Resting blood pressure (BP) was recorded using an American Diagnostic Corporation sphygmometer and stethoscope (American Diagnostic Corp, Hauppauge, NY). The BP measurement obtained at baseline was tracked using EKG input. BP measures were recorded as systolic and diastolic in mmHg.

Blood lactate

Blood lactate (LAC) was measured with a Nova Biomedical portable analyzer (Nova Biomedical, Waltham, Massachusetts) pre- and post-exercise, with the left hand and finger stick of the participant utilizing a BD 30 gauge safety lancet (BD, Franklin Lakes, NJ) in mm/dL.

Salivary nitric oxide

Salivary NO concentration test was administered pre-ingestion (Table 10) measured by (N-O Indicator Strips, HumanN, Greensboro, GA). Saliva was collected using a 15 ml disposable cup. Utilizing a 5 ml pipet with disposable tip each strip was fully saturated with at least enough saliva to repeat measures once. Results were measured as depleted, low, and optimal according to information supplied by the manufacturer.

Modified profile of mood states

The participant then completed the Modified Profile of Mood States (POMs) (Appendix K) (Mackenzie, 2001). POMs analysis collects the following cumulative mood states: Anger (0-48), Confusion (0-28), Depression (0-60), Fatigue (0-28), Tension (0-36), and Vigor (0-32). These moods states are totaled into an accumulative POMs score (-32 to 200). According to author Mackenzie (2001) vigor was associated with preparedness prior to competition among a sample of recreational athletes average (Vigor = 17.78). Behavioral analysis is a test designed to measure potential vigor and the ability for athletes to perform at international, club, and recreational levels (Mackenzie, 2001).

Exercise protocol

Utilizing the Biodex System 4, participants were measured during visit 1 for maximal strength peak torque at 0 degrees and 60 degrees for both peak elbow flexion and extension. The work load was adjusted in accordance with 40 percent of max for concentric-concentric flexion-extension. The adjusted work load was tested for 5 sets of 10 repetitions with a 60 second rest period between sets. The primary measure recorded was dynamic work. Dynamic work (Joules, J) is defined as the total work (J) for flexion and extension actions summed after each set. All participants received constructive coaching to encourage full effort throughout the exercise trials.

Restraint straps were used to maintain physical force and limit body movements in determining muscle torque and total work of muscle contraction. Each subsequent trial consisted of the same exercise protocol listed, 5 sets by 10 repetitions for all treatments.

Supplements and placebo

The pre-workout supplement (PRE) was commercially available *Iron Pump*™ (fruit punch flavored, MusclePharm, Denver, Colorado). The dose used for PRE was determined to be one scoop, i.e. 6 g, the amount recommend for pre-workout by the manufacturer. The caffeine supplement (CAF) used for the study was a commercially available caffeine pill ground, using a mortar and pestle in 350 mg doses. (PROLAB Nutrition, Chatsworth, CA).

Pills were cut in half using a prescription tablet cutter (EZY Dose Safety Shield Tablet Cutter, Apothecary Products, Inc. ®, Burnsville, MN). Those doses were then mixed with fruit punch flavored, 5 calorie sweetened drink (Crystal Light, Northfield, IL). The dosage was determined after extracting the caffeine from 600 mg of PRE, in a pure chloroform solution for 24 hours. The assumption was that all the caffeine dissolved in the chloroform solution. Then through Nuclear Magnetic Resonance (NMR) spectroscopy, the chloroform solution extract showed that the 600 mg of powder contains 35 mg of pure caffeine. The serving size of PRE is 6 g therefore the approximate dose of caffeine was 350 mg. The placebo dose used was a corn starch mix with a fruit punch flavored, 5 calorie sweetened drink (Crystal Light, Northfield, IL). Both PLA and CAF supplements were an iso-caloric match similar in taste, color and texture to the PRE supplement.

All the supplements were placed into opaque vials which were independently double-blinded into envelopes as described by Kalmar et al. (1999). During each trial the participants were randomly assigned one of the following: (PRE), (CAF), or (PLA). The subsequent trials

(T2 and T3) maintained random assignment through double-blinding procedure discussed later. The blinder was otherwise not involved with the study and each subject had their own manila envelope, handled by a research assistant, containing the double-blind, coded vials. The research assistant on the day of each trial selected one envelope containing a vial for participant consumption. The vial was then mixed in 12 oz of cold bottled water in an opaque blender bottle and mixed utilizing a blender ball (Blender Bottle, Lehi, UT). One assistant was the primary observer in mixing and ensuring full consumption of each supplement and was excluded from subsequent data collection.

Statistical analysis

Frequencies and means were estimated for age, height, and weight. Repeated measures ANOVA was used to estimate time compared to supplement. This method was related to participants measured multiple times equating to possible correlation between measures. The variance/co-variance was accessed using compound symmetry. All statistics were performed using SAS Institute Inc. 9.3, 2011 (Cary, NC).

Results

During the months in which active recruitment for the study occurred (October 2015 until March 2016), fifteen subjects were initially recruited. After completing the informed consent, HHQ, PAR-Q, and CFFQ participants with identified health problems were removed from the study. One participant was dropped due to an uncommon adverse event during exercise testing and two participants were removed due to failure to report for testing. The participant removal was related to a failure to follow fasting protocol resulting in greater than a 12 hour fast. The 12 remaining participants completed the three trials (PRE, CAF, and PLA) in random order. Participant demographics are displayed in Table 1. Two participants did not consume caffeine for the duration of the study to washout prior consumed pre-workout supplements. Participants that used supplements prior to the study met required daily habitual caffeine intake during recruitment. All subjects followed study requirements according to self-reports, no deviations reported. Trials ended when data began to illustrate comparable results suggesting that the data collected would be testable to answer research questions.

Table 5

Sex, age, caffeine use, height, and weight and 12 subjects completing pre-workout crossover study

	%	n
Sex (n= 12)		
Male	100	12
Age (n=12) 22.75 ± 4.51		
Under 18	0	0
18 – 24	75	9
25 – 35	25	3
Habitual caffeine use mg/day (n= 12)		
0 – 99	0	0
100 – 199	16.6	2
200 – 299	50	6
300 – 399	16.6	2
400 – 499	16.6	2
>500	0	0
Height (n=12)		
	183.4 ± 7.37 (166.05 to 193.99 cm)	
Weight (n= 12)		
	91.05 ± 17.77 (57.85 to 133.77 kg)	

The following sections detail the results of individual tests used for the study.

Heart rate

There were no correlations run on the EKG heart rate data. The situational heart rate data collected during exercise reached significance related to time but did not reach significance with respect to supplement. The individual data was measured as time 1, 2, 3, 4, or 5 with respect to the correlating set of exercise. Time measures for heart rate were significantly different for all treatments are as follows: time 1 to 4 (P=0.0282), time 1 to 5 (P=0.0003), time 2 to 4

(P=0.0412), time 2 to 5 (P=0.0005), and time 3 to 5 (P=0.0387) individual means are described in Figure 3. Heart rate was not recorded prior to exercise trial nor was it recorded after Post-Exercise measures were collected.

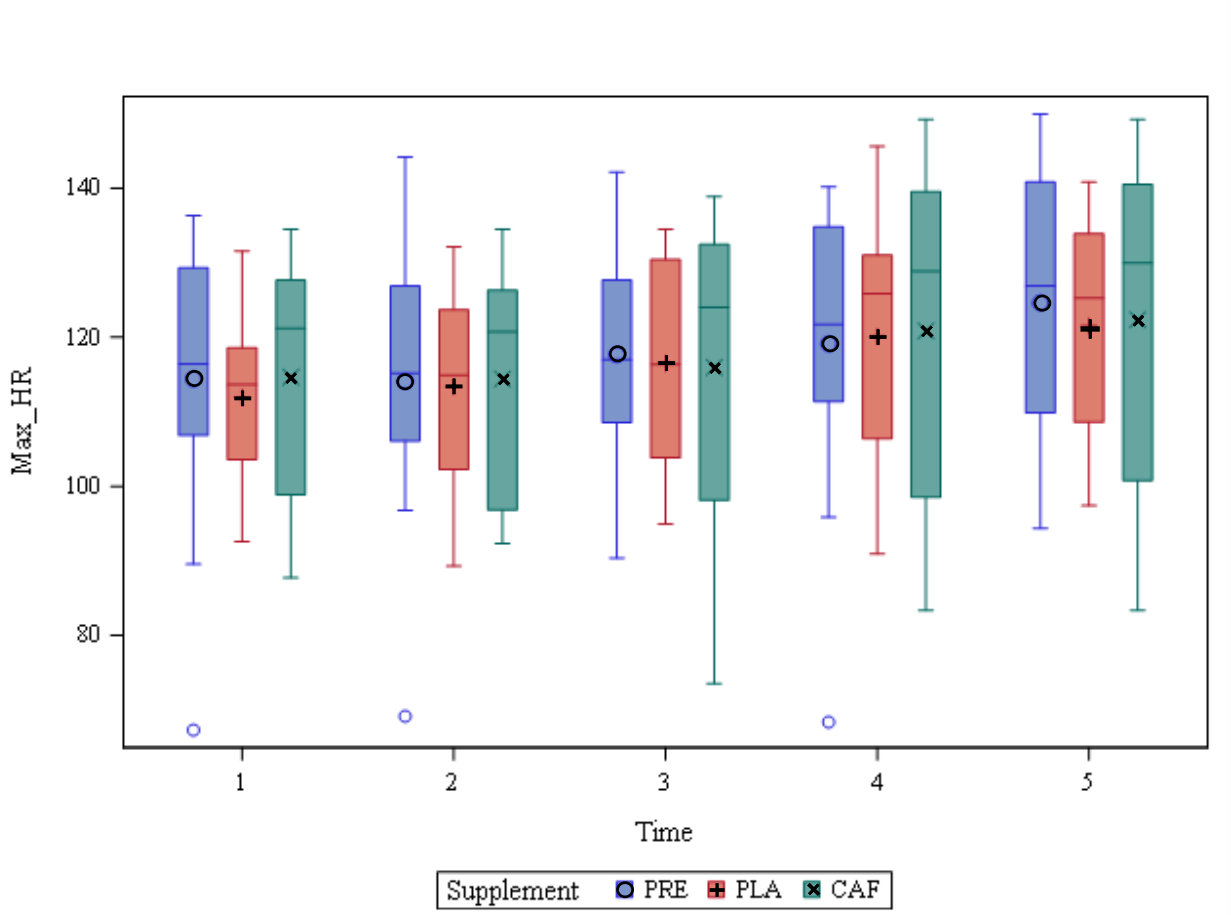


Figure 3. Max Heart Rate distribution of CAF, PLA, and PRE throughout all trials.

Ultrasound data

Vessel diameter (V-r^2) and circumference (V-*C*) and mean blood flow velocity (M-*Vel*) were all measured to test for effects of the various trials on the relationships among V-r^2, V-*C* and M-*Vel*. The measurements for each subject's V-r^2 and V-*C*, the means were 0.46766 ± 0.06826 cm (0.3260 to 0.6670) and 1.45168 ± 0.21802 cm^2 (1.010 to 2.100) with respect. M-*Vel* at baseline was 18.437 ± 8.786 m/s^2; 30-Post Supp 14.422 ± 5.812 m/s^2; Post-Ex 63.578 ±

15.683 m/s^2; 60-Post Supp 20.789 ±9.582 m/s^2. One measure was not recorded 60-Post Supp because the Post-Ex was performed at the same time point 60 minutes from supplementation.

Table 6

Mean velocity variable analyses among recreational active males (n=12)

Time	Supplement	N Obs	N	Mean	Std Dev	Minimum	Maximum
Baseline	CAF	12	12	22.384	11.474	9.710	50.100
	PLA	12	12	17.032	5.484	7.980	25.900
	PRE	12	12	15.896	7.598	8.100	35.700
30 Post Supp	CAF	12	12	18.036	6.181	7.630	30.100
	PLA	12	12	13.448	5.201	6.310	23.900
	PRE	12	12	11.782	4.396	5.690	20.800
Post Ex	CAF	12	12	65.917	18.334	26.500	93.300
	PLA	12	12	61.558	18.302	37.500	103.000
	PRE	12	12	63.258	10.047	47.600	80.400
60 Post Supp	CAF	12	11	24.600	12.067	11.400	56.800
	PLA	12	12	19.864	8.302	9.870	38.800
	PRE	12	12	18.220	7.737	1.440	33.500

The ultrasound data did not identify a correlation between M-*Vel* and V-r^2 or M-*Vel* and V-*C*. However, vessel V-r^2 and V-*C* were perfectly correlated (R=0.96944) excluding only one outlying point (Figure 4). Mean difference in velocity reached statistical significance in relation to time baseline to Post-Ex (P<0.0001), 30-Post Supp to Post-Ex (P<0.0001), and Post-Ex to 60-Post Supp (P<0.0001). The repeated supplement measures PRE to CAF were statistically different (P=0.0281).

Table 7

Vessel diameter variable analyses among recreational active males (n=12)

Time	Supplement	N Obs	N	Mean	Std Dev	Minimum	Maximum
Baseline	CAF	12	12	0.436	0.058	0.352	0.526
	PLA	12	12	0.442	0.065	0.351	0.522
	PRE	12	12	0.445	0.054	0.360	0.527
30 Post Supp	CAF	12	12	0.437	0.055	0.349	0.518
	PLA	12	12	0.448	0.065	0.355	0.524
	PRE	12	12	0.446	0.081	0.326	0.624
Post Ex	CAF	12	12	0.499	0.056	0.387	0.598
	PLA	12	12	0.531	0.069	0.407	0.655
	PRE	12	12	0.519	0.064	0.431	0.667
60 Post Supp	CAF	12	11	0.457	0.051	0.381	0.538
	PLA	12	12	0.476	0.067	0.390	0.593
	PRE	12	12	0.474	0.065	0.386	0.618

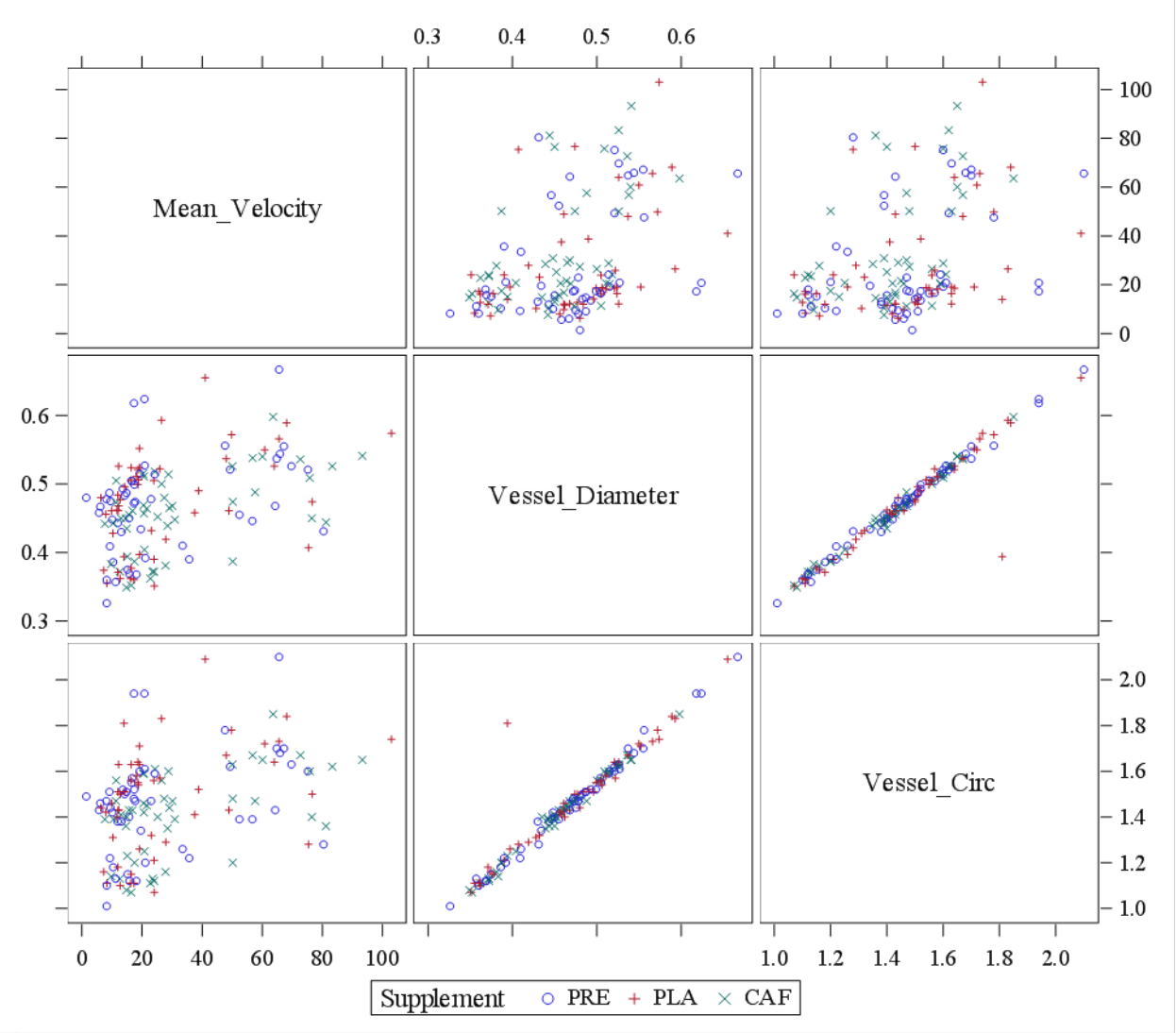


Figure 4. Blood Flow Data Correlation –Vessel and Velocity Variables Group by Supplement

The V-r^2 furthermore attained significant difference in relation to time; baseline to Post-Ex (P<0.0001), baseline to 60-Post Supp (P<0.0001), 30-Post Supp to Post-Ex (P<0.0001), 30-Post Supp to 60-Post Supp (P=0.0001), and Post-Ex to 60-Post Supp (P<0.0001). The V-r^2 did reach a significantly greater affect in relation to supplement PRE than CAF (P=0.0391) and PLA than CAF (P=0.0070).

Blood pressure data

All BP recorded were within normal range for each participant. The study did not include participants with hypertension; therefore all resting measures were required to be less than 120/80 mmHg. Both BP-sys and BP-dia illustrated a non-linear positive relationship. There were no further correlation or comparison to the previous ultrasound measures. The repeated measure mixed procedure found significance related to time but not supplement for both systolic BP and diastolic BP. Systolic BP met significant difference baseline to Post-Ex ($P<0.0001$), 30-Post Supp to Post-Ex ($P<0.0001$), and Post-Ex to 60-Post Supp ($P<0.0001$). Diastolic BP reached significance baseline to Post-Ex ($P=0.0011$) and baseline to 60-Post Supp ($P=0.0021$). The results weigh in negative effect with the exception of Post-Ex to 60-Post Supp which was positive.

Table 8

Blood Pressure-systolic variable analyses among recreational active males (n=12)

Time	Supplement	N Obs	N	Mean	Std Dev	Minimum	Maximum
Baseline	CAF	12	12	121.000	7.508	108.000	132.000
	PLA	12	12	121.500	11.446	108.000	150.000
	PRE	12	12	121.833	8.922	110.000	136.000
30 Post Supp	CAF	12	12	123.833	8.462	112.000	138.000
	PLA	12	12	121.833	10.735	112.000	140.000
	PRE	12	12	123.833	9.044	112.000	140.000
Post Ex	CAF	12	12	136.167	15.828	118.000	172.000
	PLA	12	12	134.500	14.848	114.000	160.000
	PRE	12	12	133.333	18.297	118.000	180.000
60 Post Supp	CAF	12	12	126.167	10.426	108.000	144.000
	PLA	12	12	124.667	9.847	116.000	142.000
	PRE	12	12	124.000	8.356	112.000	136.000

Table 9

Blood Pressure-diastolic variable analyses among recreational active males (n=12)

Time	Supplement	N Obs	N	Mean	Std Dev	Minimum	Maximum
Baseline	CAF	12	12	79.000	6.467	70.000	90.000
	PLA	12	12	78.833	5.357	72.000	92.000
	PRE	12	12	79.667	8.773	58.000	90.000
30 Post Supp	CAF	12	12	81.500	4.275	76.000	90.000
	PLA	12	12	80.167	6.177	72.000	92.000
	PRE	12	12	81.000	8.634	62.000	92.000
Post Ex	CAF	12	12	83.833	4.303	76.000	90.000
	PLA	12	12	81.667	5.314	72.000	90.000
	PRE	12	12	82.000	6.030	72.000	90.000
60 Post Supp	CAF	12	12	83.500	4.523	78.000	90.000
	PLA	12	12	80.333	4.579	72.000	90.000
	PRE	12	12	83.167	5.219	76.000	92.000

Blood lactate

Blood lactate (LAC) data showed no correlation to BP-sys, BP-dia, or POMs score. The mixed model of repeated measures found significance related to time but not supplement. The significant data related to time is as follows: baseline to Post-Ex ($P<0.0001$), baseline to 60-Post Supp ($P<0.0001$), 30-Post Supp to Post-Ex ($P<0.0001$), 30-Post Supp to 60-Post Supp ($P<0.0001$), and Post-Ex to 60-Post Supp ($P<0.0001$).

Table 10

Blood Lactate variable analyses among recreational active males (n=12)

Time	Supplement	N Obs	N	Mean	Std Dev	Minimum	Maximum
Baseline	CAF	12	12	0.775	0.176	0.500	1.000
	PLA	12	12	1.000	0.386	0.400	1.600
	PRE	12	12	0.858	0.268	0.400	1.300
30 Post Supp	CAF	12	12	0.967	0.427	0.400	1.800
	PLA	12	12	1.042	0.570	0.300	2.500
	PRE	12	12	0.950	0.430	0.300	1.700
Post Ex	CAF	12	12	3.125	1.223	1.700	5.200
	PLA	12	12	2.692	0.834	1.400	3.700
	PRE	12	12	3.183	1.084	1.200	5.200
60 Post Supp	CAF	12	12	2.042	0.854	0.900	4.000
	PLA	12	12	1.858	0.817	0.700	3.000
	PRE	12	11	1.809	0.793	1.000	3.000

Salivary nitric oxide

Salivary NO (NO sal) data was not run as a statistical model due to question of validity and reliability in relation to high qualitative unspecific measure. There were no correlations related to other data collected. The frequencies of the various NO strip results are shown below in Figure 5.

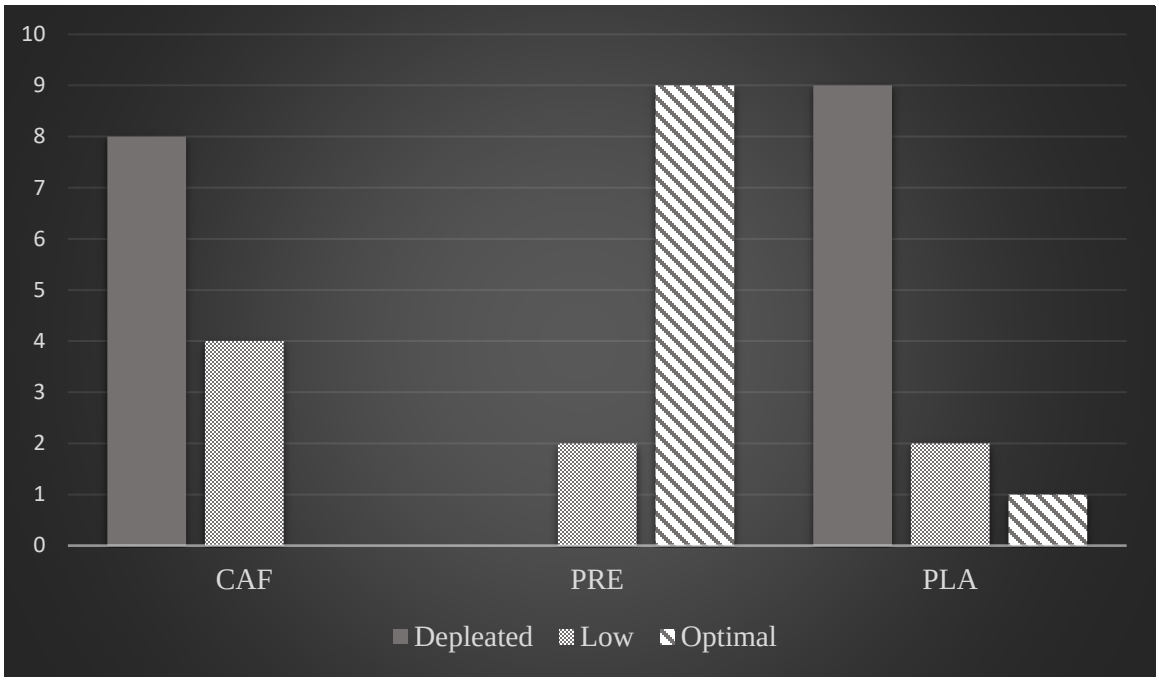


Figure 5. Salivary Nitric Oxide strips measured at 60 minutes post supplement consumption.

POMs data

Modified Profile of Mood States data did not show correlation to individual mood attributes. Measures of individual mood attributes were not statistically analyzed for the current paper. The POMs Vigor attribute means and standard deviations were: PRE 13.08 ± 6.21 and 13.67 ± 6.07; CAF 12.50 ± 7.83 and 11.58 ± 7.04; PLA 13.5 ±6.37 and 14.42 ± 5.54 with respect to 30-Post Supp and 60-Post Supp. Furthermore, there was no correlation of the totaled POMs score to blood lactate, systolic BP or diastolic BP. Modified Profile of Mood States score however reached significance with respect to both time and supplement. In relation to time baseline to 60-Post Supp was significantly different (P=0.0005). The repeated measures of supplement met significance where the PLA score was statistically greater than PRE (P=0.0358) and CAF (P=0.0426).

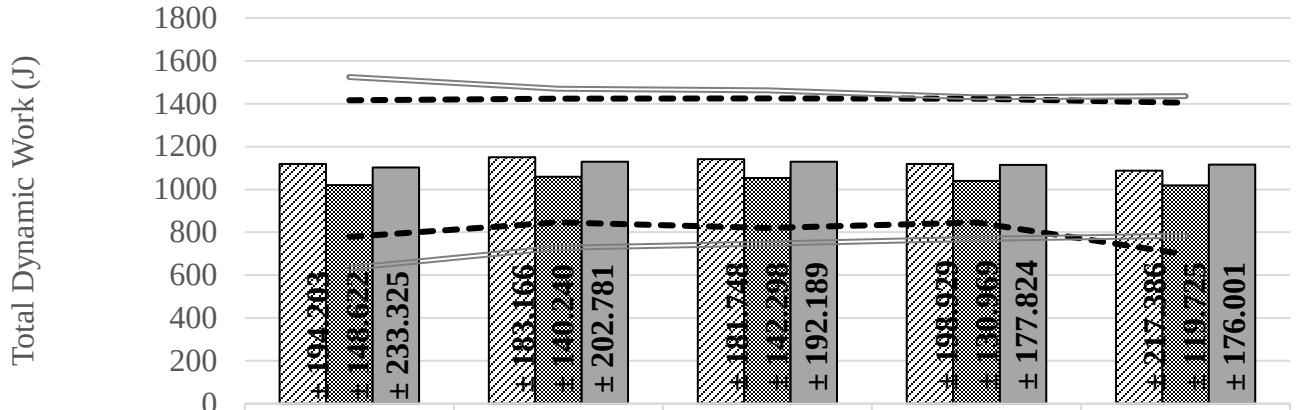
Table 11

POMS_Score variable analyses among recreational active males (n=12)

Time	Supplement	N Obs	N	Mean	Std Dev	Minimum	Maximum
Baseline	CAF	12	12	4.167	11.175	-16.000	27.000
	PLA	12	12	8.417	12.894	-9.000	32.000
	PRE	12	12	2.833	10.590	-15.000	18.000
30 Post Supp	CAF	12	12	-3.167	10.035	-21.000	11.000
	PLA	12	12	1.750	12.793	-18.000	22.000
	PRE	12	12	-0.583	10.958	-21.000	20.000
60 Post Supp	CAF	12	12	-1.500	11.751	-11.000	32.000
	PLA	12	12	1.083	11.966	-15.000	22.000
	PRE	12	12	-3.083	10.202	-21.000	20.000

Biodex data

Biodex dynamic work was not assessed for correlation with other variables. The dynamic work data reached significance for repeated measures comparison to supplement. The dynamic work J reached significant difference for PRE to PLA ($P<0.0001$) and PLA to CAF ($P<0.0001$). However the data did not present significance PRE to CAF ($P=0.9581$) nor regarding the repeated measure of time ($P=0.3933$).



	1	2	3	4	5
CAF	1119.200	1150.383	1140.583	1119.350	1087.658
PLA	1020.725	1058.733	1052.700	1040.442	1018.467
PRE	1102.758	1129.442	1129.275	1114.258	1116.417
Max_CAF	1416.4	1423.7	1424.8	1423.2	1404.7
Max_PRE	1524.7	1470.3	1463.2	1429.9	1434.9
Min_CAF	778.7	846.1	820.3	845.1	696.5
Min_PRE	632.9	727.0	746.7	769.9	783.5

Figure 6. Dynamic Work variable analyses among recreational active males (n=12).

Table 12

Dynamic Work variable analyses among recreational active males (n=12)

Time	Supplement	N Obs	N	Mean	Std Dev	Minimum	Maximum
1	CAF	12	12	1119.200	194.203	778.700	1416.400
	PLA	12	12	1020.725	148.622	784.400	1252.600
	PRE	12	12	1102.758	233.325	632.900	1524.700
2	CAF	12	12	1150.383	183.166	846.100	1423.700
	PLA	12	12	1058.733	140.240	861.200	1358.000
	PRE	12	12	1129.442	202.781	727.000	1470.300
3	CAF	12	12	1140.583	181.748	820.300	1424.800
	PLA	12	12	1052.700	142.298	836.000	1361.200
	PRE	12	12	1129.275	192.189	746.700	1463.200
4	CAF	12	12	1119.350	198.929	845.100	1423.300
	PLA	12	12	1040.442	130.969	825.500	1336.500
	PRE	12	12	1114.258	177.824	769.900	1429.900
5	CAF	12	12	1087.658	217.386	696.500	1404.700
	PLA	12	12	1018.467	119.725	861.100	1319.800
	PRE	12	12	1116.417	176.001	783.500	1434.900

Discussion

The primary focus of this study was to compare a 350 mg dose of caffeine to a cocktail supplement containing caffeine and NO, and placebo in short term effects, anaerobic performance, and blood flow effect. Specifically, the study was designed to test differences among a single dose of caffeine, a combined cocktail supplement, and placebo for short term effects, anaerobic (ergogenic) performance, and blood flow measures.

The findings from this study suggest that recreational male athletes improve performance with the acute consumption of both PRE and CAF supplements. There was no significant evidence to statistically illustrate a difference between both supplements. Nonetheless, there were pronounced trends related to supplement versus placebo groups. The following sections detailed individual measurement results.

Heart rate

There were no significant changes in HR other than those found at time measures 1-5. The HR was expected to increase as exercise increased in duration due to increased intensity of work performed from set 1 to the final set 5. Spradley et al. (2012) found similar results that supplement groups had higher HR compared to placebo. Bunswat et al. (2014) found CAF significantly increased Max HR compared to PLA. Although these researchers found significance this study did not. The results represent a noteworthy outcome that both supplements (PRE and CAF) had greater Max HR and mean HR than placebo. These results further concur with Hudson et al. (2008) that CAF increased HR compared to aspirin and PLA. The distribution of maximum heart rate can be seen in Figure 3. Maximum HR also appeared less controlled in the CAF group than PLA and PRE.

Ultrasound data

Vessel diameter and V-*C* measures were difficult points to obtain as all data points had to be collected directly from the ultrasound machine. The method of collection also demonstrated a high probability for error. The V-r^2 measures were analyzed using a point trace method preprogrammed on the ultrasound machine. This method required the vessel to be marked at the two points on the lumen (widest part of the lumen) in the cross-sectional image. This generated the diameter and each image had to be analyzed for all collected data points. Inversely V-*C* measures were recorded utilizing a preset point and circle method. This method required the lumen to be marked and expanded using a circle that encompasses the vessel. Both measures had high probability for error but values still correlated (R=0.96944, Figure 4).

Finally, ultrasound data reached significance in M-*Vel*, V-r^2 and, V-*C*. However, these increases in vessel velocity and size are expected with increased blood flow. The measures recorded illustrate a primary a correlation between pre- and post-exercise blood flow. In relation V-r^2 and V-*C* demonstrated increased blood flow from baseline to 60-Post Supp. The V-r^2 significance between baseline to 60-Post Supp ($P<0.0001$) suggests a possible trend before and after supplementation. Furthermore 30-Post Supp to 60-Post Supp ($P=0.0001$) suggests a possible lasting effect pre and post-exercise. The V-r^2 statistical significance between PRE to CAF ($P=0.0391$) and PLA to CAF ($P=0.0070$) may suggest increased vasodilation of both PRE and PLA but not CAF. This result could be related to the vasoconstriction properties of caffeine during exercise (Rosenbloom & Coleman, 2012). Similar difference was found with M-*Vel* comparing PRE to CAF ($P=0.0281$). As identified previously this could indicate improved blood flow in PRE versus CAF.

Blood pressure data

The systolic BP and diastolic BP measures met significance with respect to time however this was an expected outcome. Further the BP measures were not measured within one minute immediate post-exercise to prevent interference with the ultrasound measures collected.

Blood lactate

Blood lactate (LAC) showed significance with respect to time. These measures increased in relation to exercise when compared to pre- or post-exercise measures. The exception to this was the significant difference 30-Post Supp to 60-Post Supp ($P<0.0001$) in which the measures of LAC may have maintained higher levels due to energy system recovery post-exercise (Katch et. al., 2011). Furthermore the Post Ex and 60-Post Supp measures were collected in close proximity related to time.

Salivary nitric oxide

Salivary NO (NO sal) was not a scientifically validated test and was used merely for comparison to groups. The notable outcome was that PRE measures had an almost 82% accuracy in depicting optimal NO levels. Participants that consumed CAF had no optimal measures conversely PLA had one optimal measure. Dietary factors can further affect the data outcome. Although this data set does not validate this measure, results illustrate promise for future studies.

POMs data

POMs is a validated test used to predict the outcome performance or ready to perform in all types of athletes (Mackenzie, 2001). The POMs Vigor attribute means did not reflect those found within the larger data set (Mackenzie, 2001). However the significance difference PLA to PRE ($P=0.0358$) and PLA to CAF ($P=0.0426$) in overall score reflects a possible relationship to

altered mood states and perceived supplement use. The POMs test reflects individual motivation to complete the measure. Individuals trained on POMs perform as expected whereas untrained individuals may not test effectively. This was apparent in the variation of baseline POMs scores. The current study used an early morning protocol, which may lower subject motivation. In addition, the statistical results should include individual attributes such as Vigor for future studies. Finally two subjects reported self-identified reactions correctly identifying blinded supplements. The supplement reactions as self-reported could add impact to results and motivation.

Biodex data

Biodex dynamic work measure met significance for PRE to PLA ($P<0.0001$) and CAF to PLA ($P<0.0001$) but not PRE to CAF ($P=0.9581$). This illustrates that although muscular performance was improved compared to PLA, performance was not altered beyond the use of PRE or CAF. These data results show that caffeine may have had a primary effect of muscular performance as both supplements were presumed to contain a similar dose of caffeine. The other ingredients or possible ergogenic ingredients require further and individual testing to better understand this outcome.

The findings from this study suggest that recreational male athletes improve performance with the acute consumption of both PRE and CAF supplements. There was no significant evidence to statistically illustrate a difference between both supplements. However the dynamic work more consistent with PRE than CAF during exercise illustrating a nonsignificant trend (Figure 5). Nonetheless, there were pronounced trends related to supplement versus placebo groups.

Limitations

There were some limitations to this research study. First, a small group of participants were tested in this protocol. An increased number of participants could have revealed different findings. Participants were increasingly difficult to find for this study as individuals did not want to give up the use of other supplements for washout periods. Others did not consume enough caffeine to be considered for the study. In addition this study used only male participants and should be further investigated using female participants. Second the early time of day (0600 – 1000) was difficult for both caffeine restrictions and fasting requirements. Participants regularly consumed caffeine daily and were not able to consume breakfast prior to each trial. Third ultrasound measures collected required diverse practice with differences among participants (anatomical differences). The measures required multiple images to collect all desired data points. However, the technique was optimized by careful methods such as recording time and using a stop watch. Fourth the lumen identification, V-r^2 required two whereas V-*C* required one marker point to be manipulated to fit vessel. Both measures can only be analyzed on the ultrasound machine. Finally, the rest period could have been longer to better accommodate resting heart rate, resting blood pressure, and other baseline measures. Future studies should use 30 to 60 instead of 15 minutes rest prior to baseline measures.

Practical applications

Implications for research and practice are that there were no significant changes when using a combined cocktail supplement to a single ingredient supplement. This presents a hypothetical outcome that there may not be a benefit to beyond a single known ingredient. The supplement regulations and measured ingredients are important to investigate for purity and efficacy of use. Strength and conditioning research can benefit from knowing the effectiveness of

individual ingredient compared to multiple ingredient research. Further research is needed to comparing individual ingredients to like cocktail supplements. In addition future investigations should include male and female participants. Future research related to NO and anaerobic exercise is needed.

The results of this research do not fully articulate the total performance effects associated with these supplements in comparison to exercise and blood flow measures. Although both PRE and CAF did not greatly differ the investigation is not exclusive from further future investigations. As well the research warrants that supplement regulation by the FDA may positively impact future effects of cocktail supplements and those containing NO.

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CHAPTER 5. CONCLUSION

This study was designed to examine the short term performance enhancing effects of caffeine, compared to both a cocktail mixture including caffeine and placebo. Cocktail mixtures containing separate ergogenic substances may improve performance alone are not generally tested together. Examining the effects of individual as well as combined ingredient supplements may further the understanding of how ergogenic ingredients work together. Currently, there are no strict guidelines governing the distribution of most nutritional supplements. Along with increasing popularity, supplementing during exercise could increase risk for both safety and lack of efficacy. This apparent deficiency in guidance could ultimately result in over supplementation.

The hypothesis of this research was that a single dose of caffeine will not illustrate difference from a combined mixture including a similar dose of caffeine compared to short term effects of anaerobic (ergogenic) performance and blood flow.

The results of this research study revealed that acute effects of supplementation were similar across all three treatment groups. In addition, both PRE and CAF illustrated improved dynamic work beyond PLA with no difference between the two. Finally the blood flow measures weighed favorably to both PRE and PLA as both treatments had greater velocity and vasodilation than CAF. Caffeine is known to be a vasoconstrictor but there was no difference between PLA and PRE.

Major limitations of the study included gender specificity as a small group of male participants were examined. Participants were further unwilling to give up the use of other supplements for washout periods. Second the time of day (0600 – 1000) was difficult for both caffeine restrictions and fasting requirements. Participants regularly consumed caffeine daily and were not able to consume breakfast prior to each trial. Third the lumen identifying markers V-r^2

and V-*C* could only be analyzed on the ultrasound machine. Finally, the rest period could have been longer to better accommodate resting heart rate, resting blood pressure, and other baseline measures. Future studies should use 30 to 60 instead of 15 minutes rest prior to baseline measures.

Future research and practice identifying the effectiveness of individual ingredient compared to multiple ingredient research. Further investigations should be gender indifferent including both male and female participants. Research related to NO, l-arginine, nitrate and nitrite in anaerobic exercise.

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APPENDIX A. IRB APPROVAL

NDSU NORTH DAKOTA STATE UNIVERSITY

July 2, 2015

Kyle J. Hackney
Department of Health, Nutrition & Exercise Sciences
24B BBFH

IRB Approval of Protocol #HE15244, "Ergogenic Potential of a Pre-Workout Dietary Supplement Combined with Resistance Exercise"
Co-investigator(s) and research team: Sherri Stastny, Michael Blake, Ashlyn Nelson, Zach Wyatt, Derek VanSlyke, Mitch Hager, Mason Haley, Mason Ankenbauer, Ben Olson, Beau Gagnon

Approval period: 7/2/15 to 7/1/16
Continuing Review Report Due: 6/1/16

Research site(s): NDSU Funding Agency: n/a
Review Type: Expedited category # 2, 3, 4
IRB approval is based on original submission (dated 4/27/15), with revised: protocol and consent (received 6/30/2015).

Additional approval is required:
o prior to implementation of any changes to the protocol (Protocol Amendment Request Form).
o for continuation of the project beyond the approval period (Continuing Review/Completion Report Form). A reminder is typically sent 4-6 weeks prior to the expiration date; timely submission of the report is your responsibility. To avoid a lapse in approval, suspension of recruitment, and/or data collection, a report must be received, and the protocol reviewed and approved prior to the expiration date.

A report is required for:
o any research-related injuries, adverse events, or other unanticipated problems involving risks to participants or others within 72 hours of known occurrence (Report of Unanticipated Problem or Serious Adverse Event Form).
o any significant new findings that may affect risks to participants.
o closure of the project (Continuing Review/Completion Report Form).

Research records are subject to random or directed audits at any time to verify compliance with IRB regulations and NDSU policies.

Thank you for cooperating with NDSU IRB procedures, and best wishes for a successful study.

Sincerely,
Kristy Shirley

Kristy Shirley, CIP, Research Compliance Administrator

For more information regarding IRB Office submissions and guidelines, please consult www.ndsu.edu/irb. This Institution has an approved FederalWide Assurance with the Department of Health and Human Services: FWA00002439.

INSTITUTIONAL REVIEW BOARD
NDSU Dept 4000 | PO Box 6050 | Fargo ND 58108-6050 | 701.231.8995 | Fax 701.231.8098 | ndsu.edu/irb
Shipping address: Research 1, 1735 NDSU Research Park Drive, Fargo ND 58102
ndsu is an equal opportunity university

APPENDIX B. INFORMED CONSENT

NDSU North Dakota State University
Health, Nutrition, and Exercise Sciences
Department # 2620, PO Box 6050
Fargo, ND 58108-6050
701-231-6706

Title of Research Study: Ergogenic Potential of a Pre-Workout Dietary Supplement Combined with Resistance Exercise

This study is being conducted by: Principal Investigator- Kyle Hackney, PhD, CSCS, kyle.hackney@ndsu.edu, 701-231-6706.Co-investigator- Sherri Stastny, PhD, RD, CSSD, LRD, sherri.stastny@ndsu.edu, 701-231-7479.

Why am I being asked to take part in this research study?
We are looking to recruit 20 participants for this study.

You are being asked to participate in this study because you:

- Are a male between 18-35 years of age.
- Are apparently healthy as identified by two health questionnaires.
- Are a habitual caffeine user as identified by a nutrition questionnaire.
- Have participated in resistance exercise, weightlifting, or weight training for a minimum of 2 days per week for the past 6 months.

You should not participate in this study if you:

- Answered "Yes" to any questions on the Physical Activity Readiness Questionnaire.
- Are a current tobacco-user/ e-cigarette user
- Are currently taking any prescription medications that interact with caffeine.
- Are currently taking anabolic steroids or corticosteroids (such as Flovent for asthma).
- Have any current or previous cardiovascular, musculoskeletal, or neurological medical problems.
- If you are known to have had violent allergic reactions to drugs, chemicals, or food ingredients including milk, eggs, fish, shellfish, tree nuts, peanuts, wheat, and soybeans.
- Have consumed other dietary supplements (other than vitamins) within the past 3 weeks.

What is the reason for doing the study? Pre-workout dietary supplements containing L-arginine nitrate claim to increase blood flow and enhance one's ability to perform work. They are commonly used by many recreational exercisers in combination with resistance and aerobic exercise. This study will determine if a pre-workout supplement containing L-arginine does increase blood flow to the working muscle, alter energy metabolism, and result in greater work capacity compared to consuming caffeine alone or a placebo (inert substance).

What will I be asked to do?
We are asking you to complete four testing trials. The first will last approximately 30 minutes and the remaining three will last about 2 hours. The first visit can be scheduled at any time that works for you and the research team. At this visit we will measure your height, weight, and arm strength. For the three longer visits, we will need you to arrive at the laboratory at approximately 7:00am without having eaten food or had any liquids other than water. You are, however, free to drink as much water as needed. Will also place 10 electrodes on your chest in order to monitor your heart via an electrocardiogram. Then we will ask you to sit in a testing chair and take a survey regarding your current mood. We will then measure the blood flow occurring near your elbow using an ultrasound machine. Next, we will ask you to spit a small amount of saliva in a test tube so we can measure different physiological markers. After this measure, the finger on your left hand will be wiped with an alcohol pad and a small blood sample (an amount that would fill a contact lens) will be obtained from one of your fingers (finger prick). This will be done to examine your blood lactate levels, which is an indicator of metabolism. Following the finger stick a band-aid will be placed over the site. Finally, we will measure your blood pressure using an inflation cuff. After these measures you will drink approximately 10 oz of liquid containing either the pre-workout dietary supplement, caffeine only, or a fruit punch flavored placebo. After drinking the beverage there will be a 30 minute period of rest where you can use the restroom, check email, and/or complete homework/study. After 30 minutes, we will repeat the electrocardiogram, blood flow via ultrasound, finger stick for lactate level, and blood pressure measures.

Next, we will ask you to perform resistance exercise with your right arm. A brief warm-up will be provided prior to this effort. Once the weight is determined we will ask you to perform 5 sets of 10 repetitions with 60 seconds between each set.

Immediately post-exercise the following measures will be obtained: electrocardiogram, blood flow via ultrasound, finger stick for lactate level, and blood pressure.

At 60 minutes post-beverage consumption (approximately 20 minutes post-exercise) the following measures will be obtained: mood survey, electrocardiogram, blood flow via ultrasound, saliva sample for physiological markers, finger stick for lactate level, and blood pressure.

Once all measurements are completed you will be unhooked from all equipment and released from the study. At your next visit, everything will be the same except a different beverage will be provided. Neither you nor the researchers will know the order of the beverages.

Where is the study going to take place, and how long will it take?
This study will take place in room 14 of Bentson-Bunker Fieldhouse. The estimated time for each session is as follows:
Visit #1 = 30 minutes
Visit #2 = 120 minutes
Visit #3 = 120 minutes
Visit #4 = 120 minutes
It is estimated that the total time for this study will be ~390 minutes (6.5 hours).

What are the risks and discomforts?
It is not possible to identify all potential risks in research procedures, but the researchers have taken reasonable safeguards to minimize any known risks to the participant. If new findings develop during the course of this research which may change your willingness to participate, we will tell you about these findings. Below are examples of known risks for this study.

Muscle Strength Testing and Resistance Exercise
1. Muscle soreness following testing- Exercising with higher than accustomed resistance, performing new exercises, maximal exercises, or performing eccentric (muscle lengthening) movements (risk- moderate).
2. Muscle cramping- Inadequate warm-up or stretch may cause cramping (risk- low).
3. Musculoskeletal injury during testing- Muscle overload or improper performance of a test can cause muscle, ligament, tendon, or bone injury (risk- low).
4. Adverse cardiovascular responses- Abnormal blood pressure responses from holding ones breathe to help generate force or from the standard exercise (risk-low).
5. Lightheadedness- Quickly standing following exercise or the strain of standard exercise (risk-low).
6. General personal injury- Inadvertently walking into test stations during operations, having contact with sharp edges, pinch points, or hardware/software failure could cause injury (risk-low).

Dietary Supplement, Caffeine, or Placebo Intake
1. Nausea- Upset stomach from known or unknown ingredient in dietary supplement or placebo (risk- moderate).
2. Allergic reaction- to known or unknown ingredient in dietary supplement or placebo (risk-low).
3. Adverse cardiovascular responses- Abnormal heart rate or blood pressure responses from dietary supplement or placebo (risk-low).

Measurements
1. Mood change- caffeine is known to influence mood and may acutely elevate vigor, awareness, but also irritability (risk moderate).
2. Rash or skin irritation -At the site of application of the ultrasound gel or from alcohol preparation pads or electrodes (risk-low).
3. Infection- At the site of the finger stick (risk-low).

Risk Minimization
The study team has minimized the known risks by studying healthy, participants that are habitual caffeine users with resistance exercise experience. By being healthy and used to resistance exercise all exercise testing risks are lowered. We will also monitor heart rate and stop the session if your heart rate is raised above your age predicted heart rate (220-age) for more than 30 seconds. For lactate samples, safety lancets, gloves, proper cleaning of fingers with alcohol, and post-finger stick wound coverings will reduce the risk of infection. If you are known to have had violent allergic reactions to drugs, chemicals, or food ingredients, you should not take part in this study. We will use non-allergenic ultrasound gel to reduce the risk of rash or skin irritation. If redness, swelling, or bruising occurs at any sites during this study please contact Kyle Hackney, NDSU: 701-231-6706 or cell: 616-886-0226 or call Student Health Services directly at 701-231-7331. If you feel it is a medical emergency call 911.

What are the benefits to me?
You are not expected to get any benefit from being in this research study.

What are the benefits to other people? Pre-workout dietary supplements are sold at many stores and online; and are common practice in fitness facilities/gyms. It is important to conduct research to validate or refute the advertising claims made.

Do I have to take part in the study? Your participation in this research is your choice. If you decide to participate in the study, you may change your mind and stop participating at any time without penalty or loss of benefits to which you are already entitled.

What will it cost me to participate? There are no direct costs for participation in the study.

What are the alternatives to being in this research study? Instead of participating in this research study, you can choose not to participate.

Who will see the information that I give?
We will keep private all research records that identify you. Your information will be combined with information from other people taking part in the study. When we write about the study, we will write about the combined information that we have gathered. We may publish the results of the study; however, we will keep your name and other identifying information private.
We will make every effort to prevent anyone who is not on the research team from knowing that you gave us information, or what that information is. For example, your name will be kept separate from your research records and these two things will be stored in different places under lock and key. If you withdraw before the research is over, your information will be retained in the research record and we will not collect additional information about you.

Can my taking part in the study end early?
You can choose to not be in the study at any time, however, we ask that you please contact the researchers if you choose to do so. If you fail to show up to sessions or fail to comply with the study guidelines you may be removed from the study.

Will I receive any compensation for taking part in this study? Compensation of $40.00 after completion of this study (all 4 visits).

What happens if I am injured because of this research?
If you receive an injury in the course of taking part in the research, you should contact Kyle Hackney at the following phone number NDSU 701-231-6706 or cell 616-886-0226. If an injury should occur first aid will be administered as well as contact of emergency services (911). Payment for this treatment must be provided by you and your third party payer (such as health insurance). This does not mean that you are releasing or waiving any legal right you might have against the researcher or NDSU as a result of your participation in this research.

What if I have questions?
Before you decide whether to accept this invitation to take part in the research study, please ask any questions that might come to mind now. Later, if you have any questions about the study, you can contact the researcher Kyle Hackney, kyle.hackney@ndsu.edu, NDSU 701-231-6706 or cell 616-886-0226.

What are my rights as a research participant?
You have rights as a participant in research. If you have questions about your rights, or complaints about this research you may talk to the researcher or contact the NDSU Human Research Protection Program by:

- Telephone: 701.231.8995 or toll-free 1-855-800-6717
- Email: ndsu.irb@ndsu.edu
- Mail: NDSU HRPP Office, NDSU Dept. 4000, PO Box 6050, Fargo, ND 58108-6050.

The role of the Human Research Protection Program is to see that your rights are protected in this research; more information about your rights can be found at: www.ndsu.edu/irb .

Documentation of Informed Consent:
You are freely making a decision whether to be in this research study. Signing this form means that

1. you have read and understood this consent form
2. you have had your questions answered, and
3. you have decided to be in the study.

You will be given a copy of this consent form to keep.

_____ _____
Your signature Date

_____
Your printed name

_____ _____
Signature of researcher explaining study Date

_____
Printed name of researcher explaining study

Documentation of release of video or images:
You also have the choice to allow all images and/or video obtained during this study to be used by the research team in publications, manuscripts, poster presentations, PowerPoint presentations, and University websites. Images will only be used in a professional context when describing the study. You name will never be associated with the image/video.

Yes _____

No _____

_____ _____
Your signature Date

_____
Your printed name

APPENDIX C. CAFFEINE FREQUENCY QUESTIONNAIRE

Caffeine and Food Frequency

NDSU HNES

1340 Administration Avenue
Fargo, ND 58102

NDSU HNES requests your help. Please complete the following Caffeine and Food Frequency Survey. **Please carefully read all questions before answering.**

Please feel free to ask questions. Thank you for your time.

Project Name:
Pre-workout study

Participant Number:
_____

Project Manager:
Dr. Kyle Hackney

Date:
April 10, 2015

1. Do you use caffeine regularly?

☐ Yes ☐ No; If no proceed to question **#3**

1a. Do you drink coffee?

☐ Yes ☐ No; If no proceed to question **#2**

1b. How much coffee do you drink daily?

☐ 1 cup or less ☐ 2-4 cups ☐ More than 4 cups ☐ Other (Specify)_____

1c. What size coffee cup do you normally use?

☐ Small cup (6oz) ☐ Medium cup (8oz) ☐ Large cup (12oz) ☐ Larger (Specify)_____

2. Do you drink caffeinated soda, energy drinks, energy shots, or tea?

☐ Yes ☐ No; If no proceed to question **#3**

2a. How many cans or equivalent bottles of caffeinated soda do you drink daily? (1 can = 12oz)

☐ None ☐ 1 can or less ☐ 2-4 cans ☐ Other _____

2b. How many energy drinks do you consume daily? (1 drink ≈ 16oz)

☐ None ☐ 1 drink or less ☐ 2-3 drinks ☐ Other _____

2c. How many energy shots do you consume daily? (1 shot ≈ 2oz)

☐ None ☐ 1 shot or less ☐ 2-3 shots ☐ Other _____

2d. How many cups of tea do you drink daily? (1 cup = 6oz)

☐ None ☐ 1 cup or less ☐ 2-4 cups ☐ Other _____

2e. What type of tea do you drink?

☐ Black Tea ☐ Green Tea ☐ Non-caffeinated Herbal Tea ☐ Other _____

3. Do you currently use a supplement? **(Include all workout supplements and vitamins)**

☐ Yes; Please specify **Type**_____ ☐ No

_____

Amount_____

4. Do you consume any of the following? (If More; please specify how much)

Beets	☐	Never	☐	Monthly	☐	2-3x per month	☐	Weekly	☐ More _____
Kale	☐	Never	☐	Monthly	☐	2-3x per month	☐	Weekly	☐ More _____
Arugula	☐	Never	☐	Monthly	☐	2-3x per month	☐	Weekly	☐ More _____
Spinach	☐	Never	☐	Monthly	☐	2-3x per month	☐	Weekly	☐ More _____

Thank you very much for taking the time to complete this survey.
Your time is valued and very much appreciated!

APPENDIX D. RECRUITMENT FLYER

Pre-Workout Supplementation Study

The Department of Health, Nutrition, and Exercise Sciences is looking for volunteers to participate in a research study testing the performance and cardiovascular effects of a pre-workout supplement combined with resistance training

Participants Must Be:

- Male between 18-35 years of age
- Consume caffeine regularly
- Have be recreationally active; participated in one of the following: 1)Resistance training, 2) weightlifting, 3) or weight training exercise at least 2 days per week for the past 6 months
- Available for 4 sessions (about 2 hours per session) in Bentson Bunker Field House

Compensation of $40 will be awarded per participant.
For more information please contact Michael Blake at:
michael.blake@ndsu.edu or 701-231-9749

Pre-Workout Study- Contact: Michael Blake: By e-mail at: michael.blake@ndsu.edu Or by phone at: 701-231-9749

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APPENDIX E. RECRUITMENT EMAIL SCRIPT

Ergogenic Potential of a Pre-Workout Dietary Supplement Combined with Resistance Exercise

Principal Investigator- Kyle Hackney, PhD, kyle.hackney@ndsu.edu, 701-231-6706.

Co-investigator- Sherri Stastny, PhD, RD, sherri.stastny@ndsu.edu, 701-231-7479.

Pre-workout dietary supplements containing caffeine and L-arginine nitrate claim to increase blood flow and enhance one's ability to perform work by stimulating vessels to produce nitric oxide. Today, pre-workout supplements are commonly used by many recreational exercisers in combination with resistance and aerobic exercise. This study will determine if a pre-workout supplement containing L-arginine does increase blood flow to the working muscle, change energy metabolism, alter mood, or result in greater work capacity compared to consuming caffeine alone or a placebo (sugar pill).

We are looking to recruit 20 participants for this study. To participate you should be a male between 18-35 years of age, generally healthy, a habitual caffeine user (equivalent to about one large cup of coffee per day), have participated in resistance exercise, weightlifting, or weight training for a minimum of 2 days per week for the past 6 months.

We are asking you to complete one laboratory familiarization session and three testing trials where you will be randomly assigned to consume a pre-workout supplement, caffeine only, or placebo. During each trial, we will measure your heart rate (using electrocardiogram), arm blood flow (using ultrasound), mood (survey), nitric oxide level (saliva sample and strip), and whole blood lactate (figure stick and test strip) before and after upper body resistance exercise. Each testing trial will last approximately 2 hours.

Compensation of $40 will be awarded for full completion of the study. If interested or for more information please contact Michael Blake at E-mail- michael.blake@ndsu.edu, Phone- 701-231-9749.This research has been approved by the North Dakota State University Institutional Review Board (Protocol #HE15244).

APPENDIX F. PRE-WORKOUT DATA SHEET

Pre-Workout Data Sheet

Participant (#) _____ Date_____ Trial (#) _____

_____

Height_____ Weight_____

POMS Survey Pre-Supplementation Time _____ POMS Survey Post-Supplementation Time _____

Product Consumption Time _____ Exercise Start Time _____

Subject Discharge Time _____

Biodex Measures:

- Elbow Extension _____ 50% _____
- Elbow Flexion _____ 50% _____

Biodex Seat Measurements:	

ECG Data Collection-

Pre-Supplementation (5 minutes resting during POMS)	
30 minutes after supplementation	
Post exercise	
60 minutes post-supplementation	

POMS	**PRE**	**POST**
Anger (0-48)		
Confusion (0-28)		
Depression (0-60)		
Fatigue (0-28)		
Tension (0-36)		
Vigor		

Blood Pressure, Heart-rate, and Lactate Measures:

	Blood Pressure	Heart-Rate	Lactate
Pre-Supplementation			
30 minutes after supplementation			
Post exercise			
60 minutes post-supplementation			

Ultrasound measures:

	Pre Sup	**30 min post sup**	**Post exercise**	**60 min post exercise**	
Systolic					**Comments:**
Diastolic					
Mean Velocity					
AT					
TAVM					
S/D					
RI					
PI					
Vessel Diameter					
Vessel Circumference					

Nitric Oxide Measures:

	Color	availability of nitric oxide in system
Pre-Supplementation		
30 minutes after supplementation		
Post Exercise		
60 minutes post-supplementation		

APPENDIX G. DATA COLLECTION CHECKLIST

Subject _____Date _____ Researchers at data collection (initials)

Before subject arrives

_____ ECG “On”
_____ Biodex “On”
_____ POMS Laptop on and connected to internet
_____ Lactate analyzer and testing strips prepared
_____ Nitric oxide strips and equipment ready
_____ Ultrasound “On”
_____ Blood pressure cuff ready
_____ Supplement ready
_____ Cold water ready

Subject arrives

_____ Informed Consent
_____ PARQ
_____ Health History Questionnaire
_____ Caffeine and Nutritional Compliance Questionnaire
_____ Height
_____ Weight
_____ ECG preparation and hook-up
_____ Biodex Fitted for subject- subject playing on computer

Pre- data collections

_____ ECG data collection 5 minutes during POMS (Pre)- save as sub#_pre_date
_____ Initiate POMS survey (Pre, screen shot and save as Sub#_pre_date
_____ Ultrasound measures (Pre)- process later record on data sheet
_____ Lactate measures (Pre)- record on data sheet
_____ Nitric Oxide (Pre)- record color on date sheet
_____ Blood Pressure measurement (Pre)- record on data sheet
_____ Consume product---------- **Record time** **here**

At 30 minutes post-intake - Start data collections -Record time **here**
_____ ECG data collection 5 minutes during POMS (30)- save as sub#_ 30_date
_____ Ultrasound measures (Pre)- process later
_____ Lactate measures (Pre)- record on data sheet
_____ Blood Pressure measurement (Pre)- record on data sheet

Get ready for Exercise

_____ Biodex strength Test

_____ ECG data collection On “Exercise”

_____ Biodex Resistance Exercise

_____ ECG data collection Off “Exercise”, save as sub# exercise

Immediately Post obtain

_____ECG data collection sub# post-ex_date

_____ Ultrasound measures (Post-ex), process later

_____ Lactate measures (Post-ex)

_____ Blood Pressure measurement (Post-ex)

Wait until 60 minutes post Pre-workout intake. Start data collections Record time here

_____ ECG data collection On, save as sub#_60_date

_____ Initiate POMS survey (Post-60, screen shot and save data as sub#_60_date

_____ Ultrasound measures (Post-60), process later

_____ Lactate measures (Post-60)

_____ Nitric Oxide (Post-60)

_____ Blood Pressure measurement (Post-60)

Unhook subject from all devices

_____ Give subjects discharge instructions

_____ Subject data collection complete

APPENDIX H. DISCHARGE INSTRUCTIONS

Pre-workout Study Subject Discharge Instructions.

Date_____

Dear Study Participant:

Please be aware that you ***may*** have just completed a trial consisting of a minimum dose of 350 mg of caffeine. For your safety please avoid other foods/drugs that may also contain large doses of caffeine for 24 hours. Examples are provided below. Also, please avoid consumption of any alcoholic beverages for 24 hours as alcohol consumption may lead to dehydration.

Food or Drink	**Serving**	**Caffeine, mg (variability)***
Instant Coffee	250 mL (8oz) cup	60 (12-169)
Brewed Coffee	250 mL (8oz) cup	80 (40-110)
Expresso	1 standard serving	107(25-214)
Starbucks Breakfast Blend	600 ml (20 oz)	415(300-564)
Iced coffee	500 ml (16 oz)	30-200
Frappuccino	375 ml (12oz) cup	90
Tea	250 ml (8 oz)	27 (9-51)
Hot Chocolate	250 ml (8 oz)	5-10
Coca Cola	375 (12 oz) can	49
Red Bull Energy drink	250 ml (8 oz)	80
AMP Energy drink	500 ml (16 oz) can	143
Fixx energy drink	600 ml(20 oz) can	500
No-Doz (U.S.)	1 tablet	200
Extra Strength Excedrin	1 tablet	65

***caffeine content in some items can vary. Value estimates obtain from Appl. Physiol. Nutr. Metab. Vol. 22, 2008.**

Although the influence of a high caffeine intake is temporary (~12 hours) and we do not anticipate any study related problems, if you experience nausea, persistent rapid heart rate, chest pain, or other bothersome side effects, please seek medical attention (your personal provider) and contact the research team.

If you have any questions, do not hesitate to call or email.

Thank you,

_____ _____

Dr. Kyle Hackney, PhD, CSCS Dr. Sherri Stastny, PhD, RD

Kyle.hackney@ndsu.edu Sherri.stastny@Ndsu.edu

701 231 6706 701 231 7479

***This signature form should remain in the research lab

I was informed that I should avoid foods/drugs containing large amounts of caffeine and alcohol for 24 hours.

___ Yes

___ No

Trial 1

Participant Name (please print)

Participant Signature _____ Date _____

Trial 2

I was informed that I should avoid foods/drugs containing large amounts of caffeine and alcohol for 24 hours.

___ Yes

___ No

Participant Name (please print)

Participant Signature _____ Date _____

Trial 3

I was informed that I should avoid foods/drugs containing large amounts of caffeine and alcohol for 24 hours.

___ Yes

___ No

Participant Name (please print)

Participant Signature _____ Date _____

APPENDIX I. MODIFIED PROFILED MOOD STATES

Profile of Mood States (POMS)

POMS is a standard validated psychological test formulated by McNair et al. (1971)[1]. The questionnaire contains 65 words/statements that describe feelings people have. The test requires you to indicate for each word or statement how you have been feeling in the past week including today.

Required Resources

To undertake this test you will require:

- Questionnaire (see below)
- Assistant

How to conduct the test

- The assistant explains the test protocol to the athlete:
  - Read each word/statement below, decide how you have been feeling, in respect to the word/statement, in the past week and today, and select the appropriate statement "Not at All", "A Little", "Moderately", "Quite a Lot"or "Extremely"to indicate your feeling.
- The athlete responds to the 65 words/statements on the questionnaire below - no time limit
- The assistant determines and records the athlete's mood state scores.

Questionnaire

Feeling	How I have felt
Friendly	Not at All
Tense	Not at All
Angry	Not at All
Worn Out	Not at All
Unhappy	Not at All
Clear Headed	Not at All
Lively	Not at All
Confused	Not at All
Sorry for things done	Not at All
Shaky	Not at All
Listless	Not at All
Peeved	Not at All
Considerate	Not at All
Sad	Not at All
Active	Not at All
On Edge	Not at All
Grouchy	Not at All
Blue	Not at All
Energetic	Not at All
Panicky	Not at All
Hopeless	Not at All

Relaxed	Not at All
Unworthy	Not at All
Spiteful	Not at All
Sympathetic	Not at All
Uneasy	Not at All
Restless	Not at All
Unable to Concentrate	Not at All
Fatigued	Not at All
Helpful	Not at All
Annoyed	Not at All
Discouraged	Not at All
Resentful	Not at All
Nervous	Not at All
Lonely	Not at All
Miserable	Not at All
Muddled	Not at All
Cheerful	Not at All
Bitter	Not at All
Exhausted	Not at All
Anxious	Not at All
Ready to Fight	Not at All
Good Natured	Not at All
Gloomy	Not at All
Desperate	Not at All
Sluggish	Not at All
Rebellious	Not at All
Helpless	Not at All
Weary	Not at All
Bewildered	Not at All
Alert	Not at All
Deceived	Not at All
Furious	Not at All
Efficient	Not at All
Trusting	Not at All
Full of Pep	Not at All
Bad Tempered	Not at All
Worthless	Not at All
Forgetful	Not at All
Carefree	Not at All
Terrified	Not at All
Guilty	Not at All
Vigorous	Not at All
Uncertain about things	Not at All
Bushed	Not at All

Assessment

Select the "Analyse" button to obtain a Total Mood Disturbance (TMD) score and an analysis of your tension, depression, anger, vigour, fatigue and confusion. Your TMD is calculated by adding your scores for Tension, Depression, Anger, Fatigue and Confusion and then subtracting your Vigour score.

The scores in brackets (x-y) in the table below indicate the possible score range with lower scores indicative of people with more stable mood profiles.

Select the "Analyse" button to obtain scores for each of the mood states and the total mood disturbance. The test can be repeated by selecting the "Reset" button.

Analyse Total Mood Disturbance (-32 to 200): Reset

Mood Profile	Score
Anger (0-48)	
Confusion (0-28)	
Depression (0-60)	
Fatigue (0-28)	
Tension (0-36)	
Vigour (0-32)	

Normative Data

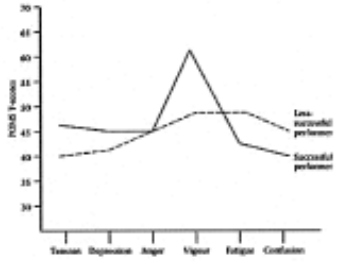
Terry (n.d.)[3] provides POMS norms for an athletic sample (n=2086) grouped by level of competition (International standard athletes, club level athletes and recreational athletes).

Group	Tension	Depression	Anger	Vigour	Fatigue	Confusion
International	5.66	4.38	6.24	18.51	5.37	4.00
Club	9.62	8.67	9.91	15.64	8.16	7.38
Recreational	6.00	3.11	3.60	17.78	6.37	4.84

Analysis

Analysis of the result is by comparing it with the results of previous tests. It is expected that, with appropriate training between each test, the analysis would indicate an improvement.

Morgan & Johnson (1978)[2] found that by plotting the mood state results of elite performers prior to competition exhibited the graph below. This graph, with a raised peak for Vigour, was termed the "Iceberg" profile.



Target Group

This test is suitable for anyone but not for individuals where the test would be contraindicated.

Reliability

Test reliability refers to the degree to which a test is consistent and stable in measuring what it is intended to measure. Reliability will depend upon how strict the test is conducted and the individual's level of motivation to perform the test. The following link provides a variety of factors that may influence the results and therefore the test reliability.

Validity

Test validity refers to the degree to which the test actually measures what it claims to measure and the extent to which inferences, conclusions, and decisions made on the basis of test scores are appropriate and meaningful. This test provides a means to monitor the mood state of an athlete.

Advantages

- No equipment required
- Simple to set up and conduct
- Can be conducted almost anywhere

Disadvantages

- Assistant required to administer the test

References

1. McNAIR et al. (1971) *Manual for the Profile of Mood States*. San Diego, CA: Educational and Industrial Testing Service.
2. MORGAN, W.P. and JOHNSON, R.W. (1978) Personality characteristics of successful and unsuccessful oarsmen. *International Journal of Sport Psychology*, 9, p. 119-133
3. TERRY, P. (n.d.) *Normative Values for the Profile of Mood States for Use with Athletic Samples*, [WWW] Available from: http://eprints.usq.edu.au/4385/2/Terry_Lane_IJASS_v12n1_Author's_version.pdf [Accessed 30/06/2013]

Related References

The following references provide additional information on this topic:

- LIN, S., HSIAO, Y. Y., & WANG, M. (2014) The Profile of Mood States 2nd Edition (POMS 2)

Page Reference

The reference for this page is:

- MACKENZIE, B. (2001) *Profile of Mood States (POMS)* [WWW] Available from: http://www.brianmac.co.uk/poms.htm [Accessed 10/4/2015]

Related Pages

The following Sports Coach pages provide additional information on this topic:

- Articles on Performance Evaluation
- Articles Psychology
- Books on Psychology
- Evaluation and Performance Tests
- Scoring for POMS

Additional Sources of Information

For further information on this topic see the following:

- BEASHEL, P. & TAYLOR, J. (1996) *Advanced Studies in Physical Education and Sport*. UK: Thomas Nelson & Sons Ltd.
- BEASHEL, P. & TAYLOR, J. (1997) *The World of Sport Examined*. UK: Thomas Nelson & Sons Ltd.
- BIZLEY, K. (1994) *Examining Physical Education*. Oxford; Heinemann Educational Publishers
- DAVIS, B. et al. (2000) *Physical Education and the Study of Sport*. UK: Harcourt Publishers Ltd.
- GALLIGAN, F. et al. (2000) *Advanced PE for Edexcel*. Oxford; Heinemann Educational Publishers
- McARDLE, W. et al. (2000) *Essentials of Exercise Physiology*. 2nd ed. Philadelphia: Lippincott Williams & Wilkins
- OHU, D. (1996) *Explosive Power and Strength*. USA; Human Kinetics Publishers, Inc.

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