EFFECTS OF INGESTING A PREWORKOUT SUPPLEMENT FOR 7 DAYS ON EXERCISE PERFORMANCE AND COGNITIVE FUNCTION

A Dissertation

by

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ABSTRACT

We examined the effects of ingesting two pre-workout supplements (PWS) on cognitive function, perceived readiness, and exercise performance related parameters. Resistance-trained participants (N=19) were randomized to a double-blind, cross-over (7-day washout), placebo-controlled study supplemented with: (1) Placebo (PLA); (2) PWS (caffeine, creatine nitrate in a 2:1 ratio as a salt, β-alanine, arginine alpha-ketoglutarate, ascorbic acid, N-acetyl tyrosine, *Mucuna pruriens*), and (3) PWS150 at ~150% of the PWS dose. Participants were tested on hemodynamic responses, resting energy expenditure, cognitive function (Stroop Color-Word test), self-perceived readiness, three sets of bench and leg press at 70% of 1RM, a 30-sec anaerobic capacity test, side effect questionnaires, and donated blood samples before and/or after acute ingestion, then after 7 days of supplementation. Data were analyzed by GLM and presented as mean (SD) or mean change (95% CI). Significant improvements in Stroop Word testing were observed for PWS (6.57 counts, 95% CI 1.36, 11.8) and PWS150 (11.5 counts, 95% CI 6.26, 16.6), but not PLA (1.31 counts, 95% CI -3.89, 6.52). Significant changes in Stroop Color testing were observed for PWS150 (8.1 counts, 95% CI 4.52, 11.6) and PLA (4.47 counts, 95% CI 0.89, 8.05), but not PWS (2.31 counts, 95% CI -1.26, 5.89). Similar results were observed for Word-Color. When all domains were summed, PWS150 (27.4 counts, 95% CI, 16.1, 38.7) and PWS (12.3 counts, 95% CI, 1.0, 23.5) showed significant improvements, but not PLA (11.3 counts, 95% CI, -.002, 22.5). We observed significant improvements in Wingate mean power for PWS150

(26.0 watt, 95 CI, 1.85, 50.3), but not PWS (-3.83 watt, 95 CI, -28.7, 21.0) and PLA (8.88 watt, 95 CI, -17.5, 35.2). No significant or adverse changes were observed for hemodynamic, thermogenic, and hematologic variables. The PW150 demonstrated consistent improvements in cognitive function; yet was unmatched by changes in self-perceived readiness or other measured parameters associated with exercise performance.

DEDICATION

I would like to dedicate my dissertation work to my loving family and wife. A special feeling of gratitude to my parents, Hashem and Nahid Koozehchian who are the reason of what I became today. My brother Vahid and my sister Sara, who have never left my side and are very special to me. My wife Mary who has been a constant source of support and encouragement during the challenges of this study.

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NOMENCLATURE

ESNL	Exercise and Sport Nutrition Laboratory
REE	Resting Energy Expenditure
RER	Respiratory Exchange Ratio
FAM	Familiarization Session
LBM	Lean Body Mass
ATP	Adenosine Triphosphate
HR	Heart Rate
SBP	Systolic Blood Pressure
DBP	Diastolic Blood Pressure
DXA	Dual X-ray Absorptiometry
FFM	Fat Free Mass
FM	Fat Mass
ECG	Electrocardiograph
VAS	Visual Analog Scale
SQQ	Sleep Quality Questionnaire
CQ	Caffeine Questionnaire
ED	Energy Drink
PWS	Preworkout Supplement
PWS150	Preworkout Supplement at 150% dosage
1RM	1-Repetition Maximum

Reps	Repetitions
ALP	Alkaline Phosphatase
AST	Aspartate Aminotransferase
ALT	Alanine Aminotransferase
BUN	Blood Urea Nitrogen
CK	Creatine Kinase
TG	Triglyceride
TC	Total Cholesterol
LDH	Lactate Dehydrogenase
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
g	Grams
PLA	Placebo
WBC	White Blood Cell
MID	Mid-Range Absolute Count
RBC	Red Blood Cell
PLT	Platelet
RCDW	Red Cell Distribution Width
HDL	High Density Lipoprotein
LDL	Low Density Lipoprotein
BCAA	Branched Chain Amino Acid

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CHAPTER I

INTRODUCTION AND RATIONALE

Background

Nutritional supplement intended for consumption before exercise may improve cognitive function and/or resistance exercise performance [1]. Consequently, several nutritional strategies such as energy drinks (ED) and preworkout supplement (PWS) developed to optimize nutrient delivery prior to exercise [2, 3]. This includes providing carbohydrate and several ergogenic nutrients to increase energy availability [4], and/or positively affect exercise capacity [5]. The composition of PWS vary widely, but the principle ingredients consistently include carbohydrates, electrolytes, and caffeine [1]. In addition, some PWS also contain other nutrients like nitrates which cause some exercise benefits such as reducing blood pressure (BP) and oxygen cost of submaximal exercise [6]. Several studies indicate that caffeinated supplements improve performance which has increased interest in caffeinated sport drinks [7-9]. There are some other evidence showing that ingesting caffeine-containing supplements prior to exercise improves memory, concentration, and complex reaction times in athletes [10-14]. Caffeine could permit athletes to train at a greater power output and/or to train longer. It has also been shown to increase speed and/or power output in simulated race conditions [15]. Caffeine acts on the central nervous system as an adenosine antagonist, but may also have an effect on substrate metabolism and neuromuscular function [16].

Numerous studies have examined the safety and efficacy of individual PWS's such as creatine monohydrate [17], carbohydrate [18], protein [19], and amino acids [20] as well as whether adding potentially ergogenic nutrients to PWS's may promote additive benefits [1]. For example, several studies have reported that ingestion of PWS's can cause improvements in exercise capacity [21], body composition [22], muscle mass and strength [23], reaction time [24], and/or subjective feelings of focus and energy [25].

While many ingredients used in PWS studies have had their safety and efficacy assessed, the interactions when combined are less understood. The present study examined the thermogenic, hemodynamic, and ergogenic effects of acute ingestion or 7 days of PWS supplementation (two doses) in healthy individuals. Thermogenic means tending to produce heat, and the term is commonly applied to nutrients which increase heat through metabolic stimulation [26]. Hemodynamics is the dynamics of blood flow such as heart rate and blood pressure [27]. Ergogenic means able to improve work or performance [28]. The formulation of the two doses of PWS and a PLA which was used for a 7 day duration contained: (1) Flavored dextrose PLA (12g); (2) PWS [β-alanine (3.2g), arginine α-ketoglutarate (2.0g), creatine nitrate (2.0g), ascorbic acid (500mg), N-acetyl tyrosine (300mg), caffeine (300mg), tetramethyluric acid (10mg), *Mucuna pruriens* (1.0g), dextrose (2.6g)]; (3) PWS at ~150% dosage (PWS150), interspersed by 7 day of washout period. Formulation decision was made by the supplement provider company based on previous studies and results from Texas A&M University. The formula that we used in the current study is a PWS formula so the results should be

immediate. If a supplement user does not see a result immediately or within 7 days, then the supplement is not effective.

Several studies show that ingesting supplements containing caffeine, creatine, L-arginine, and β-alanine prior to exercise can affect acute physical performance and/or cognitive function [21, 23, 24, 29, 30]. Caffeine is one of the most widely consumed ergogenic aids, with acute caffeine ingestion improving aerobic exercise endurance and reducing fatigue [31-33]. When ingested before exercise, caffeine (~3-6mg/kg) [16] has shown to improve exercise performance [34-36] and cognitive function [37, 38]. Plausible theories for the beneficial impact of caffeine include an increase in central nervous system activity [39, 40], enhancing calcium release and uptake to the sarcoplasmic reticulum [41], increasing plasma epinephrine concentrations [42], and functioning as an adenosine receptor antagonist [43, 44]. Caffeine is a receptor antagonist at all adenosine receptor subtypes. Antagonism at adenosine receptors stimulates the medullary vagal, vasomotor, and respiratory centers, which increases respiratory rate, reduces heart rate, and constricts blood vessels. In addition, adenosine receptor antagonism promotes neurotransmitter release (e.g., monoamines and acetylcholine), which endows caffeine with its stimulant effects; adenosine functions as an inhibitory neurotransmitter that suppresses activity in the central nervous system. Heart palpitations are caused by blockade of the adenosine A_1 receptor [45]. Supplementation with nitrate is commonly consumed as beetroot juice or sodium nitrate [46]. The doses ranging from 300 to 600mg nitrate have shown to improve exercise performance [46-48]. Dietary nitrate supplementation has been reported to be in

association with a lower ATP cost of muscle force production [49] and an increase in the mitochondrial ratio of phosphate radicals esterified to atoms of O_2 consumed [50]. The ingestion of nitrate can increase muscle oxygenation in contracting skeletal muscle [51]. During the nitrate reduction process, nitrate is reduced to nitrite [52]. Nitrite anion is recognized as a transient species that acts in the physiological regulation of blood flow and blood pressure [53, 54]. Nitrite exerts these effects in the body via its conversion to functional nitrogen oxides, including nitric oxide [54]. These physiological effects possibly account for the increased exercise tolerance [49, 51, 55] and exercise performance that has been reported following nitrate supplementation [56, 57].

Statement of the Problem

Will consuming a PWS containing caffeine, creatine nitrate, β-alanine, arginine α-ketoglutarate, and N-acetyl tyrosine affect acute exercise or cognitive function?

Purpose of the Study

The purpose of this study was to determine whether acute ingestion or 7 days of daily consumption of PWS's affect exercise and/or cognitive function. The secondary purpose of this study was to determine whether PWS and 1.5 times recommended dose is safe or not.

General Study Overview

This study was conducted in a randomized, double-blind, placebo-controlled trial and cross-over manner. The independent variable was the nutritional supplementation. Dependent variables included resting energy expenditure; HR and BP; readiness to perform visual analogue scale (VAS); cognitive function test; strength endurance and

anaerobic sprint capacity; standard clinical chemistry panel; sleep quality questionnaire; and caffeine tolerance questionnaire. Subjects signed a study consent form and underwent medical examination and familiarized to the study protocol. At day 0 testing session, participants were randomized to one of double-labeled supplement treatments; PLA, PWS, or PWS150, and then they received alternate supplement in a counter-balanced fashion following a 7-day washout period. Participants were instructed to ingest 12g of supplement immediately prior to exercise on training days and around noon on non-training days and maintain their current diet throughout the study. The data were collected prior to, following acute ingestion, after 7 days of ingestion, and following an acute dose after 7 days of supplementation. In addition, participants recorded a 4-day food-log including three weekdays and one weekend day during each washout period. The volunteers were asked to check-in the Exercise and Sport Nutrition Lab at Texas A&M University at days 2 and 4 to conduct a Stroop Color Test, Readiness to Perform VAS, sleep quality questionnaire, and caffeine tolerance questionnaire. On day 7, participants performed same tests as performed during the day 0 testing session.

Hypotheses

$H_0$1: PWS supplementation will increase resting energy expenditure.

$H_0$2: PWS supplementation will increase heart rate and blood pressure.

$H_0$3: PWS supplementation will improve perceptions of readiness to perform.

$H_0$4: PWS supplementation will improve markers of cognitive function.

$H_0$5: PWS supplementation will affect exercise performance.

$H_0$6: There will be no significant differences in markers of health.

$H_0$7: There will be no significant differences in sleep quality inventory.

$H_0$8: There will be no significant differences in caffeine tolerance inventory.

Delimitations

This study was conducted under the following guidelines:

1. Nineteen (N = 19) recreationally active men and women between the ages of 18 – 40 years were recruited for the study.
2. Subjects had at least 6 months immediate prior history of resistance training including bench press and leg press/squat.
3. Eligible participants took part in familiarization (FAM) session during which time they were informed of the study protocol, filled out necessary forms including an informed consent form and a general screening form. Then, they completed a one repetition max bench press and leg press test and a practice anaerobic sprint test on a cycle ergometer.
4. Participants were advised to maintain a consistent workout and dietary regimen throughout the duration of the study.
5. Participants had to refrain from exercise, caffeine, and use of over-the-counter stimulants for 48-hrs prior to each testing session.
6. Participants were fasted for at least 12-hrs prior to each testing session.
7. Participants performed to their maximal ability on all strength and anaerobic sprint tests.

8. Participants were instructed to consume all supplements and report any disorder(s) in weekly side-effects questionnaires.

Limitations

1. The participants were individuals from the Texas A&M University community and surrounding fitness facilities that responded to recruitment fliers and emails; therefore the selection process was not truly random.
2. There were some variations in testing times and dietary intake, although all efforts were made to conduct testing sessions at the same approximate time to account for diurnal variations and subjects were instructed to maintain a consistent diet throughout the duration of the study.
3. Motivation and effort during performance throughout testing may not have been 100% at each testing session.
4. All laboratory equipment was calibrated according to manufacturer guidelines and all samples were run in duplicate to reduce likelihood or error. However, there are innate limitations of the laboratory equipment that are used for data collection and analysis.

Assumptions

1. Participants followed the protocol that was explained to them during the FAM session.
2. Participants answered the entrance questionnaires accurately and honestly prior to being accepted into the study.

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

CHAPTER II

REVIEW OF THE LITERATURE

Introduction

Scientific research on ergogenic supplements has led manufacturers to introduce preworkout energy drinks (ED) to the market. ED are a unique category within the beverage industry and typically contain glucose, caffeine, and/or nutrients that are often used to improve energy metabolism, mental focus, strength, and power [19, 29, 58]. Numerous studies have shown that some of the individual ingredients contained in PWS improve physical performance as each individual ingredient is associated with a different physiological mechanism [16, 59, 60]. For example, acute use of caffeine as the main active ingredient has shown to increase muscular strength, power, and endurance [36, 61, 62]. In addition, caffeine produces mild central nervous system stimulation, reducing fatigue and increasing concentration and alertness [44, 63]. Except for sugar-free versions, all ED contain sugars in the form of monomers glucose and fructose, the dimer sucrose, and the synthetic polymer maltodextrins, also known as glucose polymers [64, 65]. Investigators have documented the improved exercise performance in athletes using carbohydrate beverages, compared to water or other PLA beverages [66-68].

Some studies have shown that using diverse multi-ingredient PWS mediates improving time to fatigue [69, 70], increasing mental alertness [71], and enhancement of muscular strength [24]. However, the combined effects of nutritional ingredients needs to be more investigated despite increasing the popularity of multi-ingredient PWS. The

following scientific support serves as the basis for specific ingredients used in the most PWS.

Caffeine

Table 2.1 provides the summary of some studies investigating the effects of caffeine supplementation. In the current study, we planned to use 300mg/day and 400mg/day caffeine anhydrous in two doses of PWS. Caffeine has shown to be an effective ergogenic aid for enhancing sport performance in trained athletes when consumed in low-to-moderate dosages (~3-6mg/kg) [16]. For example, acute anhydrous caffeine ingestion (2-5mg/kg^{-1}) improved resistance exercise performance (5-8%) by allowing the consumer to enhance exercise intensity, leading to an increased training volume [60]. In the brain, adenosine is an inhibitory neurotransmitter. This means, adenosine functions as a central nervous system depressant. In normal conditions, it promotes sleep and suppresses arousal. Adenosine generally decreases the concentration of major neurotransmitters, including serotonin, dopamine, acetylcholine, norepinephrine and glutamate. Caffeine functions as an antagonist to A_1 and A_{2A} adenosine receptors, and increases the concentration of these major neurotransmitters [72]; thus, caffeine can improve mental and physical performance [73-75] and reducing pain and fatigue [60, 76-78] associated with high-intensity resistance training. Caffeine has been broadly studied as an ergogenic aid for increased energy, improved exercise performance, and increased fat oxidation [79-82]. The breadth of research spans across several decades and is fairly unequivocal. What remains to be somewhat elucidated is the exact physiological mechanism that makes this ingredient beneficial to sport

performance. Ivy et al. [82] indicated that caffeine ingestion (160mg) 40 min prior to exercise improves endurance performance in PWS vs. PLA group (3,690 ± 64 sec vs. 3,874 ± 93 sec). Graham et al. [83] examined the effect of ingesting caffeine (4.45mg/kg) in coffee or in water 1 hr prior to exercise. The endurance time showed a significant improvement in the supplement group (7.5-10 min). Based on these findings, the investigators concluded: 1) caffeine has the potential to increase endurance performance and 2) caffeine in isolation from coffee can prominently improve endurance capacity.

Exercise physiologists have also investigated the caffeine phenomenon, and tested for the possibility that it might improve high intensity relatively short-term performance. Doherty et al. [7] indicated that caffeine ingestion (5mg/kg^{-1}) increased mean power output in high-intensity cycling. Green et al. [84] found caffeine (~6mg/kg) to moderately enhance resistance training performance. The findings of similar rating of perceived exertion concurrent with higher repetitions suggested that caffeine can blunt pain responses, possibly delaying fatigue in high-intensity resistance training. Therefore, other mechanisms were cited to explain the effect of caffeine on performance improvement, such as the central nervous system, a direct effect on skeletal muscle, and increased motor unit recruitment [85]. Kalmar and Cafarelli [85], suggested that caffeine functions as an antagonist on adenosine receptors to block the inhibitive actions of adenosine on neurotransmitter (e.g., dopamine) release. Davis et al. [44] also concluded that caffeine may function to impede fatigue by the action of blocking adenosine receptor sites and increasing the release of excitatory neurotransmitters, especially

dopamine. Therefore, where the central nervous system is concerned, caffeine may act to increase excitability of the motor neuron pool by binding to competing adenosine receptor sites. In another study, Beck et al. [36] examined the acute effects of caffeine supplementation on strength, muscular endurance, and anaerobic capacity on resistance trained males. Caffeine ingestion was effective for increasing bench press 1RM (2.1 kg = 2.1%). Daniels et al. [86] examined the effects of caffeine ingestion, 45 min prior to exercise, on BP, HR, and forearm blood flow during dynamic leg exercise on trained caffeine-naive cyclists. Before exercise, caffeine increased both SBP (17%) and mean arterial pressure (11%). During dynamic exercise, caffeine attenuated the increase in forearm blood flow (53%) and forearm vascular conductance (50%). SBP and mean arterial pressure were also higher during exercise plus caffeine; however, these increases were secondary to the effects of caffeine on resting BP. No significant differences were observed in HR. The authors concluded that the caffeine-induced alterations were probably due to antagonism of adenosine receptors. Caffeine has been shown to inhibit both A_1 and A_2 adenosine receptors. The A_2 receptors are primarily responsible for adenosine's vasodilatory effects. The observation that caffeine attenuated the increase in forearm blood flow during exercise suggested that caffeine may offset adenosine-provoked dilation in other regional circulations under these circumstances. Astorino et al. [87] examined the effect of caffeine (6mg/kg) ingestion 1 hr prior to exercise, on cardiovascular function in resistance-trained males during resistance training. The HR was significantly higher at rest and bench press, in the treatment compared to PLA group (+10 beats/min). No difference was detected for DBP for time or treatment. SBP was

significantly increased in both the caffeine and PLA treatments, as a result of an acute bout of resistance exercise (+8-10 mmHg).

There is a wealth of research showing that the ingestion of ED containing caffeine can benefit cognitive performance, particularly alertness and vigilance, mood, and perception of fatigue [88-92]. In addition, many athletes believe supplementation prior to training will result in greater focus, quicker reaction time, and increased power [24, 93, 94]. It is possible that the addition of caffeine as a psychopharmacologically active substance affects memory processes even under non-demanding task conditions [24, 95]. Smith and Rogers [38] studied the effects of 0, 12.5, 25, 50, and 100mg caffeine on cognitive performance, mood and thirst in adults with low and moderate to high habitual caffeine intakes. Effects on performance and mood confirmed psychostimulant action of caffeine. All doses of caffeine significantly affected cognitive performance ($p < 0.05$). They concluded that after overnight caffeine abstinence, caffeine can significantly affect cognitive performance, mood, and thirst at doses within and even lower than the range of amounts of caffeine contained in a single serving of popular caffeine-containing drinks. Giles et al. [96] studied the effects of caffeine, taurine, and glucose alone and in combination on cognitive performance and mood in 24-hrs caffeine-abstained habitual caffeine consumers. Using a randomized, double-blind, mixed design, habitual caffeine consumers who were 24-hrs caffeine deprived received one of four treatments (200mg caffeine/0mg taurine, 0mg caffeine/2,000mg taurine, 200mg caffeine/2,000mg taurine, 0mg caffeine/0mg taurine), on each of four separate days, separated by a 3-day washout period. Between-participants treatment was

a glucose drink (50g glucose, PLA). Salivary cortisol, mood, and HR were measured. An attention task was administered 30-min post-treatment, followed by a working memory and reaction time task 60-min post-treatment. Caffeine enhanced executive control (caffeine + taurine = 84.2 ± 26.1, caffeine = 87.4 ± 26.5, taurine = 96.3 ± 30.3, PLA = 97.1 ± 22.3) and reduced simple and choice reaction time. Taurine increased choice reaction time but reduced reaction time in the working memory tasks. Glucose alone slowed choice reaction time (caffeine + taurine = 181.3 ± 13.6, caffeine = 185.7 ± 14.3, taurine = 195.1 ±17.1, PLA = 213.8 ± 19.9). Glucose in combination with caffeine, enhanced object working memory and in combination with taurine, enhanced orienting attention (caffeine + taurine = 44.0 ± 19.9, caffeine = 29.7 ± 14.2, taurine = 39.4 ± 17.7, PLA = 36.0 ± 13.6). No effects were found for salivary cortisol or HR. Caffeine, not taurine or glucose, was likely responsible for reported changes in cognitive performance following consumption of ED.

Some studies reported that caffeine had no effect on exercise performance. For example, Paton et al. [97] investigated the effect of caffeine ingestion on performance in a test that simulated the repeated sprints of team sports. In a randomized double-blind cross-over experiment, team-sport athletes ingested either caffeine ($6mg/kg^{-1}$) or a PLA 60 min before performing a repeated 20-m sprint test. The observed effect of caffeine ingestion on mean sprint performance and fatigue over 10 sprints was negligible. Glaister et al. [98] evaluated the effects of caffeine supplementation on sprint cycling performance and to determine if there was a dose-response effect using a randomized, double-blind, placebo-controlled design. Subjects ingested caffeine or PLA 1 hr prior to

each exercise performance. To examine dose-response effects, caffeine doses of 2, 4, 6, 8, and 10mg/kg were used. The results of this study show that caffeine supplementation has no effect on short-duration sprint cycling performance, irrespective of the dosage used.

While numerous studies have supported the efficacy of caffeine supplementation for delaying fatigue [44], strength [36], power [7], aerobic performance [83, 99], and cognitive function [95, 96, 100], there have been conflicting results. Based on the literature, the doses used in our study would seemingly affect exercise performance [61, 101] and/or cognitive function [102, 103].

Creatine

Table 2.2 provides the summary of some studies investigating the effects of creatine (Cr) supplementation. In the present study, we planned to use 1.34g/day and 2.01g/day Cr nitrate in two doses of PWS. Cr is a nitrogenous amino acid derivative naturally found in skeletal muscle, brain, testes, and other organs [104]. Human body can endogenously synthesize Cr from three amino acids–glycine, arginine, and methionine, primarily in the liver, pancreas, and kidneys [105]. Animal products such as fresh fish and meat and various synthetic Cr supplements are sources of dietary Cr. Approximately, 90-95% of Cr in the body is stored in the skeletal muscle, where it serves as an energy source and a small amounts found in the brain and testes (~5%) [106]. In an average 70 kg adult, the total Cr pool in the body amounts to about 120g; this pool is subject to continuous degradation to creatinine, which is excreted in

Table 2.1 Effect of Caffeine Supplementation

Study (year)	Design	Population	Protocol	Diet control	Dosage/Duration	Additional supplements	Measurements	Major Findings
Ivy et al. [82] (2009)	Randomized, double-blind, cross-over	6 male (23 yr) and 6 female (27 yr) trained cyclists	60 min of cycling at 70% W_{max}	No	160mg taken 45 min before exercise	Niacin, carbohydrate, taurine, glucuronolactone vitamins B_6 and B_{12}	Ventilation, $\dot{V}O_2$, $\dot{V}CO_2$ production, and RER	Improved endurance performance
Graham et al. [83] (1998)	Double-blind, placebo-controlled	9 endurance runner young males (21-47 yr)	VO_2 max test and 20–30 min run at 85% (5 trials) in a separate day	No	4.45mg/kg taken 60 min before exercise	No	VO_2 max and blood variables	No effect on caffeine bioavailability. No increase in endurance
Beck et al. [36] (2006)	Randomized, double-blind, placebo-controlled, parallel design	37 resistance-trained males (20 ± 2 yr)	Wingate test, strength tests	No	201mg taken 60 min before exercise	Vitamins B_6, C, niacin, pantothenic acid	Anaerobic capacity, upper and lower body strength, peak power	Increase in bench press lRM, No effect on leg extension l-RM, leg extension total volume, bench press total volume, peak power, and mean power
Daniels et al. [86] (1998)	Double-blind, placebo-controlled	10 trained cyclists, 3 males, 7 females (30 ± 0.3 yr)	55 min of cycle ergometry at 65% of VO_2 max (4 trials)	No	6mg/kg taken either 45 min or 100 min before exercise	No	Hemodynamic and blood variables	Alter in cardiovascular response to dynamic exercise in a manner that may modify regional blood flow and conductance
Astorino et al. [60] (2010)	Randomized, single-blind, counterbalanced, cross-over	15 active males (26 ± 3 yr)	Maximal knee extension and flexion of the dominant leg (3 trials separated by 48 h)	No	2 or 5mg.kg taken 60 min before exercise	No	Knee extension/flexion torque, power, and total work	Ergogenic for maximal knee extension/ flexion exercise
Smith and Rogers [38] (2000)	Double-blind, within-subjects, placebo-controlled	11 males and 12 females (18-56 yr)	Two performance tests, a long duration simple reaction time task and a rapid visual information processing task, and a mood questionnaire	No	5 treatments (0, 12.5, 25, 50, and 100mg caffeine) administered once per week	No	Cognitive performance, mood, and thirst	Improved cognitive performance, mood, and thirst at doses within and lower than range of amounts of caffeine
Giles et al. [96] (2012)	Randomized, double-blind, mixed design	18 males and 30 females (20 ± 1 yr)	Multiple measures of caffeine withdrawal symptoms, mood, and cognitive performance using questionnaires	No	One of four treatments (200mg caffeine/0mg taurine, 0mg caffeine/2,000mg taurine, 200mg caffeine/2,000 mg taurine, 0mg caffeine/0mg taurine), on each of four separate days, 60 min before test	Taurine	Executive control, working memory, and reaction time	Improved cognitive performance
Paton et al. [97] (2001)	Randomized, double-blind, cross-over	16 male sport-science students (22 ± 3 yr)	10 sprints, each performed within 10 sec, followed by rest for remainder of each 10 sec	No	$6mg \cdot kg^{-1}$, 60 min before performing the test	No	Exercise performance	Caffeine ingestion on mean sprint performance and fatigue was negligible
Glaister et al. [98] (2012)	Randomized, double-blind, placebo-controlled	17 well-trained men (24 ± 6 yr)	7 maximal 10-sec sprint trials on a cycle ergometer	No	2, 4, 6, 8, and 10mg/kg, 60 min before exercise	No	Exercise performance and blood variables	No effect on short-duration sprint cycling performance, irrespective of the dosage used

the urine at a rate of about 2g/day. Replenishment of Cr at a similar rate is achieved by a combination of dietary intake and endogenous synthesis. Once in muscle cells, nearly 65% of Cr is phosphorylated to produce phosphocreatine (PCr), while the remaining content of Cr is stored as free Cr [107]. PCr as a high energy compound can donate a phosphate group to adenosine diphosphate (ADP) to create adenosine triphosphate (ATP) via the enzymatic reaction of creatine kinase (CK). ATP is the initial source of energy for muscle contraction [108].

Cr obtained either from endogenous synthesis or by ingestion of Cr-containing food is transported into muscle, brain, heart, and many other tissues with high and fluctuating energy demands by a specific Cr transporter [109]. Imported Cr is charged to the high energy compound PCr by the action of either strictly soluble, cytosolic CK, by CK coupled to glycolysis or by mitochondrial CK coupled to oxidative phosphorylation. In a resting cell, this results, at equilibrium, in a distribution of the total Cr pool into approximately two-thirds (PCr) and one-third (Cr) and in a very high ATP/ADP ratio (≥ 100:1). A fraction of cytosolic isoforms of CK are specifically associated with ATP-consuming processes such as the myofibrillar actomyosin ATPase, the sarcoplasmic reticulum Ca^{2+}-ATPase, the plasma membrane Na^{+}/K^{+}-ATPase, and the ATP-gated K^{+}-channel or ATP-requiring constituents for cell signaling [108].

Within these functional micro-compartments, CK regenerates the utilized ATP, drawing from the large PCr pool. These micro compartments with associated CK represent the ATP/PCr-consuming side of the CK/PCr system. At the ATP/PCr-generating side of the system, there are the glycogenolytic/glycolytic CK-g micro

compartments and the mitochondrial CK micro compartment connected to oxidative phosphorylation and energy channeling reactions inside the mitochondrion [110]. Mitochondrial CK is located in the intermembrane space of mitochondria with preferential access to ATP generated by oxidative phosphorylation via adenine nucleotide translocator of the mitochondrial inner membrane. This mitochondrial ATP is trans-phosphorylated into PCr that then leaves the mitochondria. This route of ATP generation is most important for refilling the PCr energy store in oxidative tissues, e.g. upon extensive stimulation of muscle contraction and thus is relevant for recovery after exhausting exercise [108].

Goldberg and Bechtel [111] evaluated speed, strength, and power before, during, and after a low dose (3g/day) of Cr or PLA supplementation among trained athletes involved in off-season weight training program. The varsity football and track athletes ingested three gel capsules, each containing 1g Cr or PLA once a day, for 14 consecutive days. Participants were tested on day 0, 7, and 14 for 1RM bench press, vertical jump, 40-yard sprint, leg sled, and leg extension. Results from this study suggested that 3g/day of oral Cr supplementation in trained athletes over a 14-day period can improve vertical jump but no other activities. Harris et al. [112] examined two doses of Cr to determine whether Cr supplementation can increase in the total Cr pool in muscle. An additional effect of exercise upon uptake into muscle was also investigated. Low doses of Cr (1g or less in water) produced only a modest rise in the plasma Cr concentration, whereas 5g Cr resulted in a mean peak after 1 hr. Repeated dosing with 5g every 2 hrs sustained the plasma concentration at around 1,000μmol/l. Supplementation with 5g of Cr four or six

times a day for 2 or more days resulted in a significant increase in total Cr content of the quadriceps femoris muscle. Thompson et al. [113] examined the effect of a relatively low dose of Cr (2g/day) during a six weeks course on skeletal muscle metabolism and oxygen supply in a group of training athletes. There was no effect of Cr on metabolite ratios at rest or on metabolism during exercise and recovery from exercise. Muscle oxygen supply and exercise performance were not improved by Cr when compared to PLA treated subjects. They concluded that oral Cr supplementation at 2g daily has no effect on muscle Cr concentration, muscle oxygen supply or muscle aerobic or anaerobic metabolism during endurance exercise.

It seems that chronic supplementation with Cr at low-dose might cause ergogenic effects. For example, Rawson et al. [114] examined the effects of low-dose Cr supplementation (2.3g/day) on body composition, muscle function, and body Cr retention for 6 weeks in a double-blind placebo-controlled fashion. Participants were tested before supplementation and retested after supplementation. Testing included body composition, maximal strength, muscle fatigue, and plasma Cr concentration. The results from this study indicated that chronic ingestion of a low-dose of Cr significantly increased plasma Cr concentration and enhanced resistance to fatigue during repeated bouts of high-intensity contractions. Based on the literature, the doses used in our study would seemingly have little to no ergogenic effect.

Nitrate

In the present study, we planned to use 660mg and 990mg nitrate in two doses of PWS. Nitrate (NO_3^-) is a naturally occurring anion in the human body with an inert

oxidative end product of endogenous nitric oxide (NO) metabolism [115]. Nitrate may be reduced to nitrite and NO in vivo, particularly in environments of hypoxia and acidosis [46]. The physiological actions of nitrate are reduction of BP [116], improvement of vascular compliance [117], and attenuation of oxidative stress [118] following consumption. Beetroot juice (BRJ) contains high amounts of nitrate (>250mg/100g of fresh weight), among other foods rich in nitrate include spinach, lettuce, celery, and carrot juice [119]. Circulating nitrate can be reduced to nitrite by commensal bacteria in the oral cavity and by specific enzymes (e.g., xanthine oxidase) within tissues. Nitrite can be metabolized by mitochondria to form NO in competition with oxygen [120]. NO is a signaling molecule that can diffuse through biological membranes. NO can be formed in the endothelium by NO synthase, which stimulates vasodilatation by interacting with vascular smooth muscle resulting in increased blood flow [121, 122]. BRJ would seem likely to improve markers of exercise performance. The impact of BRJ ingestion on power output, VO_2, and cycling time trial performance was investigated by Lansley et al. [57] using competitive cyclists who consumed either 0.5L BRJ (384mg of NO_3^-) or PLA containing nitrate-depleted BRJ (0.29mg of NO_3^-) before each time trial of 4 km or 16 km. BRJ consumption increased plasma nitrite by 138% and resulted in significantly reduced time to completion and increased power output during both the 4 km (2.8% and 5%, respectively) and 16 km time trials (2.7% and 6%, respectively) compared to PLA. Bailey et al. [51] supplemented healthy men with 0.5L of BRJ (341mg of NO_3^-) or a low-calorie blackcurrant juice cordial (negligible NO_3^- content) for 6 days, and they performed moderate (80% gas exchange

threshold) and intense cycling (70% of the difference between the power output at the gas exchange threshold and VO_2 peak) protocols during the last 3 days. BRJ ingestion increased the average plasma nitrite by 96% and reduced muscle deoxyhemoglobin amplitude by 13%, suggesting that fractional oxygen extraction was reduced. In addition, BRJ consumption reduced the amplitude of the VO_2 slow component, defined as a delayed onset of VO_2 consumption during high intensity exercise. The authors concluded that increased dietary inorganic nitrate consumption from BRJ has the potential to improve high-intensity exercise tolerance.

Only few studies have been published that investigated the effect of BRJ on resistance exercise performance [123-125]. The results of these BRJ studies are equivocal, with the overarching theme being a moderate increase in resistance exercise performance. Bailey et al. [49] recruited recreationally active men to consume either 0.5L/day of BRJ (316mg NO_3^-) or a PLA (blackcurrant juice cordial with negligible NO_3^- content) for 6 days. During the last 3 days of supplementation, participants completed low and high (15% and 30% maximal voluntary isometric contractions, respectively) intensity "step" knee extension tests. The increase in pulmonary VO_2 from rest to low-intensity exercise was reduced by 25% following dietary nitrate supplementation. Furthermore, BRJ ingestion lead to a 36% reduction in the amount of PCr degraded during low-intensity exercise (knee extensions) and a 59% reduction during high-intensity exercise compared to PLA. These reductions in PCr usage were accompanied by a reduction in the total ATP utilization during both high and low-density exercise. Several studies indicated either an improvement in exercise

performance or exercise efficiency resulting from an acute dose of nitrate, 75-150 min before exercise, suggesting effects may occur in a relatively short time frame [57, 126, 127]. Nitrate has been prescribed in absolute (300–600mg) and relative (6.2mg/kg) contents. In relative terms, a 70 kg person prescribed 6.2mg/kg would ingest approximately 435mg nitrate [46, 120, 128]. Studies have demonstrated that chronic (3-15 day) and acute BRJ intake (e.g., ~300mg, 2-2.5 hrs prior to exercise) are associated with a consistent enhancement of exercise economy [129]. Therefore, it is likely that the doses used in our study would seemingly have an ergogenic effect.

β-Alanine

Table 2.3 provides the summary of some studies investigating the effects of β-alanine supplementation. In the current study, we planned to use 3.2g/day and 4.8g/day β-alanine in two doses of PWS. β-alanine is a non-proteogenic amino acid that is produced endogenously in the liver. The binding of β-alanine to histidine is the rate-limiting step in the formation of carnosine within muscle fibers [130]. Carnosine (β-alanyl-L-histidine) is a cytoplasmic dipeptide, which plays a role in the intracellular buffer of hydrogen ions. Carnosine is found in high concentrations in the skeletal muscle of both vertebrates and non-vertebrates as well as in the central nervous system [131]. In human blood, carnosine is rapidly hydrolyzed to its constituent amino acids due to the presence of carnosinase, a specific hydrolyzing enzyme. Following this, β-alanine and histidine can then be transported to other organs and tissues. Whilst the carnosinase enzyme has been identified in several tissues including the blood, liver and kidney;

Table 2.2 Effect of Creatine Supplementation

Study (year)	Design	Population	Protocol	Diet control	Dosage/Duration	Additional supplements	Measurements	Major Findings
Goldberg and Bechtel [111] (1997)	Randomized, double-blind, placebo-controlled	34 varsity football and track athletes (18-22 yr)	1RM bench press, vertical jump, 40-yard sprint, leg sled using twice body weight and leg extension	No	3g/day before and during for 14 consecutive days	No	Exercise performance	Anaerobic performance was enhanced in the vertical jump but not in other activities
Harris et al. [112] (1992)	Randomized, dose dependent	5 females and 12 males (20-62 yr)	60 min hard exercise per day using one leg	No	Two doses, low dose (1g or less), high dose (5g)	No	Needle biopsy, blood variables, exercise performance	Supplementation with 5g resulted in a significant increase in total Cr content of the quadriceps femoris muscle
Thompson et al. [113] (1996)	Randomized, placebo-controlled	10 female athletes	Plantar flexion of the right ankle, lifting a weight of 10% lean body mass a distance of 7 cm at a rate of 30 min^{-1}	No	2g/day for 6 weeks	No	Muscle biopsy	No effect on muscle creatine concentration, muscle oxygen supply or muscle aerobic or anaerobic metabolism
Rawson et al. [114] (2011)	Randomized, double-blind, placebo-controlled	8 females, 12 males (21 ± 2 yr)	Three-repetition maximum concentric knee extension test at 180 degrees/sec and five sets of 30 concentric knee extensions at 180 degrees/sec with a 1 min rest between sets	No	~ 2.3g/day for 6 weeks	No	Blood variables, Exercise performance	Significant increase in plasma Cr concentration and enhanced resistance to fatigue during repeated bouts of high-intensity contractions

critically it is not found in skeletal muscle. Therefore, oral carnosine supplementation is an inefficient method of augmenting muscle carnosine levels in humans, as ingested carnosine is ultimately metabolized before reaching skeletal muscle [132, 133]. β-alanine as the rate-limiting precursor to carnosine synthesis has been shown to augment carnosine and may therefore improve exercise performance [134].

It typically takes a number of weeks (at least 2 weeks) for β-alanine supplementation to yield meaningful increases in muscle carnosine content. As such, it is unlikely that β-alanine is the primary ingredient improving performance outcomes in studies utilizing acute, one-time supplementation [132]. For example, Glen et al. [135] evaluated the effects of acute β-alanine supplementation (1.6g) on anaerobic performance among athletes in a randomized, double-blind study. Participants completed two supplement trials: 1) PLA (34g dextrose) and 2) β-alanine (1.6g β-alanine + 34g dextrose). Thirty minutes after supplementation, participants performed three repeated Wingate anaerobic capacity tests with 2 min of active rest after each. Fatigue index, mean power, and peak power were measured during each Wingate test. Lactate, HR, and rating of perceived exertion were measured at rest, immediately after each Wingate test, and after each active rest period. Findings suggested that an acute dose of β-alanine decreased rating of perceived exertion during anaerobic power activities in athletes; however, no significant supplementation effect was observed for any performance or physiological variable. Invernizzi et al. [136] investigated the acute effects of carnosine and β-alanine ingestion on anaerobic intermittent running performance on healthy, young, active males in a randomized, cross-over design. Participants performed running-

based anaerobic test twice (with 30 min recovery in between) on two separate occasions. The test consisted of 6 × 35-m sprints interspersed with 10 sec rests after acute ingestion (4 hrs before the test) of either 2g L-carnosine + 2g β-alanine or PLA. The overall performance decreased (test 1 vs test 2, carnosine + β-alanine: 32.8 ± 1.3 sec, 33.4 ± 1.2 sec; PLA: 32.9 ± 1.0 sec, 33.6 ± 1.2 sec), pain after the test increased almost in the same way in both groups, and rating of perceived exertion did not show any difference. In conclusion, these findings suggest that acute administration of carnosine + β-alanine might have a very small enhancing effect on anaerobic sprint performance.

In studies extending over 4 to 8 weeks, the likelihood of β-alanine contributing to improvements in performance is greater [132]. For example, Smith et al. [137] evaluated the effects of combining β-alanine supplementation with high-intensity interval training on endurance performance and aerobic metabolism in recreationally active college-aged men. All subjects supplemented four times per day (total of 6g/day) for the first 21-days, followed by two times per day (3g/day) for the subsequent 21 days. The results indicated that β-alanine supplementation in combination with high-intensity interval training resulted in significant improvements in VO_2 peak and time to fatigue during exercise. Kern et al. [130] studied the effects of β-alanine supplementation (4g/day) on performance for 8 weeks in collegiate wrestlers and football players. Performance improvements were greatest in the football players' supplement group, decreasing 300 shuttle time by 1.1 sec (vs. 0.4 sec PLA) and increasing flexed-arm hang (3.0 vs. 0.39 sec). They concluded that β-alanine has the ability to improve performance in previously

trained athletes. Based on the literature, it is not likely that β-alanine supplementation for a 7-day period to have an ergogenic effect.

L-Arginine

Table 2.4 provides the summary of some studies investigating the effects of L-arginine supplementation. In the present study, we planned to use 2g/day and 3g/day L-arginine in two doses of PWS. The semi-essential amino acid L-arginine is critical factor in a variety of physiological conditions. L-arginine is available in several nutritional supplements and food such as nuts, fish, pork, chicken, dairy products, and egg. It is one of the building blocks of proteins in the body. Among many important proteins in which L-arginine is included are hormones, histones, collagen, and intracellular structural proteins. It serves as a substrate for Cr, urea, and NO synthesis. L-arginine regulates interorgan metabolism of energy substrates and the function of multiple organs. L-arginine is not considered as an "essential" amino acid since human body can synthesize it "de novo" from glutamine, glutamate, and proline. This means the body produces adequate functional contents on its own to meet most normal physiological demands; however, during times of stress or increased demand, for example, in exercise or disease, further amounts of L-arginine is needed from external dietary sources [138-142]. Wax et al. [143] examined the efficacy of acute ingestion of L-arginine on muscular strength and endurance in resistance trained and untrained men in a randomized, double-blind cross-over design. Participants ingested either 3g of L-arginine or PLA (microcrystalline cellulose), 45 min prior to a resistance exercise protocol. The results from this study indicated that acute L-arginine supplementation provides no ergogenic benefit on 1RM

Table 2.3 Effect of β-Alanine Supplementation

Study (year)	Design	Population	Protocol	Diet control	Dosage/Duration	Additional supplements	Measurements	Major Findings
Glen et al. [135] (2015)	Randomized, double blind, placebo-controlled, cross-over	12 trained, competitively active female cyclists (26 ± 1 yr)	3 repeated Wingate cycling tests with 2 min of active rest after each	No	1.6g, 30 minutes before exercise	No	Exercise performance, HR, blood variables, rating of perceived exertion	Acute dose of β-alanine decreased rating of perceived exertion during anaerobic power activities. No effects on any performance or physiological variable
Invernizzi et al. [136] (2013)	Randomized, double blind, counterbalanced, cross-over, placebo-controlled	12 healthy males (21 ± 4 yr)	6 × 35-m sprints interspersed with 10 sec rests	No	2g, 4 hrs before the test	L-carnosine Carnosine	Exercise performance, blood variables,	Acute ingestion of carnosine + β-alanine did not influence the cytokine response to exercise but had a very small enhancing effect on anaerobic sprint performance
Smith et al. [137] (2009)	Randomized, placebo-controlled, double-blind	46 recreationally active males (22 ± 2 yr)	Four, 2-min work bouts on a cycle ergometer	No	First 3 weeks: 6g/day, second 3 weeks: 3g/day	No	VO_2 Peak, time to fatigue, ventilatory threshold, total work done	Improved high intensity interval training, endurance performance
Kern et al. [130] (2011)	Placebo-controlled, double-blind	37 male collegiate athletes (19 ± 1 yr)	Timed 300-yd shuttle, 900 flexed-arm hang	Yes	$4g/day^{-1}$ for 8 weeks	Vitamin E	Blood variables body composition	Improvement in sprinting time, but no sufficient power to show significant benefits

or total load volume as measured by bench press and leg press, regardless of the participants' training status. Olek et al. [144] assessed the effect of an acute dose of L-arginine in a low dose (2g) 60 min before exercise. They showed that this amount of L-arginine did not induce any increase in the total work performed or mean power output during Wingate cycle tests (30 sec), or VO_2 either. Additionally, plasma levels of nitrate/nitrite were unchanged after L-arginine supplementation compared to PLA. Schaefer et al. [145] investigated the acute effect of L-arginine supplementation (3g) on physiological and metabolic changes during exercise. The results indicated that the specific L-citrulline increase and the inverse relationship observed between L-citrulline and plasma lactate after L-arginine might support that L-arginine supplementation enhances the L-arginine-NO pathway during exercise. Nagaya et al. [5] assessed the effects of L-arginine supplementation (0.5g/10 kg body weight) for 7 days on hemodynamics and exercise capacity in patients with pulmonary hypertension. These results indicated that oral supplementation of L-arginine produced a 9% decrease in mean pulmonary arterial pressure and a 16% decrease in pulmonary vascular resistance. They concluded that L-arginine supplementation may have beneficial effects on hemodynamics and exercise capacity. Zajac et al. [146] investigated the effects of L-arginine (3g) plus L-ornithine (2.2g) and resistance exercise for 3 weeks on serum biomarkers including growth hormone and insulin-like growth factor-1. Resting hormonal levels did not differ, but increases in growth hormone and insulin-like growth factor-1 were observed following exercise and after 1 hr of recovery, suggesting that the combination of L-arginine–L-ornithine supplements has potential ergogenic effect.

Table 2.4 Effects of L-Arginine Supplementation

Study (year)	Design	Population	Protocol	Diet control	Dosage/Duration	Additional supplements	Measurements	Major Findings
Wax et al. [143] (2012)	Randomized, double-blind, placebo-controlled, cross-over	8 resistance trained and 8 untrained healthy males	60 % 1RM repetition to failure on bench press and leg press	No	3g single dose taken before exercise	No	Exercise tests	Acute ingestion of L-arginine provided no ergogenic benefit on 1RM or total load volume
Olek et al. [144] (2010)	Randomized, double-blind, placebo-controlled	16 healthy active subjects	Three 30 sec all-out supramaximal Wingate tests with 4 min rest between tests	No	2g single dose taken 60 min before exercise	No	Blood variables and exercise test	Neither induced effect on NO concentration, nor improved physical performance
Schaefer et al. [145] (2002)	Randomized, double-blind, placebo-controlled, cross-over	8 healthy men (29 ± 4 yr)	Two tests including 2-min period at 50 W, then workload increased by 25 W each 2 min to reach the maximal power output	No	3g single dose taken 60 min before exercise	No	Hemodynamic, respiratory, blood variables, and exercise test	Intravenous L-arginine reduced significantly exercise-induced increase in plasma lactate and ammonia
Nagaya et al. [5] (2001)	Randomized, double-blinded	4 men and 15 women with pulmonary hypertension; (49 ± 4 yr)	Cardiopulmonary exercise tests before and 1 week after supplementation	No	1.5g/10 kg body weight taken for 1 week	No	Blood variables and cardiopulmonary exercise tests	Beneficial effects on hemodynamics and exercise capacity in patients with precapillary pulmonary hypertension
Zajac et al. [146] (2010)	Randomized, double-blind, placebo-controlled	17 strength-trained male athletes (22 ± 1 yr)	6 weeks of high volume and 3 weeks of high-intensity strength training	No	3g twice daily taken for 3 weeks	L-ornithine	Blood variables and exercise tests	Elevations in growth hormone and insulin like growth factor-1 in response to an acute resistance exercise test after a short-term resistance training program

Based on the literature, the doses used in our study would seemingly have little to no ergogenic effect.

N-Acetyl Tyrosine

In this study, we planned to use 300mg/day and 460mg/day N-acetyl tyrosine in two doses of PWS. N-acetyl tyrosine is a non-essential amino acid that is used by cells to synthesize proteins. The physiological basis of tyrosine's beneficial effects on cognitive function is attributed to its role as a precursor for catecholamine neurotransmitters, dopamine, and norepinephrine. [147]. These neurotransmitters have a key role in a variety of stress-related behaviors. Norepinephrine as a neurotransmitter has shown to be critical for modulating the central stress response [148]. During acute stress, norepinephrine is depleted, and when further substrate in the form of tyrosine is available, the release of norepinephrine increases [149, 150]. Tyrosine influences the same neurotransmitter system as the amphetamines that are effective performance-enhancing compounds, yet they cause several side effects [149]. Acute tyrosine ingestion is thought to improve aerobic endurance, muscle strength and endurance, and anaerobic power of individuals undergoing severe physiologic stress [151]. In addition, tyrosine has the potential as an acute treatment to prevent stress-related decline in cognitive function [152]. For example, Deijen and Orlebere [153] evaluated the effect of tyrosine on mood, cognitive function, performance, and hemodynamics under stress. Participants were tested on two separate days, one test session after ingestion of 100mg/kg tyrosine and the other test session after PLA in a random order. While performing a number of stress sensitive tasks, volunteers were exposed to a noise stressor. Tyrosine was found to

improve the performance on two cognitive tasks which were performed 60 min after administration of the medication and which could be characterized as highly sensitive to stress. In addition, tyrosine decreased DBP 15 min after ingestion, while 60 min after ingestion DBP was the same with tyrosine and PLA. No effects on mood, SBP, and HR were found. Grevet et al. [154] evaluated the effects of acute phenylalanine and tyrosine depletion on memory, attention, and mood in normal subjects in a randomized, double-blind, and cross-over study. Participants ingested a nutritionally balanced mixture or a similar mixture deficient in phenylalanine and tyrosine. Before and 5 hrs after ingestion of the drink, volunteers underwent tests on mood, memory, and attention. Results of the memory tests showed that the mixture deficient in phenylalanine and tyrosine impaired word recall. The assessment of changes in mood showed that the balanced mixture improved scores of as alertness and the mixture deficient in phenylalanine and tyrosine induced an opposite effect, increased scores of anxiety. These results suggested that tyrosine plasma levels and catecholamines may be important factors in regulating mood and memory. Based on the literature, the doses used in our study would seemingly have little to no ergogenic effect.

Pterostilbene

In the current study, we planned to use 12.5mg/day pterostilbene in PWS150. Pterostilbene (trans-3, 5-dimethxy-4-hydroxystilbene) is a stilbenoid chemically similar to resveratrol found in blueberries and grapes. It is closely related structurally to resveratrol which is a naturally occurring dimethyl ether analogue of resveratrol and shows many of the same characteristics as well as its own unique therapeutic potential

[155]. Pterostilbene has shown to have several pharmacological benefits for the prevention and treatment of many diseases such as cancer [156], diabetes [157], cardiovascular degeneration [158], and pain [159]. Antioxidant and anti-inflammatory effect of effects of pterostilbene are also shown [160]. The antioxidant activity of pterostilbene was demonstrated by methyl linoleate oxidation [160]. It prevents generation of hydroxyl radicals [161]. In addition, pterostilbene downregulates inflammatory tumor necrosis alpha, interleukin-6, cycloogygenase-2, inducible NO synthase, interleukin-1β, monocyte chemotactic protein-1, C-reactive protein, and plasminogen activator inhibitor-1 expression by inhibiting the activation of nuclear factor kappa B [162]. Pterostilbene has also shown to decrease BP, cholesterol, and blood glucose, reverse cognitive decline and it has shown anticancer effects in rats [163-165]. Human studies using up to 250mg/day have shown the decrease in BP and body weight [166, 167]. Apparently, to enhance glycogen replenishment, pterostilbene is included in the ingredient profile. Pterostilbene appears to increase insulin sensitivity by enhancing hepatic enzymes associated with glucose uptake [168]. Based on our knowledge, to date no study has evaluated the impact of pterostilbene supplementation on exercise performance, exercise-induced oxidative stress and inflammatory response in both trained and sedentary subjects. There is no established recommended dose for pterostilbene. Therefore, it not clear whether the doses used in our study would be effective or not.

Theacrine

In this study, we planned to use 10mg/day and 20mg/day theacrine in two doses of PWS. Theacrine (TeaCor™ Tetramethyluric acid) is a purine alkaloid metabolite of caffeine produced as plants mature from young (stage 1) to a more mature plant (stage 3) [169]. Abundantly present in *Camellia assamica var. kucha*, theacrine has been shown to increase excitability and locomotor activity in rats similar to caffeine [170, 171]. However, theacrine appears to affect the central nervous system different to caffeine. Xu et al. [171] indicated that theacrine, co-administered with pentobarbital, significantly prolonged sleep time in rats while caffeine and theobromine exhibited the opposite effect. Likewise, Wang et al. [172] demonstrated that theacrine has anti-inflammatory and analgesic effects, where caffeine does not. Habowski et al. [173] examined the effects of theacrine on subjective measures of cognitive function, psychometric indices. The 200mg dose of theacrine caused significant improvements in energy (+8.6% vs. -5.7%) and reductions in fatigue (-6.7% vs. +5.8%). The cohort study demonstrated moderate to large effect sizes (0.50-0.71) with the supplement over a 7-day period of assessment for the following subjective measures: energy, fatigue, concentration, anxiety, motivation to exercise and libido. Tylor et al. [174] investigated the safety and non-habituating effects of TeaCrine®, (200mg/day) a nature-identical, chemically equivalent bioactive version of theacrine. Results indicated that all values fell within normal limits and no group × time interactions were noted for clinical safety markers. No evidence of habituation was noted as day 0 values for energy, focus, concentration, anxiety, motivation to exercise, and Profile of Mood States remained stable in subjects

across the 8-week study protocol. Theacrine would act in a similar manner to caffeine on exercise performance when administered in the dose of ≥ 200mg [175]. Based on the literature, the doses used in our study would seemingly have little to no ergogenic effect.

Niacinamide

In this study, we planned to use 60mg/day and 90mg/day niacinamide in two doses of PWS. Niacinamide, also known as nicotinic acid amide, is the amide of nicotinic acid (vitamin B_3/niacin). Niacinamide is a water-soluble B complex vitamin [176]. In cells, niacin is incorporated into nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate [177]. Murray et al. [178] assessed how selected physiological and performance responses are affected when the normal increase in plasma free fatty acid concentration during exercise is blunted by nicotinic acid. The result of the study indicated that blunting the normal rise in free fatty acid alters the hormonal response to exercise and reduces the capacity to perform high-intensity exercise. On the contrary, Koh et al. [179] examined the responses of blood lipids and lipoproteins to extended-release niacin and exercise. They concluded that the combined effects of extended-release niacin and a single bout of exercise did not provide a synergistic effect on blood lipids and lipoproteins. Based on the literature, the doses used in our study would seemingly have little to no ergogenic effect.

Mucuna Pruriens

In this study, we planned to use 1g/day and 1.5g/day *Mucuna pruriens* in two doses of PWS. L-DOPA (L-3,4-dihydroxyphenylalanine), found within the seeds of *Mucuna pruriens*, is a naturally occurring non-protein amino acid, synthesized from L-

tyrosine. The most important function of L-DOPA is to act as the precursor to the neurotransmitter dopamine, as well as the catecholamines epinephrine and norepinephrine [180]. *Mucuna pruriens* is recognized as an aphrodisiac, and has been shown to improve mental alertness and coordination [181]. Shah and Goyal [182] investigated the neuropsychopharmacological effect of a polyherbal formulation supplement containing *Mucuna pruriens* and other ingredients (500mg/kg) on learning and memory processes in rats. The supplement activity on memory acquisition and retention was studied using passive avoidance learning and elevated plus maze model. The animals treated with polyherbal formulation supplement showed a significant decrease in transfer latency as compared to the control group. Polyherbal formulation supplement also produced significant improvement in passive avoidance acquisition and memory retrieval, as compared to the controls and reduced the latency to reach the shock free zone after 24 hours. The authors found that the supplement resulted in significant improvement in passive avoidance acquisition and memory retrieval in rats. Based on the literature, *Mucuna pruriens* supplementation at doses used in our study would seemingly have little to no effect on cognitive function.

Vitamin B_6

In this study, we planned to use 1.48mg/day and 2mg/day vitamin B_6 in two doses of PWS. Vitamin B_6 refers to all biologically active forms of vitamin B_6. The forms include pyridoxine, pyridoxal, pyridoxamine, pyridoxine phosphate, pyridoxal phosphate, and pyridoxamine phosphate. Of those, pyridoxine, pyridoxal, and pyridoxamine are the most common forms in foods [183]. A major function of vitamin

B_6 is the metabolism of proteins and amino acids. The most biologically active form of vitamin B_6 is pyridoxal 5'-phosphate which acts as a cofactor for transaminases, decarboxylases, and other enzymes used in the metabolic transformations of amino acids and nitrogen-containing compounds. During exercise, the gluconeogenic process involves the breakdown of amino acids for energy in the muscle and the conversion of lactic acid to glucose in the liver. Various pyridoxal 5'-phosphate-containing enzymes are involved in this metabolically driven conversion [184, 185]. Few studies have examined the vitamin B_6 status in athletes. Three studies indicated that 40% to 60% of athletes have reduced vitamin B_6 based on the enzyme stimulation test [186-188]. Only one study reported more than one indicator of B_6 nutriture, and it found less (17-35%) deficiency among the athletes than when only one indicator of status was used, indicating the need to use independent biochemical measures of B_6 nutritional status and dietary B_6 intakes to assess the interaction between diet and performance [186]. Coburn et al. [189] examined the influence of dietary B_6 in men. The men received a B_6-depletion diet (1.8μM of pyridoxine HCl) for 6 weeks followed by a self-selected diet supplemented with 980μM/d of pyridoxine HCl for 6 weeks. Depletion resulted in a significant decrease in plasma pyridoxal and pyridoxal phosphate concentrations and a significant increase in the enzyme stimulation test. Dietary B_6 did not affect muscle pyridoxal phosphate. Despite alterations in dietary B_6, skeletal muscle concentrations of B_6 are recalcitrant to depletion and plasma B_6 concentrations are a rapidly responding pool that may not reflect tissue stores. Based on the literature, vitamin B_6 used in our study would seemingly have little to no ergogenic effect.

Vitamin B_9

In this study, we planned to use 0.5mg/day and 0.76mg/day vitamin B_9 in two doses of PWS. Vitamin B_9 (Folate) serves as a coenzyme in single-carbon transfers in the metabolism of nucleic and amino acids. It is required for synthesis of purines and pyrimidines that are needed for DNA production and erythropoiesis. A deficiency of folate causes abnormal cell replication, particularly in the erythropoietic system, and results in megaloblastic anemia. This type of anemia also is caused by vitamin B_{12} deficiency. Supplemental folate in the presence of B_{12} deficiency can correct megaloblastic anemia but not the B_{12} deficiency [190, 191]. Singh et al. [192] found adequate serum folate concentrations in ultra-marathoners. Folate supplementation of folate-deficient, but not anemic, athletes did not improve physical performance. Female marathoners with low serum folate concentrations (<4.5ng/mL) supplemented with folate (5mg/day for 10 weeks) did not experience a significant improvement in treadmill performance, cardiorespiratory function, or metabolic response during exercise as compared to PLA treated, folate-depleted controls [193]. The lack of a physiologic benefit of folate supplementation contrasted with a significant increase in serum folate. Thus, changes in circulating folate may not reflect changes in tissue or cellular folate status [183]. Based on the literature, vitamin B_9 used in our study would seemingly have little to no ergogenic effect.

Vitamin B_{12}

In this study, we planned to use 1.8mg/day and 2mg/day vitamin B_{12} in two doses of PWS. Vitamin B_{12} (Cyanocobalamin) is a general term that describes a group of

cobalt-containing compounds, the corrinoids. This vitamin contains cobalamin with or without a cyanide group, both of which are biologically active for humans. Cyanocobalamin acts as a coenzyme for the methyl transfer reaction that converts homocysteine to methionine and another reaction that converts L-methyl-malonyl coenzyme A to succinyl coenzyme A. It also is required for normal erythrocyte production and neurologic function [191]. Cyanocobalamin is involved in carbohydrate metabolism and maintaining nerve cell transmission which is fundamental for muscle contraction, coordination, and muscle growth. In addition, cyanocobalamin is involved in the metabolism of every cell in the body, especially affecting DNA synthesis and regulation, fatty acid synthesis and energy production [194]. General cyanocobalamin supplementation apparently has no beneficial effect on performance. In well-nourished athletes, no ergogenic effect has been reported. Based on the literature, vitamin B_{12} used in our study would seemingly have little to no ergogenic effect.

Vitamin C

In this study, we planned to use 500mg/day and 760mg/day vitamin C in two doses of PWS. Vitamin C (ascorbic acid) is a micronutrient with numerous crucial physiological functions and acts well in the aqueous environment. It is found in high contents in leukocytes, adrenal and pituitary glands. In addition to scavenging of free radicals, vitamin C recycles the tocopherol radical, thereby being reduced to dehydroascorbic acid that can be regenerated, for example by glutathione-glutathione reductase system. This vitamin is also an enzyme cofactor in the biosynthesis of carnitine, histamine, collagen, norepinephrine, and epinephrine [195-197]. Some decades

ago, the intake of vitamin C was inefficient; however, nowadays, vitamin C intake has increased due to the abundance of fruits and vegetables. Vitamin C is enriched in every food, particularly meat-containing foods as antioxidant [198]. Pharmacokinetic data indicate a plasma steady state after the ingestion of 200mg vitamin C, whereas ascorbic acid contents of neutrophils, monocytes, and lymphocytes are saturated at a daily intake of 100mg [199]. Ascorbic acid has a multiplicity of antioxidant properties, but the high dose supplementation can exert pro-oxidant effects in vitro, usually by interaction with transition metal ions [200]. Based on the literature, vitamin C used in our study would seemingly have little to no ergogenic effect.

Summary

As it has been discussed, PWS supplementation is a relatively recent and growing area of research. PWS has shown to cause beneficial effects on cognitive function and high intensity exercise including anaerobic sprints and resistance training. Over time, these improvements could potentially increase training adaptations; future studies will assist further explanation of the precise effects of PWS and ED to increase alertness as well as improvement in athletic performance under a variety of conditions in both men and women. The main body of research has focused on the effects of either individual PWS or those containing a few ingredients in young athletes. It seems that studying the effects of multi-ingredient PWS can potentially open the door to more research of the effects in athletes. The two doses of PWS used in the present study contain caffeine and nitrate likely to affect cognitive and/or exercise performance. Other ingredients used in the supplement were lower than those recommended in previous

studies. Rather than offering a watered down product, the supplement provider company tries to realize if their product functions in light of having to make business/economic decisions on cost to benefit return ratios. Further research in the area of multi-ingredient PWS and ED may potentially promote more improvements in high intensity exercise, sport performance, and cognitive function in a wide range of individuals.

CHAPTER III

METHODS

Experimental Design

Table 3.1 presents the experimental design of the study and what was required by participants during each testing session throughout the 35 days. Participants repeated the sessions two times after 1-week washout while randomly ingesting the alternate supplements. Figure 3.1 outlines an individual testing session within the study. In a randomized, double-blind, placebo-controlled trial and cross-over manner, we examined the acute and after 7 days effects of consuming PWS containing caffeine, Cr nitrate, β-alanine, arginine α-ketoglutarate, N-acetyl tyrosine, *Mucuna pruriens*, and ascorbic acid, at two doses on cognitive function and exercise performance. The independent variable was the nutritional supplementation. The outcome variables were exercise performance including upper body and lower strength, and anaerobic power; resting energy expenditure responses; heart rate and blood pressure; readiness to perform; cognitive function; hematologic variables; general health markers; and side effect questionnaires.

Study Site

All laboratory testing took place in the Exercise & Sport Nutrition Laboratory. This laboratory is located in the Department of Health and Kinesiology and College of Education and Human Development at Texas A&M University in College Station, Texas.

Table 3.1 Experimental Design

Familiarization Session	Day 0	Day 2	Day 4	Day 7
Physical Exam Body Weight Body Composition 1RM[a] Determination on the Bench Press and Leg Press Anaerobic Sprint Practice Test Schedule Testing Refrain from exercise, caffeine and use of over-the-counter stimulants for 48-hours and fasted for 12 hours prior to days 0 and 7 testing sessions	Fasting Blood Sample Resting HR[b]/BP[c]/ECG[d] – 5 min.; start REE (0 min.) Ingest supplement in a randomized and counterbalanced manner (30 min.) Stop REE[e] (60 min.) Stroop Word & Color Test Ready to Perform VAS[f] Sleep Quality and Caffeine Questionnaires Resting HR/BP/ECG/BIA[g] – 5 min. Bench Press Warm-Up Bench Press (3 sets of 10 reps @ 70% of 1RM with 2-min rest btw. sets. Total reps to failure on last set) Resting HR/BP – 1 min after bench press 5 min. rest Leg Press Warm-up Leg Press (3 sets of 10 reps @ 70% of 1RM with 2-min rest btw. sets. Total reps to failure on last set) Resting HR/BP – 1 min after leg press 5 min. rest Wingate Anaerobic Capacity Test Resting HR/BP – 1 min after Wingate test Ingesting supplement for six additional days	Stroop Word & Color Test Ready to Perform VAS Sleep Quality and Caffeine Questionnaire	Stroop Word & Color Test Ready to Perform VAS Sleep Quality and Caffeine Questionnaire	Fasting Blood Sample Resting HR/BP/ECG – 5 min.; start REE (0 min.) Ingest supplement in a randomized and counterbalanced manner (30 min.) Stop REE (60 min.) Stroop Word & Color Test Ready to Perform VAS Sleep Quality and Caffeine Questionnaires Resting HR/BP/ECG/BIA – 5 min. Bench Press Warm-Up Bench Press (3 sets of 10 reps @ 70% of 1RM with 2-min rest btw. sets. Total reps to failure on last set) Resting HR/BP – 1 min after bench press 5 min. rest Leg Press Warm-up Leg Press (3 sets of 10 reps @ 70% of 1RM with 2-min rest btw. sets. Total reps to failure on last set) Resting HR/BP – 1 min after leg press 5 min. rest Wingate Anaerobic Capacity Test Resting HR/BP – 1 min after Wingate test 4 – Day Food Record-One week washout

[a] 1-Repetition Maximum, [b] Heart rate, [c] Blood pressure, [d] Electrocardiogram, [e] Resting Energy Expenditure, [f] Visual Analogue Scale,

[g] Bioelectrical Impedance Analyzer

Recruiting Methods

Recruitment fliers were posted around the Health & Kinesiology building as well as emailed to students enrolled in physical education activity classes. People expressing interest in the study were interviewed via phone screening or by on-site attendance to determine if they were qualified to participate. All eligible subjects participated in a morning FAM session with the investigator during which the study design, testing procedures, and supplementation protocols were explained in detail. This included a brief description of the blood collection procedure and a brief explanation of the tests

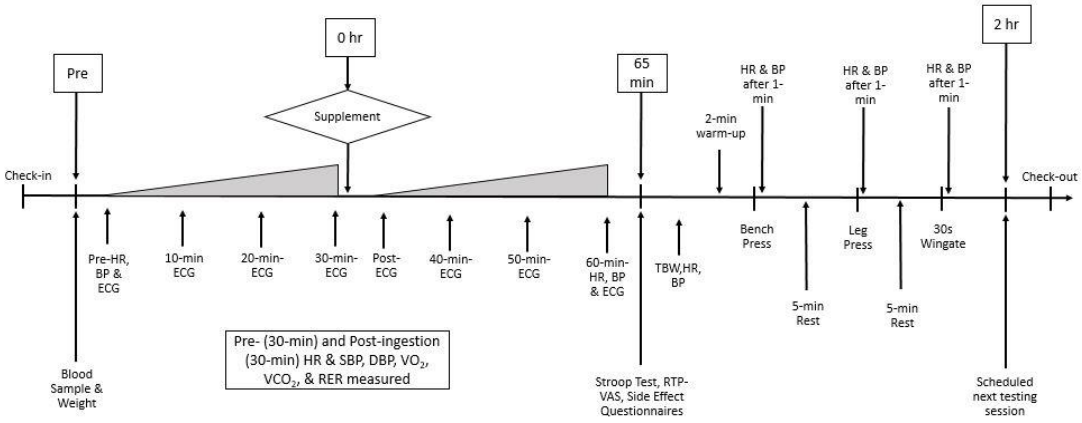

Figure 3.1 Testing Session Timeline

and equipment that were used. Information related to dietary records and side effect questionnaires were also explained during the FAM session. Subjects meeting all criteria were asked to sign an informed consent approved by the Institutional Review Board of Texas A & M University (*College Station, TX*, IRB number: IRB2014-0795). They were also asked to fill out a medical history questionnaire to eliminate those with any possible

contraindications to participate in the study. A research nurse reviewed medical history documents and performed a physical exam on each subject to ensure safety and participation eligibility. Participants were asked to continue their training without initiating any new exercise or diet regime. They were weighed and assessed for body mass, BP, HR, and dual energy X-ray absorptiometry (DXA) body composition (excluding cranium). Then, participants performed a 1RM test on the bench press and leg press. Finally, participants performed a Wingate 30 sec anaerobic capacity test on a cycle ergometer. Following baseline measurements, participants were randomly assigned into three treatments. At the end of the session, participants were scheduled to return to the lab for the first week of supplementation.

Inclusion and Exclusion Criteria

Participants were allowed to take part in the study if they were between the ages of 18-40 and if they had at least six months immediate prior history of resistance training including bench press and leg press/squat. The exclusion criteria of the study were a history of treatment for metabolic disease (e.g., diabetes); hypertension; hypotension; thyroid disease; symptomatic arrhythmias and/or cardiovascular disease; current use of any prescription medication; intolerance to caffeine and/or other natural stimulants; pregnant or lactating females; a history of smoking; or excessive drinking (>12 drinks per week).

Procedures

Participants reported to the lab for day 0 and day 0 testing after a 12-hr fast and refrained from exercise training and ingestion of caffeine and over the counter

medication with a known stimulant use for 48 hrs prior to testing sessions. All testing sessions were started in the morning. Participants were instructed to maintain their normal resistance training program on study day where physical activity was not restricted (e.g. 48-hrs prior to each long testing session). Participants were also asked to not change their current diet level during the course of the study. Participants recorded their dietary intake prior to each supplementation week. Once at the lab, participants turned in their food records. Next, they donated approximately 20ml of unsupplemented and fasting blood sample via venipuncture. Then, body mass was measured and participants were assessed for HR, BP, and resting energy expenditure (REE) for two separate 30-min periods. Following the first 30-min, the REE measurement participants ingested their assigned supplement. Then, REE measurement continued for another 30 min. Resting HR were measured immediately prior to REE and every 10 min during the REE measurement. Then, HR was measured 1 min after bench press test, 1 min after leg press test, and 1 min after Wingate anaerobic power test. Resting BP was measured prior to and following REE. BP was also be measured immediately after determining HR at the end of each exercise test.

Upon supplementation, participants continued the REE for another 30 min. Following REE measurement, participants performed a series of cognitive function tests as well as readiness to perform, sleep quality, and caffeine questionnaires using VAS. Participants then warmed up for 5 min and performed three sets of 10 repetitions at 70% of 1RM on the bench press and leg press with 2 min recovery between sets and 5 min recovery from exercise modes. Participants performed as many repetitions as possible

during the last set. Then, they performed a 30 sec anaerobic capacity test on a cycle ergometer. Participants were asked to pedal as fast as possible prior to application of the workload and sprint at an all-out maximal capacity for 30-sec Wingate test.

Supplementation Protocol

Participants were matched for age, body mass, and FFM and randomly assigned to ingest in a double-blind cross-over manner either: (1) flavored dextrose PLA (12g); (2) PWS [caffeine (300mg), creatine nitrate in a 2:1 ratio as a salt (2.0g), β-alanine (3.2g), arginine α-ketoglutarate (2.0g), N-acetyl tyrosine (300mg), *Mucuna pruriens* (1.0g), ascorbic acid (500mg), dextrose (2.6g) (Table 3.2)]; (3) PWS at ~150% dosage (PWS150) (Table 3.3) (*Cellucor®, Woodbolt International, Bryan, TX*). In order to balance the 150% dosage, 2.6g dextrose PLA was added. Participants were required oral administration of one serving of the supplement mixed in approximately eight ounces of plain water. Supplements were provided by the funding agency, and were independently packaged into coded single foil packets for double-blind administration following Good Manufacturing Practices and certified to contain the aforementioned ingredients by VMI Nutrition (*Salt Lake, UT*). All supplements had similar color and powdered texture. Participants were instructed to ingest one foil packet per day immediately prior to exercise on training days and around noon on non-training days. On testing days, ingesting followed after 30 min REE measurement. Supplement compliance was verified by weekly compliance verification and collecting and counting empty packets. Participants repeated the experiment after a one week washout period for two more times with alternate supplements in a randomized manner.

Table 3.2 PWS Preworkout Supplement Ingredients and Dosages

PWS Formula	**G**	**mg**
β-Alanine	3.2	3,200
Arginine AKG [1] 2:1	2.0	2,000
Creatine Nitrate (1.34g CR; 0.66g N)	2.0	2,000
Ascorbic Acid	0.5	500
N-Acetyl Tyrosine	0.3	300
Caffeine Anhydrous	0.3	300
Mucuna Pruriens 15% L-Dopa [2]	1.0	1,000
Niacinamide USP [3]	0.06	60
Theacrine	0.01	10
Pyridoxal 5-Phosphate	0.00148	1.48
Folic Acid USP	0.0005	0.5
Methylcobalamin	0.0018	1.8
Dextrose	2.62	2,620
TOTALS	12	12,000

1. α-ketoglutarate
2. L-3,4-dihydroxyphenylalanine
3. The United States Pharmacopeia

Table 3.3 PWS150 Preworkout Supplement Ingredients and Dosages

PWS150 Formula	**G**	**mg**
β-Alanine	4.8	4,800
Arginine AKG [1] 2:1	3.0	3,000
Creatine Nitrate (2.01g CR; 0.99g N)	3.0	3,000
Ascorbic Acid	0.76	760
N-Acetyl Tyrosine	0.46	460
Caffeine Anhydrous	0.4	400
Mucuna Pruriens 15% L-Dopa [2]	1.5	1,500
Niacinamide USP [3]	0.09	90
Purenergy™ (43%) caffeine (12mg), (57%) pterostilbene (12.5mg)	0.05	50
Theacrine	0.02	20
Pyridoxal 5-Phosphate	0.002	2.0
Folic Acid USP	0.00076	0.76
Methylcobalamin	0.002	2.0
TOTALS	14	14,000

1. α-ketoglutarate
2. L-3,4-dihydroxyphenylalanine
3. The United States Pharmacopeia

Dietary Recording and Analysis

After the FAM session and prior to day 0 testing, participants completed a dietary record to include three weekdays and one weekend day. Participants were asked to precisely record all the food item (brand if applicable), preparation method, and total quantity consumed as well as beverage consumption except water. The next two dietary recordings then took place during the washout period prior to each supplementation week. All food logs were entered and analyzed by a registered dietitian using dietary analysis software (*ESHA Food Processor Version 8.6, Salem, OR, USA*). Appendix D shows the daily food-log table.

Body Composition Testing

Height and body mass were measured according to standard procedures using a self-calibrating electronic scale (*Cardinal Detecto Scale Model 8430, Webb City, MO*) in socks or bare feet with an accuracy of ±0.02 kg and ±0.25 cm, respectively. Total body water was determined at day 0 and day 7 testing sessions under standardized conditions using an ImpediMed DF50 bioelectrical impedance analyzer (*BIA, ImpediMed, San Diego, CA, USA*). Participants were stationed in a supine position with four electrodes placed on the wrists and ankles. Bioelectrical impedance analysis has been shown to be a valid method to determine total body water, intracellular water, and extracellular water [201, 202]. Body composition testing took place once at the FAM session. Body composition was measured by Hologic Discovery DXA technology (*Hologic Inc., Waltham, MA, USA*) equipped with APEX. Software (*APEX Corporation Software, Pittsburg, PA, USA*). Previous studies indicate DXA to be an accurate and reliable

means to assess changes in body composition [203, 204]. Participants were asked to remove all metal from their bodies or clothing, including items such as jewelry and body piercings. The existence of any metal on a person may interfere with the accuracy of the DXA scan, although the extent of this error is unknown. Participants were then positioned in the supine position based on manufacturer's guidelines by a trained technician. DXA measurement was then performed, taking approximately 6-8 min. Analysis of body composition was immediately performed by a trained technician to determine body composition. Test/retest reliability studies performed on male and female athletes with DXA yielded mean deviation for total bone mineral content and total fat-free/soft tissue mass of 0.31-0.45%, with a mean intra-class correlation of 0.985 [203].

Resting Energy Expenditure Measurement

REE was measured by indirect calorimetry using a ParvoMedics *TrueOne 2400* Metabolic Measurement System (*ParvoMedics, Inc., Sandy, UT*). A large plastic "bubble" was placed over the participant's head while a plastic sheet covers the participant's upper body, preventing external air from entering the bubble. Oxygen flows into the bubble from a valve at the top. The calorimeter measures the amount of O_2 consumed ($\dot{V}O_2$) and the amount of CO_2 produced ($\dot{V}CO_2$) while at rest by comparing the concentrations of O_2 and CO_2 in the air inspired by the participant with the concentration in the air expired by the participant.

The participant lied on his/her back on the bed for 30 min pre- and 30 min post-ingestion. The 1-hr REE measurement was conducted in order to assess the plausible

changes in $\dot{V}O_2$, $\dot{V}CO_2$, and respiratory exchange ratio (RER) following supplementation. The participant relaxed but not fall to sleep during the measurement. The lab technicians looked in from time to time to make sure he/she is awake. The participant was free to remove the bubble if he/she felt uncomfortable.

Heart Rate Measurement

During the testing sessions, participants were assessed for several HR measurements. HR was measured in two forms: resting electrocardiographs (ECG) and resting HR using pulse palpation. Resting ECG was assessed using a CARDIO System 12-lead interpretative ECG (*Nasiff Associates, Inc., Brewerton, NY*). HR was also measured by palpitation of the radial artery using standard procedure in the seated position [205]. Trained technicians were count the pulse for 15 sec to determine the number of beats per minute.

Blood Pressure Measurement

BP was determined at each time point by auscultation of the brachial artery using a mercurial sphygmomanometer using standard clinical procedure [206]. This proceeded immediately after determining HR. The BP measurement was performed before, during, and after 60 min REE measurement as well as following Stroop Color test in the supine position. The remaining BP measurements occurred in the seated position 1 min after each exercise test.

Cognitive Function (Stroop Word-Color Test)

The cognitive function was tested by Stroop Word-Color test which was standardized by Golden and others [207-209]. The test consists of three pages (Appendix

E). Each page contains 100 items, presented in 5 columns of 20 items. Items on Page 1 (Word) are the color words RED, GREEN, and BLUE in black ink. On Page 2 (Color) the items are XXX's colored in red, green, or blue ink. Items on Page 3 (Word-Color) are the words RED, GREEN, and BLUE printed in red, green, or blue ink with the limitation that word and ink do not match. Participants read loudly each page (Word, Color, and Word-Color page in order) for 45 sec as fast as they can. The number of correct responses obtained during the time period is used to assess cognitive function. In neuropsychology, Stroop Word-Color test measures three basic domains. Word measures post left hemisphere function and reading skills. The Color section determines disorders of the dominant temporal-occipital areas of the posterior right hemisphere. The Word-Color section measures prefrontal cortex, executive functions [210, 211]. Day-to-day test reliability of administering this test in our lab has yielded a standard error of mean (SEM) ranging from 1.8-1.86 counts, an SEM as a percent of grand mean ranging from 2-3%, coefficient of variation (CV) ranging from 0.14-0.25, and intraclass correlation coefficients of 0.90, 0.68, 0.57 for Word, Color and Word-Color, respectively. Jensen [212] reported test/retest reliabilities of 0.88, 0.79, and 0.71 for the three Word, Color, and Word-Color raw scores. Golden [213] reported test-retest reliabilities of 0.86, 0.82, and 0.73 for the individual version. As can be seen, there are little differences between these correlations, despite using different forms of test.

Readiness to Perform Visual Analogue Scale

Readiness to Perform was measured by VAS with five subjective feeling (strongly disagree to strongly agree) on 20 cm dotted bar (Appendix F). The VAS has

five questions; (1) "*I am looking forward to today's workout*", (2) "*I am optimistic about my future performance*", (3) "*I feel vigorous and energetic*", (4) "*My appetite is great*", (5) "*I have little muscle soreness*". Participants circled the number or dotted between numbers that best indicated how they currently feel. Day-to-day test reliability of administering this test in our lab has yielded a SEM's ranging from 0.9-0.13, an SEM as a percent of grand mean ranging from 2-4%, CV's ranging from 0.19-0.34, and intraclass correlation coefficients of 0.66, 0.88, 0.70, 0.90, and 0.67 for questions 1 through 5, respectively.

Sleep Quality and Caffeine Ingestion Assessment

Participants were also given a short sleep quality questionnaire (SQQ) (Appendix G) at each testing session to determine how well they tolerated supplementation; and if they experienced any symptoms as a result of the supplement. The SQQ is an effective instrument that was used to measure the quality and patterns of sleep in participants. It differentiates "poor" from "good" sleep quality by measuring several areas (components): sleep duration, subjective sleep quality, enthusiasm quality, and sleep disturbances over the past 48 hrs. The SQQ also contains a 10-item "yes" or "no" question regarding the probable sleeping troubles for the past 48 hrs: cannot get to sleep within 30 min, wake up in the middle of the night or early morning, have to get up to use the bathroom, cannot breathe comfortably, cough or snore loudly, feel too cold, feel too hot, had bad dreams, have pain, and other reasons. Day-to-day variability in SQQ in our lab yielded an SEM of 0.38, an SEM as a percent of grand mean of 8.2%, a CV range of 0.22-2.2, and an intraclass correlation coefficient range of -1.3-0.92.

Participants were also be given a caffeine questionnaire (CQ) (Appendix H) at each testing session and they were asked to rank the severity of their symptoms - drowsiness, tiredness, irritability, etc. Participants were asked to rank their symptoms with 0 (not at all), 1 (a little), 2 (moderately), 3 (quite a bit), and 4 (extremely). During day 0 and day 7 testing sessions, the questionnaires were completed immediately after the REE measurement. In addition, participants referred to the lab for two screening sessions to complete the questionnaires during the supplementation week. Day-to-day variability in caffeine inventory questions in our lab yielded an SEM of 0.02, an SEM as a percent of grand mean of 2.5%, a CV range of 0.22-5.2, and an intraclass correlation coefficient range of -0.2-0.88.

Strength Testing with 1RM

Maximal strength was determined following a standard warm-up consisting of 10 repetitions using 50% of their estimated 1RM, 5 repetitions using 70% of their estimated 1RM, and 1 repetition using 90% of their estimated 1RM. Participants continued increasing weight until their 1RM's were determined. Participants were encouraged to reach 1RM during the FAM trials. With 1RM that was determined at the FAM session, participants performed three sets of bench and leg press test. At the first and second sets, participants were required to lift 10 repetitions at 70% of 1RM on the bench press and leg press interspersed by 2-min of rest between sets and 5-min recovery between each exercise testing modality. During the third set, we asked participants to complete as many repetitions as possible. Total lifting volume was calculated by multiplying the amount of weight lifted times the number of successful repetitions completed. The

concurrent verbal encouragement was applied during the tests as an extrinsic motivational factor to encourage maximal performance. All strength testing took place on an isotonic Olympic bench press and hip/leg sled (*Nebula Fitness, Versailles, OH*) using standard procedures [214]. Upper body power output was measured using a Tendo Fitrodyne (*Tendo Sport Machines, Slovak Republic*). Tendo Power Analyzer is a portable training tool that determines power output and bar speed in training and testing. Peak power, average power, and average velocity were measured during each repetition of the three sets. Day-to-day test reliability of performing this endurance test in our lab on resistance-trained participants has yielded a standard error of measurement (SEM) of 86 kg, a SEM as a percent of grand mean of 4%, a CV of 0.34, and an intraclass correlation coefficient of 0.99 for 3 sets of bench press total lifting volume and an SEM of 480 kg, an SEM as a percent of grand mean of 4%, a CV of 0.32, and an intraclass correlation coefficient of 0.96 for 3 sets of leg press total lifting volume.

Wingate Anaerobic Capacity Test

The Wingate test requires the participant to pedal a mechanically braked bicycle ergometer for 30 sec, at an "all out" pace. Wingate testing was assessed using a Lode Excalibur Sport Ergometer (*Lode BV, Groningen, The Netherlands*) and work rate was set at of 7.5 J/kg/rev for everyone. Participants were asked to pedal as fast as possible 10 sec prior to application of the workload and sprint at an all-out maximal capacity for 30-sec Wingate test. The concurrent verbal encouragement was applied during the test as an extrinsic motivational factor to encourage maximal performance. Day-to-day variability in performing Wingate anaerobic capacity tests in our laboratory yielded an SEM of 18

W, an SEM as a percent of grand mean of 3%, a CV 0.26, and an intraclass correlation coefficient of 0.89 for mean power.

Blood Collection

Participants refrained from exercise, caffeine, and use of over-the-counter stimulants 48 hrs prior to days 0 and 7 testing sessions. They were required to be fasted for 12 hrs prior to donating approximately 20mL of venous blood from an antecubital vein in the forearm according to standard phlebotomy procedures. Blood sample was collected via venipuncture (catheterization) in two 7.5mL BD Vacutainer® serum separation tubes (*Becton, Dickinson and Company, Franklin Lakes, NJ, USA*), leave at room temperature for 15 min, and then was centrifuged at 3500 rpm for 10 min using a standard, refrigerated (4°C) bench top Thermo Scientific Heraeus MegaFuge 40R Centrifuge (*Thermo Electron North America LLC, West Palm Beach, FL, USA*). Serum supernatant was removed and stored at -80°C in polypropylene microcentrifuge tubes for later analysis. Serum was analyzed for liver enzymes including alkaline phosphatase (ALP), AST, and alanine transaminase (ALT); kidney enzymes including creatinine and blood urea nitrogen (BUN); muscle enzymes including CK and LDH; glucose; and blood lipids including total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), and triglycerides (TG) using a Cobas® C 111 (*Roche Diagnostics, Basel, Switzerland*). Calorimetric assay kits were used to measure serum creatine concentrations (*Sigma-Aldrich, St. Louis, MO*). The Cobas® automated clinical chemistry analyzer was calibrated according to manufacturer guidelines. This analyzer has been known to be highly valid and reliable in previously published studies [215].

The internal quality control for the Cobas c 111 was performed using two levels of control fluids purchased from manufacturer to calibrate acceptable SD and coefficients of variation values for all aforementioned assays. Samples were re-run if the observed values were outside control values and/or clinical norms according to standard procedures. Test-to-test reliability (10-days) assessment of assays evaluated in this study yielded reliability CV's ranging 0.4–2.4% for low control samples and 0.6-1.9% for high controls with precision ranging 0.8–2.4% on low and 0.5–1.7% for high controls.

Blood was also collected in a single 3.5mL BD Vacutainer® lavender top tube containing K_2 EDTA (*Becton, Dickinson and Company, Franklin Lakes, NJ, USA*), leave at room temperature for 15 min, and was refrigerated for approximately 3-4 hrs before complete blood count analysis. Complete blood count with platelet differential was run on whole blood [hemoglobin, hematocrit, red blood cell (RBC) counts], mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RCDW), white blood cell counts, lymphocytes, granulocytes, and mid-range absolute count (MID) using a Abbott Cell Dyn 1800 (*Abbott Laboratories, Abbott Park, IL, USA*) automated hematology analyzer. The internal quality control for the Abbott Cell Dyn 1800 was performed using three levels of control fluids purchased from manufacturer to calibrate acceptable CV values for all whole blood cell parameters (±6.3%).

Statistical Analysis

All data were analyzed using a repeated measured multivariate analysis of variance (MANOVA) with Wilks' Lambda and Green-Geisser adjustments using SPSS

24.0 for Windows (*IBM Corporation, Armonk, NY, USA*). Participant baseline demographic data were analyzed using one-way ANOVA. Overall MANOVA effects were examined as well as MANOVA univariate treatment effects for certain variables when significant interactions were seen. Greenhouse-Geisser univariate tests of time, treatment and treatment x time effects were reported for each variable analyzed within the MANOVA model. Data were considered statistically significant when the probability of error is 0.05 or less. Post-hoc LSD pairwise comparisons using partial eta squared was used to determine effect magnitude. In REE, area under the curve (AUC) of $\dot{V}O_2$, $\dot{V}CO_2$, and REE were calculated by summing up response by every 1-min for 30-min pre and 30-min post-ingestion of supplement. AUC was computed on REE data by GraphPad Prism 6 software (*GraphPad Software Inc., La Jolla, CA, USA*) calculating the trapezoid rule and analyzed by ANOVA. In addition, 95% confidence intervals was constructed for Stroop Word/Color test, readiness to perform VAS, and exercise outcomes. When the 95% confidence interval did not include zero, the change was considered significant ($p < 0.05$) [216]. Regarding Stroop test and VAS results, the baseline values were considered as covariates. Delta and percent change values were calculated and used to determine changes from day 0 which were analyzed by repeated measures analysis of variance (ANOVA). In regard to hematology, we also pursued analyses denoting changes from normal to exceeding normal clinical limits from day 0 to day 7 using a Chi-square analysis. With regard to SQQ, we used Cochran's Q test to determine if there are differences on a dichotomous dependent variable among three treatments.

CHAPTER IV

RESULTS

Subject Demographics

Figure 4.1 presents a CONSORT schematic for study participation. Twenty two participants were initially recruited for the current study, all completed consent forms and participated in the required familiarization session. However, of the original 22 participants, 19 completed the study (16 men and 3 women). One participant dropped out due to time constraints and two participants could not continue the study, because ingesting PWS150 resulted in jittery and lightheadedness. The baseline demographics for the participants are listed in Table 4.1. We did not observe any significant changes in body weight and hydration status during the course of study ($p > 0.05$) (Table 4.2).

Diet

Table 4.3 presents absolute and relative macronutrient intake data. MANOVA analysis revealed overall Wilks' Lambda treatment ($p = 0.95$). One-way ANOVA did not reveal any statistically significant differences among dietary intake from session to session of testing ($p > 0.05$).

Resting Energy Expenditure

Figures 4.2, 4.3, 4.4, and 4.5 present the day 0 and day 7 minute-by-minute comparison of (a) $\dot{V}O_2$, (b) $\dot{V}CO_2$, and (c) RER for 30-min pre- and 30-min post supplementation. Table 4.4 presents the results for $\dot{V}O_2$, $\dot{V}CO_2$, RER, and REE before and after supplementation. MANOVA analysis revealed overall Wilks' Lambda

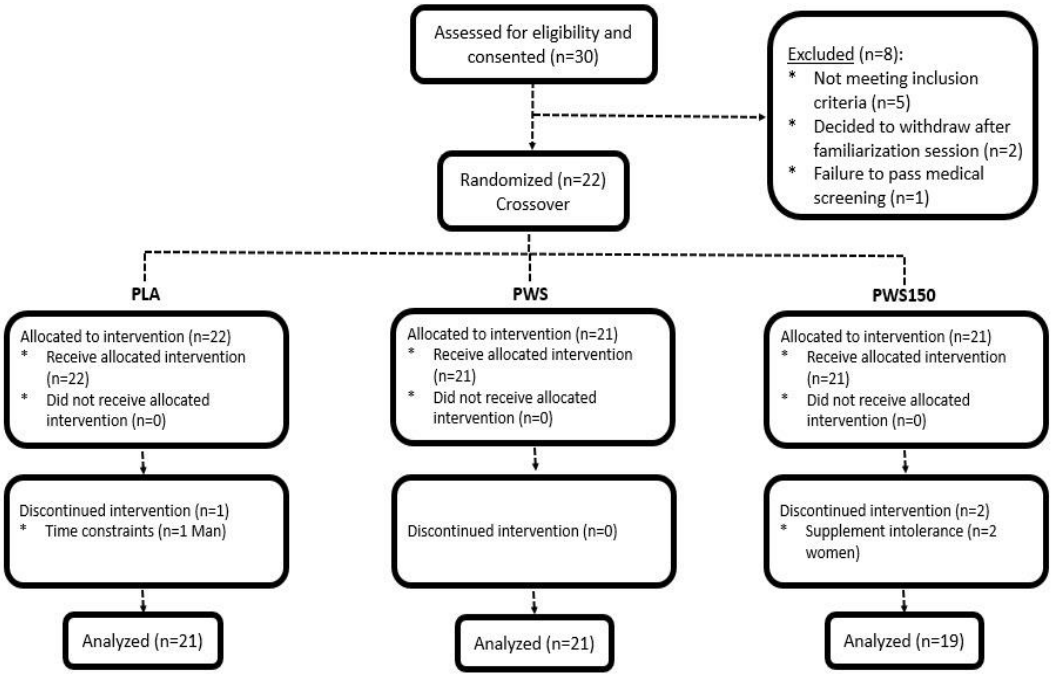

Figure 4.1 Consort Diagram for Study Participation

Table 4.1 Participant Demographics

N	Age (yrs)	Height (cm)	Body Weight (kg)	BMI	Body Fat (%)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
19	21.8 ± 2.1	175.7 ± 9.5	83.9 ± 18.2	27.1 ± 3.9	21.6 ± 8.5

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

treatment x time ($p = 0.76$). There were no significant changes for $\dot{V}O_2$ ($p = 0.15$), $\dot{V}CO_2$ ($p = 0.08$, partial $\eta^2 = 0.07$ – medium effect), RER ($p = 0.24$), and REE ($p = 0.61$). Table 4.5 presents the AUC results for $\dot{V}O_2$, $\dot{V}CO_2$, RER, and REE before and after supplementation. The AUC did not show any significant interaction for REE

Table 4.2 Body Weight and Hydration Status

Variable	Treatment	Day 0	Day 7			
		Mean ± SD	Mean ± SD	Mean ± SE		p-level
Body Weight (kg)	PLA	83.5 ± 18.1	83.8 ± 17.9	83.7 ± 2.8	Treatment	0.99
	PWS	83.8 ± 17.9	84.1 ± 17.9	84.0 ± 2.8	Time	0.08
	PWS150	84.2 ± 18.3	84.3 ± 18.3	84.3 ± 2.9	T x T	0.51
	Overall	83.9 ± 17.8	84.1 ± 17.7			
Total Body Water (L)	PLA	38.8 ± 6.0	39.9 ± 5.9	39.3 ± 0.9	Treatment	0.83
	PWS	40.8 ± 7.9	39.9 ± 6.2	40.3 ± 1.1	Time	0.75
	PWS150	40.4 ± 7.9	40.7 ± 7.3	40.6 ± 1.2	T x T	0.25
	Overall	40.0 ± 7.2	40.2 ± 6.4			
Intracellular Body Water (L)	PLA	22.4 ± 3.3	22.7 ± 3.2	22.5 ± 0.5	Treatment	0.48
	PWS	24.4 ± 8.2	22.7 ± 3.4	23.6 ± 1.0	Time	0.42
	PWS150	22.2 ± 3.5	21.9 ± 3.6	22.1 ± 0.5	T x T	0.44
	Overall	23.0 ± 5.5	22.4 ± 3.3			
Extracellular Body Water (L)	PLA	17.4 ± 4.3	17.2 ± 3.5	17.3 ± 0.6	Treatment	0.99
	PWS	17.4 ± 3.8	17.1 ± 3.6	17.2 ± 0.6	Time	1.0
	PWS150	17.1 ± 3.9	17.7 ± 4.2	17.4 ± 0.6	T x T	0.46
	Overall	17.3 ± 4.0	17.3 ± 3.7			

Values are means ± standard deviations. Data were analyzed by MANOVA with repeated measures. Greenhouse-Geisser Treatment (T), time (T), and Treatment x time (T x T) interaction p-levels are reported with univariate treatment p-levels. MANOVA analysis revealed overall Wilks' Lambda treatment ($p = 0.80$), time ($p = 0.22$), and treatment x time ($p = 0.39$).

Table 4.3 Dietary Characteristics

	Treatment				
	PLA	PWS	PWS150		Treatment
Dietary Characteristics	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SE	p-level
Absolute Amount					
Energy Intake (kcals/day)	1,655 ± 230	1,532 ± 232	1,613 ± 282	1,600 ± 33	0.31
Protein (g)	101.9 ± 27.8	97.3 ± 30.2	101.2 ± 28.3	100.1 ± 3.7	0.87
Fat (g)	59.2 ± 13.1	52.6 ± 12.5	59.8 ± 15.0	57.2 ± 1.8	0.20
Carbohydrate (g)	160.4 ± 36.0	152.1 ± 39.5	152.2 ± 48.0	154.9 ± 5.4	0.77
Relative Amount					
Energy Intake (kcals/kg/day)	20.2 ± 3.4	18.9 ± 4.2	19.9 ± 4.7	19.7 ± 0.5	0.60
Protein.per.kg	1.26 ± 0.41	1.20 ± 0.43	1.25 ± 0.41	1.24 ± 0.05	0.90
Fat.per.kg	0.72 ± 0.16	0.66 ± 0.21	0.73 ± 0.22	0.70 ± 0.02	0.44
Carbohydrate.per.kg	1.95 ± 0.47	1.86 ± 0.51	1.86 ± 0.62	1.89 ± 0.07	0.82

Values are means ± standard deviations (SD). Differences in macronutrient intake among treatments were tested using one-way ANOVA.

measurements. The results from the $\dot{V}O_2$, $\dot{V}CO_2$, RER, and REE analysis rejects the directional hypothesis 1 which states that PWS supplementation will increase resting energy expenditure.

Hemodynamic Characteristics

Table 4.6 presents the results for HR, SBP, and DBP. MANOVA analysis for HR and BP revealed Wilks' Lambda treatment x time ($p = 0.13$). We observed a significant interaction for SBP after bench press between PWS and PWS150 at day 0 and between PLA and both supplement treatments at day 7 ($p = 0.02$). The post-bench press SBP decreased in PLA, while it increased in PWS and PWS150 treatments. No significant interaction was seen for HR ($p = 0.08$, partial $\eta^2 = 0.06$ – medium effect) and DBP ($p = 0.49$) at day 0 and day 7 for rest and 1 min after each exercise test. The results from Hr and BP analysis rejects the directional hypothesis 2 which states that PWS supplementation will increase HR and BP responses.

Readiness to Perform and Cognitive Function

Readiness to Perform was measured by visual analogue scale with five subjective feeling (strongly disagree to strongly agree) on 20 cm dotted bar. The results are presented in Table 4.7. MANOVA analysis revealed overall Wilks' Lambda treatment x time ($p = 0.36$). No significant interactions were observed among treatments regarding the perceived readiness to perform as determined by the VAS ($p > 0.05$). The results from readiness to perform analysis rejects the directional hypothesis 3 which states that PWS supplementation will improve perceptions of readiness to perform.

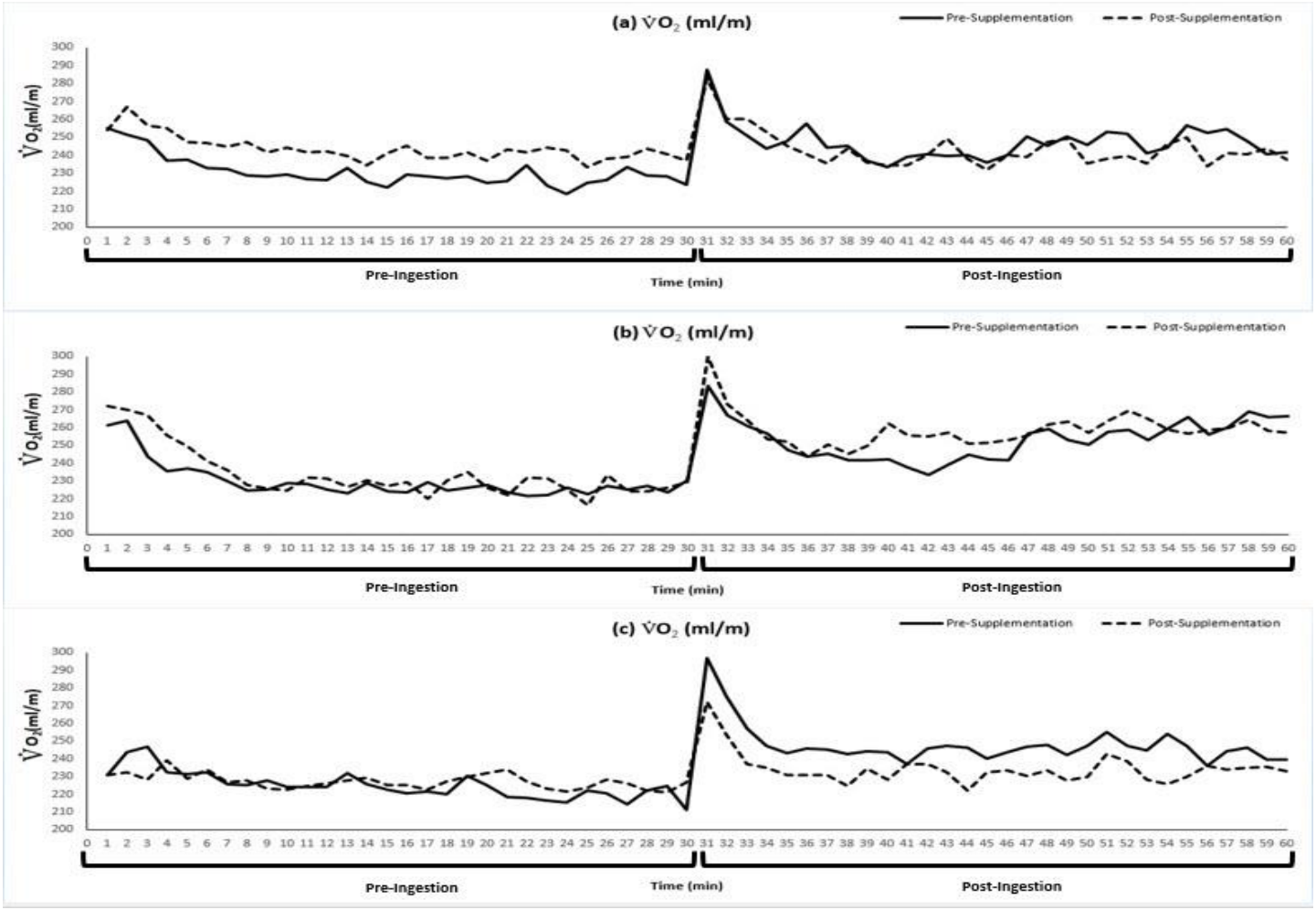

Figure 4.2 Minute-by Minute Comparison of $\dot{V}O_2$ Changes During 30-min Pre- and 30-min Post-Supplementation for (a) PLA, (b) PWS, and (c) PWS150 Treatments

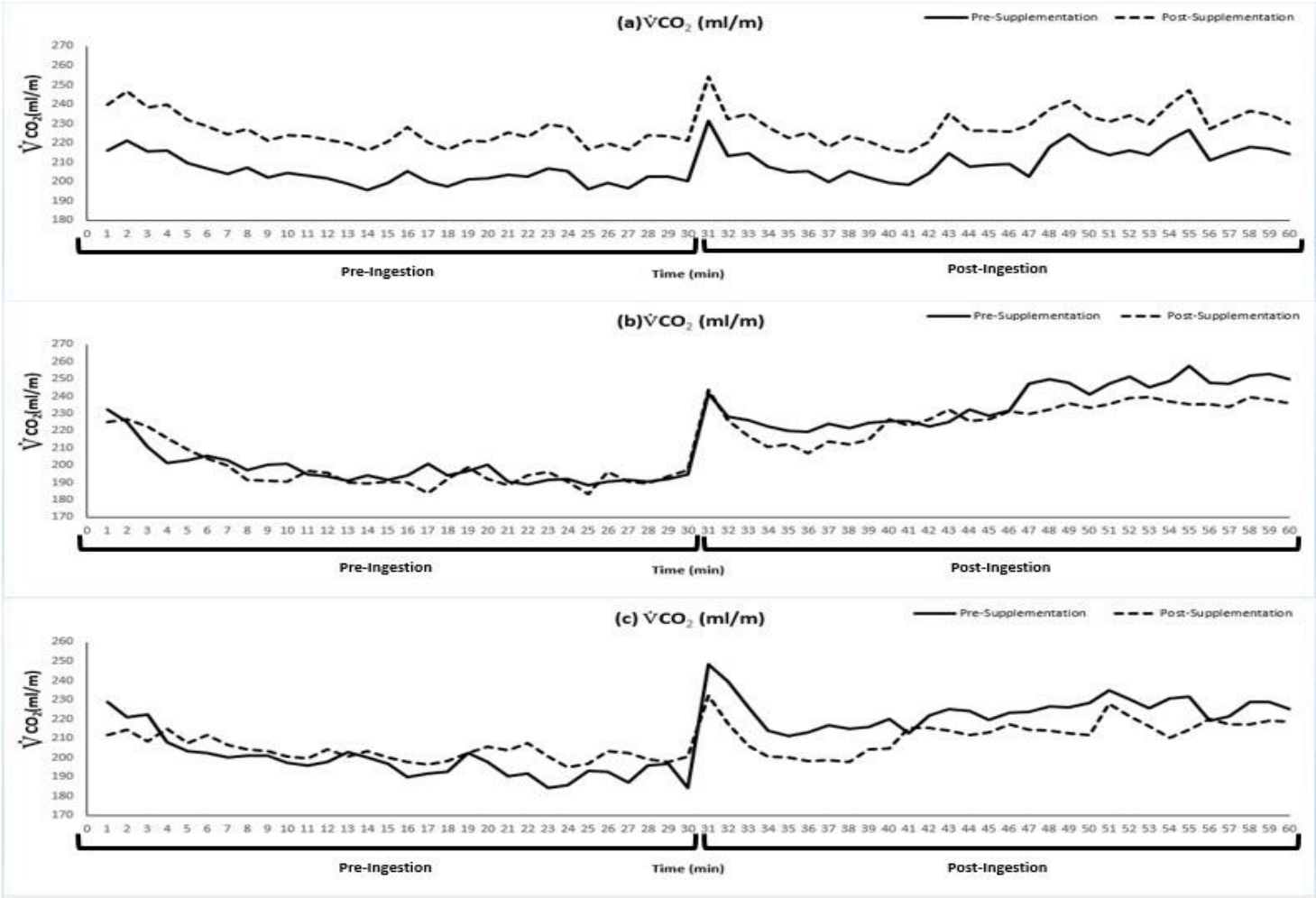

Figure 4.3 Minute-by Minute Comparison of $\dot{V}CO_2$ Changes during 30-min Pre- and 30-min Post-Supplementation for (a) PLA, (b) PWS, and (c) PWS150 Treatments

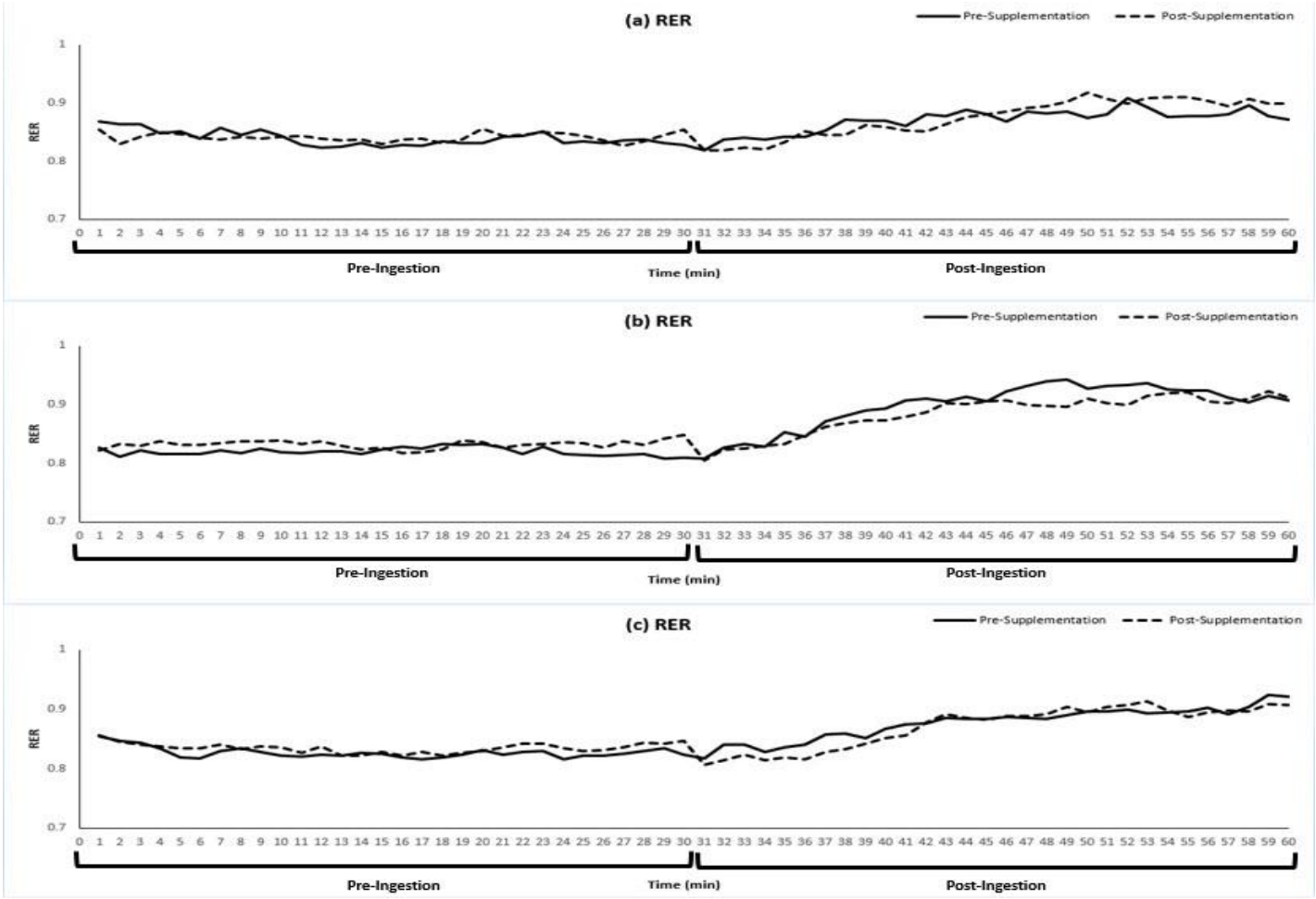

Figure 4.4 Minute-by Minute Comparison of RER Changes during 30-min Pre- and 30-min Post-Supplementation for (a) PLA, (b) PWS, and (c) PWS150 Treatment

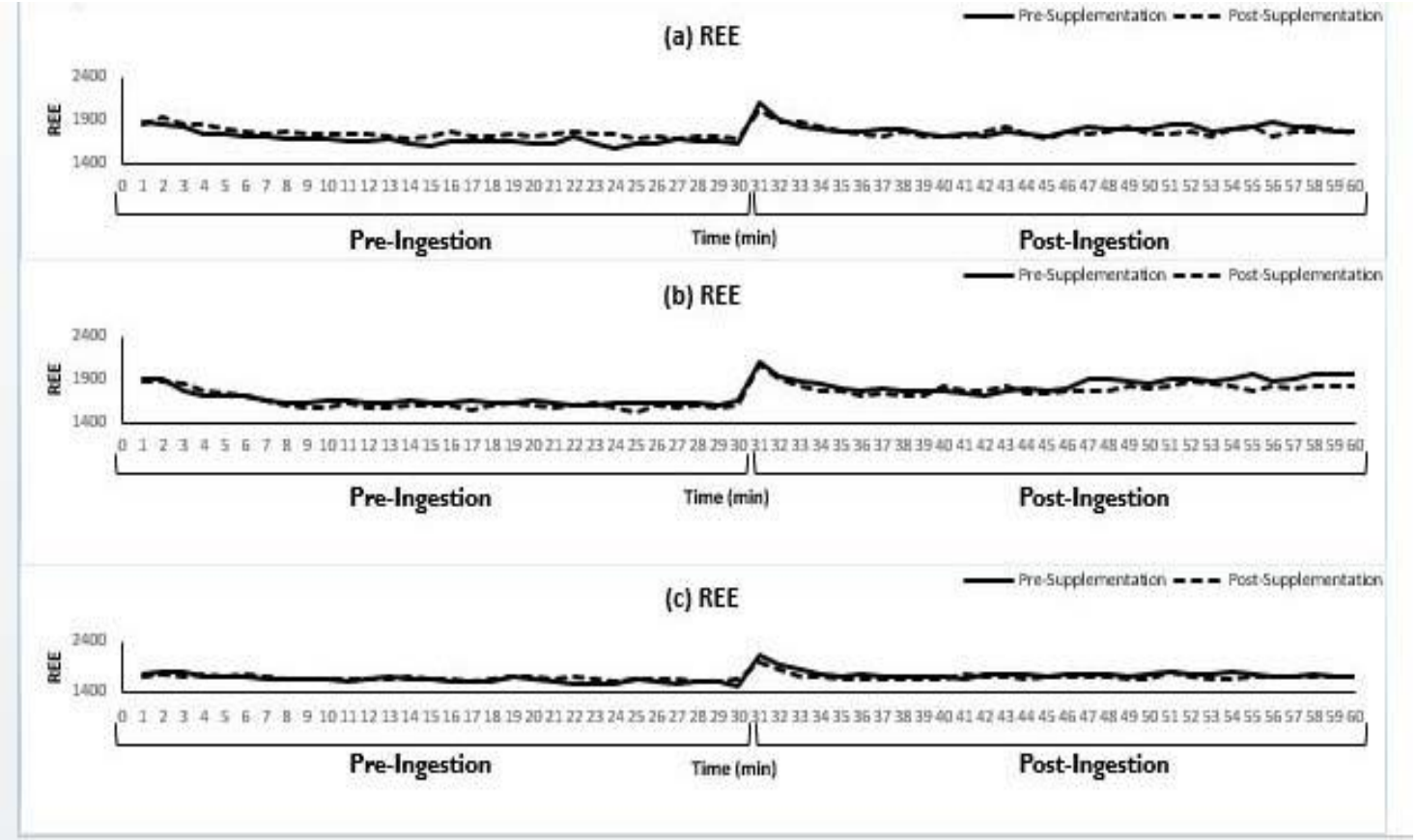

Figure 4.5 Minute-by Minute Comparison of REE Changes during 30-min Pre- and 30-min Post-Supplementation for (a) PLA, (b) PWS, and (c) PWS150 Treatment

Table 4.4 REE Measurement Before and After Supplement Ingestion

Variable	Treatment	Day 0 Pre-Ingestion	Day 0 Post-Ingestion	Day 7 Pre-Ingestion	Day 7 Post-Ingestion			
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SE		p-level
VO_2 (ml/min)	PLA	239 ± 56	256 ± 61	244 ± 63	247 ± 58	247 ± 6	Treatment	0.94
	PWS	237 ± 59	253 ± 62	251 ± 59	263 ± 66	250 ± 7	Time	0.002
	PWS150	251 ± 59	270 ± 54	247 ± 51	252 ± 52	252 ± 6	T x T	0.15
	Overall	242 ± 57	260 ± 58*	247 ± 57	254 ± 58*			
VCO_2 (ml/min)	PLA	199 ± 44	221 ± 47	204 ± 52	220 ± 50	211 ± 5	Treatment	0.95
	PWS	198 ± 51	225 ± 55	213 ± 55	231 ± 61	216 ± 6	Time	< 0.001
	PWS150	207 ± 50	238 ± 46	204 ± 45	216 ± 47	212 ± 6	T x T	0.08
	Overall	201 ± 48	228 ± 49*	207 ± 50	222 ± 53*			
RER	PLA	0.83 ± 0.05	0.86 ± 0.06	0.84 ± 0.05	0.89 ± 0.06	0.86 ± 0.01	Treatment	0.57
	PWS	0.83 ± 0.05	0.89 ± 0.05	0.84 ± 0.06	0.87 ± 0.05	0.86 ± 0.01	Time	< 0.001
	PWS150	0.82 ± 0.04	0.88 ± 0.05	0.82 ± 0.04	0.85 ± 0.04	0.85 ± 0.01	T x T	0.24
	Overall	0.83 ± 0.05	0.88 ± 0.05*	0.83 ± 0.05	0.87 ± 0.05*			
REE (Kcal/day)	PLA	1,686 ± 423	1,848 ± 369	1,718 ± 371	1,769 ± 359	1,713 ± 46.4	Treatment	0.94
	PWS	1,683 ± 426	1,795 ± 401	1,605 ± 329	1,763 ± 406	1,726 ± 44.4	Time	< 0.001
	PWS150	1,672 ± 389	1,783 ± 420	1,712 ± 432	1,755 ± 465	1,747 ± 48.1	T x T	0.61
	Overall	1,680 ± 405	1,808 ± 391*	1,678 ± 376	1,762 ± 405*			

Values are means ± standard deviations. REE values were analyzed by MANOVA with repeated measures. Greenhouse-Geisser treatment (T), time (T), and treatment x time (T x T) interaction p-levels are reported with univariate treatment p-levels. MANOVA analysis revealed overall Wilks' Lambda treatment ($p = 0.97$), time ($p < 0.0001$), and treatment x time ($p = 0.76$). * represents $p < 0.05$ difference from pre-ingestion day 0.

Table 4.8 presents the results for the Stroop Word-Color test. MANOVA analysis revealed overall Wilks' Lambda treatment x time ($p = 0.41$). The cognitive function test results indicate a significant interaction among treatments for the Word test between PWS150 and PLA ($p = 0.027$). The results of our study indicate that there was a significant change from day 0 in cognitive function as determined by the Stroop Word-Color Test (Fig. 4.6). In the regard, we observed significant changes in Word count, Color recognition, Word-Color assessment, and total Stroop results. There no other

Table 4.5 Cumulative Values for REE Measurement (AUC)

Variable	Treatment	Day 0 Pre-Ingestion	Day 0 Post-Ingestion	Day 7 Pre-Ingestion	Day 7 Post-Ingestion			
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SE		p-level
VO_2 (ml/30 min)	PLA	7,064 ± 1,827	7,448 ± 1,582	7,228 ± 1,509	7,338 ± 1,500	7,121 ± 187	Treatment	0.99
	PWS	6,865 ± 1,547	7,398 ± 1,595	7,226 ± 1,825	7,578 ± 1,881	7,267 ± 195	Time	0.002
	PWS150	7,135 ± 1,705	7,620 ± 1,891	7,081 ± 1,717	7,169 ± 1,724	7,251 ± 199	T x T	0.26
	Overall	7,020 ± 1,664	7,490 ± 1,671*	7,176 ± 1,665	7,363 ± 1,693*			
VCO_2 (ml/30 min)	PLA	5,823 ± 1,431	5,925 ± 1,324	6,530 ± 1,202	6,312 ± 1,303	6,002 ± 163	Treatment	0.96
	PWS	5,694 ± 1,194	6,075 ± 1,434	6,584 ± 1,400	6,754 ± 1,709	6,277 ± 169	Time	< 0.001
	PWS150	5,979 ± 1,554	5,988 ± 1,671	6,614 ± 1,638	6,270 ± 1,591	6,213 ± 183	T x T	0.26
	Overall	5,832 ± 1,379	5,998 ± 1,463*	6,578 ± 1,406	6,450 ± 1,540*			
RER	PLA	23.8 ± 1.60	23.9 ± 1.52	23.9 ± 1.52	25.0 ± 1.68	24.5 ± 0.21	Treatment	0.46
	PWS	24.1 ± 1.18	24.4 ± 1.66	24.4 ± 1.66	25.9 ± 1.64	25.0 ± 0.19	Time	< 0.001
	PWS150	24.1 ± 2.73	24.3 ± 1.64	24.3 ± 1.64	25.3 ± 1.54	24.7 ± 0.20	T x T	0.76
	Overall	24.0 ± 1.50	24.2 ± 1.60*	24.2 ± 1.60	25.4 ± 1.63*			
REE (Kcal/30 min)	PLA	48,831 ± 12,375	52,790 ± 9,952	49,785 ± 10,786	51,248 ± 10,383	49,452 ± 1,324	Treatment	0.99
	PWS	48,827 ± 11,773	52,486 ± 11,462	49,550 ± 12,512	51,441 ± 11,582	50,576 ± 1,341	Time	< 0.001
	PWS150	48,270 ± 10,818	51,927 ± 11,873	49,853 ± 12,247	51,914 ± 13,839	50,491 ± 1,387	T x T	0.98
	Overall	48,636 ± 11,431	52,387 ± 10,966	49,727 ± 11,690	51,545 ± 11,857			

Values are means ± standard deviations. Cumulative REE values (AUC) were analyzed by MANOVA with repeated measures. Greenhouse-Geisser treatment (T), time (T), and treatment x time (T x T) interaction p-levels are reported with univariate treatment p-levels. MANOVA analysis revealed overall Wilks' Lambda treatment ($p = 0.95$), time ($p < 0.0001$), and treatment x time ($p = 0.78$). * represents $p < 0.05$ difference from pre-ingestion day 0.

significant interaction for Color count ($p = 0.10$, partial $\eta^2 = 0.06$ – medium effect) and total Stroop results ($p = 0.07$, partial $\eta^2 = 0.07$ – medium effect). The results from cognitive function test accepts the directional hypothesis 4 which states that PWS supplementation will improve markers of cognitive function.

Exercise Performance

Upper and Lower Body Strength

Table 4.9 presents the results of bench press and leg press performance including lifting volume during the 3rd set and total lifting volume. Figures 4.7 and 4.8 present the changes from day 0 in bench and leg press 3rd set lifting volume and total lifting volume.

Participants performed three sets of bench press and leg press using a load equivalent to70% of their 1RM. The first two sets consisted of ten repetitions each. The third set consisted of maximal repetitions performed. MANOVA analysis for bench and leg press lifting volume revealed overall Wilks' Lambda treatment x time ($p = 0.74$). Univariate analysis did not show any significant interaction for bench and leg press performance. However, the percent change from day 0 in bench press lifting volume during the 3rd set was 13.7%, 4.6%, and 0.2% with PLA, PWS, and PWS150, respectively; bench press total lifting volume changed by 6.8%, -0.5%, and 12.8% for PLA, PWS, and PWS150, respectively; leg press lifting volume during the 3rd set was 15.4%, 13.4%, and 5.5% with PLA, PWS, and PWS150, respectively; and the leg press total lifting volume increased by 8.7%, 7.4%, and 4.8% with PLA, PWS, and PWS150, respectively. Table 4.10 presents the results for upper body power output including mean power, peak power, and mean velocity during the 3rd set. Figure 4.9 presents the changes from day 0 in bench press mean power, peak power, and mean velocity. The upper body power output was measured by Tendo Fitrodyne. MANOVA analysis for upper body power output revealed overall Wilks' Lambda treatment x time ($p = 0.96$). Overall, there was no significant differences among treatments in upper body mean power, peak power, and mean velocity; however, the percent change from day 0 in peak power was 3.3%, -0.83, and 7.3% with PLA PWS, and PWS150, respectively; mean power increased by 1.5%, 2.7%, and 6.3% with PLA, PWS, and PWS150, respectively; and mean velocity increased with by 4.7%, 1.8%, and 5.4% with PLA, PWS, and PWS150, respectively.

Table 4.6 Heart Rate and Blood Pressure Measurement Before and After Supplement Ingestion

Variable	Treatment	Day 0 Pre-ingestion	Day 0 Post-ingestion	Day 0 Post-Bench press	Day 0 Post-Leg press	Day 0 Post-Wingate	Day 7 Pre-ingestion	Day 7 Post-ingestion	Day 7 Post-Bench press	Day 7 Post-Leg press	Day 7 Post-Wingate			
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SE		p-level
HR (b/min)	PLA	62.8 ± 7.8	62.4 ± 9.0	93.6 ± 18.1	109.4 ± 16.8	126.6 ± 30.3	61.5 ± 10.0	58.7 ± 10.2	94.7 ± 13.9	103.5 ± 27.3	120.5 ± 17.3	89.4 ± 2.2	Treatment	0.02
	PWS	60.4 ± 10.6	61.8 ± 10.3	99.6 ± 18.8	105.3 ± 20.3	131.2 ± 13.6	57.9 ± 7.5	58.1 ± 7.1	102.6 ± 11.4	107.6 ± 32.8	131.7 ± 12.9	91.6 ± 2.3	Time	< 0.001
	PWS150	59.3 ± 9.1	59.2 ± 7.9	110.2 ± 13.8	122.3 ± 22.1	137.2 ± 15.6	58.6 ± 9.7	62.2 ± 12.9	109.4 ± 15.2	108.7 ± 27.4	136.9 ± 12.1	96.4 ± 2.5	T x T	0.08
	Overall	60.8 ± 9.2	61.1 ± 9.0	101.1 ± 18.1*	112.3 ± 20.8*	131.7 ± 21.3*	59.3 ± 9.1	59.7 ± 10.3	102.2 ± 14.7*	106.6 ± 28.8*	129.7 ± 15.6*			
SBP (mmHg)	PLA	115.6 ± 7.1	116.4 ± 8.2	130.2 ± 10.7	136.4 ± 17.4	143.8 ± 17.5	117.7 ± 9.5	116.4 ± 8.5	125.7 ± 14.3[a,b]	134.7 ± 16.4	144.0 ± 17.4	128.1 ± 1.2	Treatment	0.93
	PWS	114.1 ± 8.7	113.3 ± 6.9	128.3 ± 11.0[a]	135.4 ± 14.4	143.1 ± 17.2	113.7 ± 7.8	116.1 ± 8.0	138.9 ± 13.1[c]	145.1 ± 15.3	151.6 ± 17.2	130.0 ± 1.3	Time	< 0.001
	PWS150	114.8 ± 5.9	115.7 ± 7.5	136.6 ± 9.9[b]	140.1 ± 15.8	149.3 ± 17.2	116.8 ± 7.3	114.9 ± 6.7	137.3 ± 13.2[c]	140.6 ± 15.6	149.6 ± 17.2	131.6 ± 1.3	T x T	0.02
	Overall	114.8 ± 7.2	115.1 ± 7.5	131.7 ± 11.1*	137.3 ± 15.7*	145.4 ± 17.2*	116.1 ± 8.3	115.8 ± 7.7	134.0 ± 14.5*	140.1 ± 16.1*	148.4 ± 17.3*			
DBP (mmHg)	PLA	71.1 ± 7.3	69.0 ± 5.8	70.6 ± 7.5	69.0 ± 8.8	67.0 ± 10.0	71.3 ± 7.9	68.7 ± 7.9	67.2 ± 8.6	65.0 ± 9.7	67.9 ± 10.0	69.0 ± 0.62	Treatment	0.99
	PWS	69.5 ± 7.2	68.5 ± 6.6	70.7 ± 7.7	66.8 ± 8.4	66.0 ± 10.4	71.5 ± 7.2	67.0 ± 7.6	62.2 ± 8.6	62.9 ± 7.8	61.7 ± 9.3	66.7 ± 0.63	Time	< 0.001
	PWS150	67.1 ± 5.3	66.5 ± 6.2	66.3 ± 9.8	63.1 ± 10.2	65.2 ± 8.7	68.2 ± 6.6	68.5 ± 5.7	65.4 ± 9.0	64.1 ± 9.7	62.7 ± 9.7	65.7 ± 0.61	T x T	0.49
	Overall	69.2 ± 6.7	68.0 ± 6.2	69.2 ± 8.5	64.0 ± 9.0*	67.0 ± 10.0	70.3 ± 7.3	68.1 ± 7.0	64.9 ± 8.8*	64.0 ± 9.0*	64.1 ± 9.9*			

Values are means ± standard deviations. Hemodynamic data were analyzed by MANOVA with repeated measures. Greenhouse-Geisser treatment (T), time (T), and treatment x time (T x T) interaction p-levels are reported with univariate treatment p-levels. MANOVA analysis revealed overall Wilks' Lambda treatment ($p = 0.058$), time ($p < 0.0001$), and treatment x time ($p = 0.13$). [a] denotes a significant difference from PWS150. [b] denotes a significant difference from PWS. [c] denotes a significant difference from PWS. * represents $p < 0.05$ difference from pre-ingestion day 0.

Table 4.7 Readiness to Perform-Visual Analogue Scale

	Treatment	Time (wks) Day 0	Day 2	Day 4	Day 7			
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SE		p-level
Looking forward to today's workout	PLA	3.89 ± 0.65	3.89 ± 0.73	3.47 ± 0.61	3.52 ± 0.90	3.70 ± 0.09	Treatment	0.79
	PWS	3.84 ± 0.76	3.68 ± 0.58	3.36 ± 0.83	3.68 ± 0.88	3.64 ± 0.09	Time	0.01
	PWS150	3.68 ± 0.74	3.52 ± 0.61	3.47 ± 0.84	3.63 ± 0.89	3.58 ± 0.09	T x T	0.65
	Overall	3.80 ± 0.71	3.70 ± 0.65	3.43 ± 0.75*§	3.61 ± 0.88			
Optimistic about my future performance	PLA	3.89 ± 0.99	3.89 ± 1.04	3.84 ± 0.89	3.52 ± 0.84	3.70 ± 0.11	Treatment	0.97
	PWS	3.78 ± 0.78	3.73 ± 0.87	3.73 ± 0.73	3.68 ± 0.88	3.71 ± 0.10	Time	0.22
	PWS150	3.78 ± 0.78	3.73 ± 0.80	3.73 ± 0.93	3.68 ± 1.10	3.70 ± 0.11	T x T	0.79
	Overall	3.82 ± 0.84	3.78 ± 0.90	3.77 ± 0.84	3.63 ± 0.93			
Feel vigorous and energetic	PLA	3.31 ± 1.00	3.47 ± 0.90	3.63 ± 0.89	3.05 ± 0.97	3.37 ± 0.11	Treatment	0.78
	PWS	3.26 ± 0.99	3.52 ± 0.96	3.15 ± 0.68	3.15 ± 0.83	3.28 ± 0.10	Time	0.08
	PWS150	3.31 ± 0.74	3.21 ± 0.78	3.31 ± 1.00	3.05 ± 0.84	3.22 ± 0.10	T x T	0.48
	Overall	3.29 ± 0.90	3.40 ± 0.88	3.36 ± 0.87	3.08 ± 0.87§†			
Appetite is great	PLA	3.57 ± 1.01	3.78 ± 0.91	3.73 ± 0.73	3.68 ± 1.00	3.70 ± 0.10	Treatment	0.92
	PWS	3.84 ± 0.83	3.89 ± 0.87	3.63 ± 0.83	3.68 ± 1.00	3.76 ± 0.10	Time	0.09
	PWS150	3.84 ± 1.06	4.00 ± 1.00	3.78 ± 1.18	3.57 ± 1.01	3.80 ± 0.12	T x T	0.60
	Overall	3.75 ± 0.96	3.89 ± 0.91	3.71 ± 0.92	3.64 ± 0.99§			
Have little muscle soreness	PLA	3.31 ± 1.00	3.15 ± 0.89	3.31 ± 0.94	2.94 ± 1.22	3.18 ± 0.12	Treatment	0.89
	PWS	3.57 ± 0.96	2.89 ± 0.93	2.68 ± 1.20	3.15 ± 1.11	3.08 ± 0.13	Time	0.11
	PWS150	3.21 ± 1.22	3.05 ± 1.12	3.10 ± 1.32	2.94 ± 1.22	3.08 ± 0.14	T x T	0.28
	Overall	3.36 ± 1.06	3.03 ± 0.98	3.03 ± 1.17	3.01 ± 1.17*			

Values are means ± standard deviations. Readiness to perform data were analyzed by MANOVA with repeated measures. Greenhouse-Geisser treatment (T), time (T), and treatment x time (T x T) interaction p-levels are reported with univariate treatment p-levels. MANOVA analysis revealed overall Wilks' Lambda treatment ($p = 0.99$), time ($p = 0.08$), and treatment x time ($p = 0.36$). * represents $p < 0.05$ difference from Day 0. § represents $p < 0.05$ difference from Day 2.† represents $p < 0.05$ difference from Day 4.

Table 4.8 Cognitive Function

	Treatment	Time (wks) Day 0	Day 2	Day 4	Day 7			
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SE		p-level
Word (counts)	PLA	114.7 ± 14.2	118.2 ± 16.0	118.1 ± 16.5	116.0 ± 14.9 [a]	116.7 ± 1.7	Treatment	0.68
	PWS	112.1 ± 19.0	119.8 ± 18.2	118.6 ± 18.9	118.6 ± 16.9	117.3 ± 2.0	Time	< 0.001
	PWS150	115.4 ± 16.3	119.9 ± 18.6	121.7 ± 15.6	126.9 ± 20.9 [c]	121.0 ± 2.0	T x T	0.02
	Overall	114.1 ± 16.4	119.3 ± 17.4*	119.5 ± 16.8*	120.5 ± 18.0*			
Color (counts)	PLA	84.4 ± 11.4	88.4 ± 13.2	87.3 ± 12.8	88.9 ± 11.6	87.3 ± 1.4	Treatment	0.60
	PWS	83.2 ± 11.9	86.2 ± 15.1	85.4 ± 12.4	85.5 ± 12.4	85.0 ± 1.4	Time	< 0.001
	PWS150	85.7 ± 13.8	87.1 ± 15.6	89.7 ± 13.7	93.8 ± 15.2	89.1 ± 1.6	T x T	0.10
	Overall	84.4 ± 12.5	87.2 ± 14.5*	87.5 ± 12.9*	89.4 ± 13.4*			
Word-Color (counts)	PLA	59.8 ± 12.6	63.1 ± 13.2	62.0 ± 14.0	63.3 ± 12.8	62.5 ± 1.5	Treatment	0.82
	PWS	60.0 ± 16.1	63.1 ± 16.0	61.9 ± 15.1	63.4 ± 12.2	62.1 ± 1.6	Time	< 0.001
	PWS150	59.8 ± 12.6	64.1 ± 14.6	65.3 ± 14.1	68.3 ± 14.2	64.5 ± 1.6	T x T	0.70
	Overall	60.1 ± 13.7	63.4 ± 14.4*	63.1 ± 14.3*	65.6 ± 13.0*†			
Total Stroop Results (counts)	PLA	259.0 ± 33.9	269.8 ± 38.0	267.5 ± 39.7	270.3 ± 34.5	266.6 ± 4.15	Treatment	0.67
	PWS	255.3 ± 42.5	269.2 ± 44.2	266.0 ± 42.3	267.6 ± 39.3	264.5 ± 4.78	Time	< 0.001
	PWS150	261.7 ± 40.8	271.2 ± 44.0	276.9 ± 39.6	289.1 ± 47.0	274.7 ± 4.97	T x T	0.07
	Overall	258.7 ± 38.6	270.0 ± 41.4*	270.1 ± 40.1*	275.7 ± 41.0*†			

Values are means ± standard deviations. Cognitive function data were analyzed by MANOVA with repeated measures. Greenhouse-Geisser treatment (T), time (T), and treatment x time (T x T) interaction p-levels are reported with univariate treatment p-levels. MANOVA analysis revealed overall Wilks' Lambda treatment ($p = 0.87$), time ($p < 0.001$), and treatment x time ($p = 0.41$). [a] denotes a significant difference from PWS150. [b] denotes a significant difference from PWS. [c] denotes a significant difference from PLA.* represents $p < 0.05$ difference from day 0. represents $p < 0.05$ difference from Day 4.

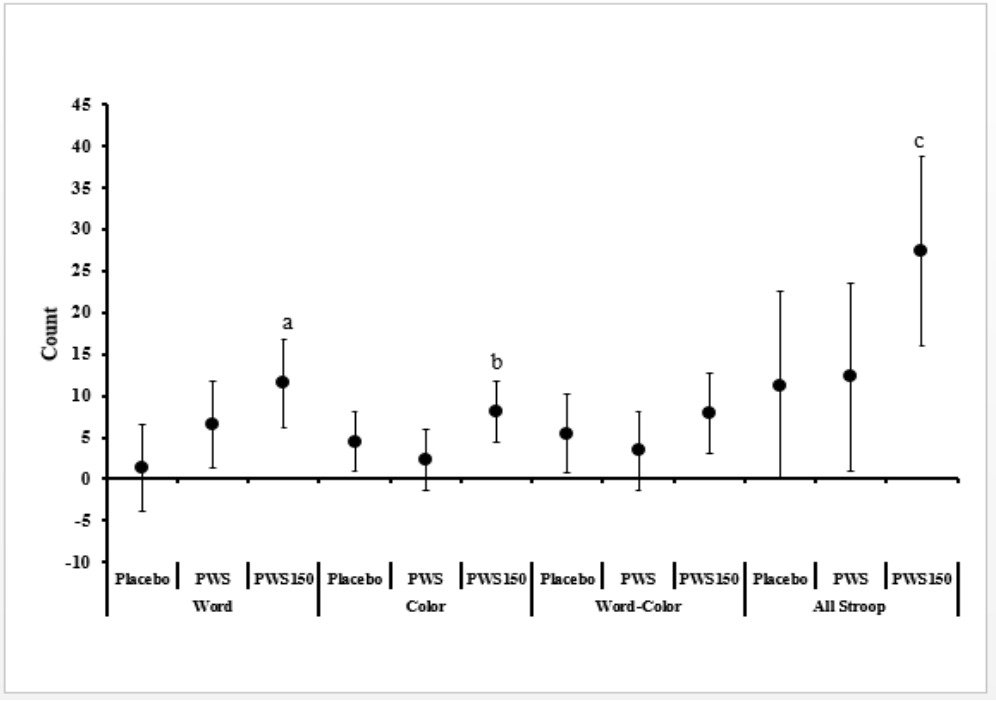

Figure 4.6 Data Represent the Change from Day 0 to Day 7 in Stroop Testing Associated Treatment. Statistical Significance is Noted as: (a) Significant vs. Placebo ($p = 0.008$), (b) Significant vs. PWS ($p < 0.03$) and (c) Significant vs. PWS ($p < 0.05$).

Table 4.9 Bench Press and Leg Press Endurance

Variable	Treatment	Day 0	Day 7			
		Mean ± SD	Mean ± SD	Mean ± SE		p-level
Bench Press 3rd Set Lifting Volume (kg)	PLA	803 ± 310	806 ± 321	794 ± 53	Treatment	0.72
	PWS	856 ± 318	888 ± 320	872 ± 51	Time	0.36
	PWS150	866 ± 371	892 ± 320	879 ± 55	T x T	0.86
	Overall	842 ± 329	862 ± 317			
Bench Press Total Lifting Volume (kg)	PLA	2,166 ± 664	2,551 ± 1,768	2,358 ± 216	Treatment	0.88
	PWS	2,241 ± 578	2,226 ± 565	2,234 ± 91	Time	0.27
	PWS150	2,251 ± 663	2,276 ± 630	2,264 ± 103	T x T	0.33
	Overall	2,219 ± 626	2,351 ± 1,120			
Leg Press 3rd Set Lifting Volume (kg)	PLA	6,401 ± 2,173	7,472 ± 2,627	6,936 ± 395	Treatment	0.12
	PWS	7,459 ± 1,932	8,146 ± 3,093	7,802 ± 416	Time	0.08
	PWS150	8,604 ± 3,403	8,510 ± 2,573	8,557 ± 482	T x T	0.31
	Overall	7,488 ± 2,695	8,043 ± 2,758			
Leg Press Total Lifting Volume (kg)	PLA	13,090 ± 3,487	14,161 ± 3,445	13,625 ± 561	Treatment	0.31
	PWS	14,210 ± 3,147	14,835 ± 4,110	14,523 ± 588	Time	0.75
	PWS150	16,276 ± 9,127	15,199 ± 3,665	15,737 ± 1,116	T x T	0.39
	Overall	14,525 ± 5,970	14,732 ± 3,708			

Values are means ± standard deviations. Bench press and leg press performance were analyzed by MANOVA with repeated measures. Greenhouse-Geisser treatment (T), time (T), and treatment x time (T x T) interaction p-levels are reported with univariate treatment p-levels. MANOVA analysis revealed overall Wilks' Lambda treatment ($p = 0.38$), time ($p = 0.10$), and treatment x time ($p = 0.64$).

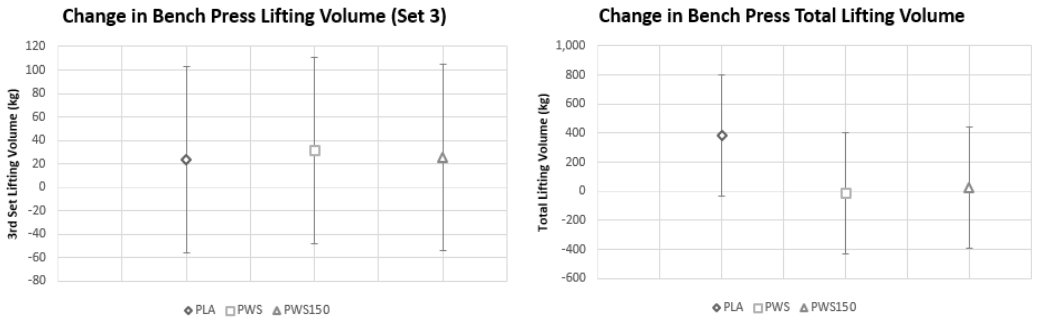

Figure 4.7 Changes from Day 0 (95% CI) in Bench Press 3rd Set Lifting Volume and Total Lifting Volume.

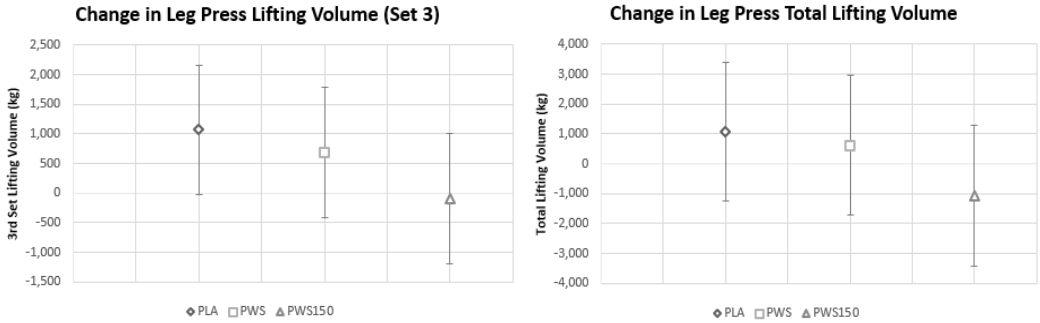

Figure 4.8 Changes from Day 0 (95% CI) in Leg Press 3rd Set Lifting Volume and Total Lifting Volume.

Anaerobic Capacity Test

Results of the Wingate testing are presented in Table 4.11. Figure 4.10 presents the changes from day 0 in Wingate mean power, peak power, total work, minimum power, and rate of fatigue. A MANOVA analysis was run in order to assess changes in anaerobic capacity variables. MANOVA analysis revealed overall Wilks' Lambda treatment x time ($p = 0.44$). Overall, no significant interactions were found for Wingate mean power ($p = 0.23$); peak power ($p = 0.87$); total work ($p = 0.16$); minimum power ($p = 0.09$, partial $\eta^2 = 0.07$ – medium effect); and rate of fatigue ($p = 0.55$). However, we

observed a significant change in Wingate mean power at day 7 only in PWS150 (26.0 95% CI 1.85, 50.3) but not PWS (-3.83 95% CI -28.7, 21.0), and PLA (8.88 95%

Table 4.10 The 3rd Set of Bench Press Tendo Performance

Variable	Treatment	Day 0	Day 7			
		Mean ± SD	Mean ± SD	Mean ± SE		p-level
Tendo Mean Power	PLA	251 ± 83	265 ± 89	259 ± 13	Treatment	0.91
(Watt)	PWS	247 ± 67	254 ± 73	247 ± 11	Time	0.08
	PWS150	258 ± 80	264 ± 70	261 ± 12	T x T	0.80
	Overall	252 ± 76	261 ± 76			
Tendo Peak Power	PLA	331 ± 116	344 ± 121	334 ± 19	Treatment	0.97
(Watt)	PWS	341 ± 110	346 ± 115	339 ± 18	Time	0.24
	PWS150	333 ± 111	340 ± 101	337 ± 17	T x T	0.88
	Overall	335 ± 110	343 ± 110			
Tendo Mean Velocity	PLA	0.37 ± 0.06	0.39 ± 0.07	0.38 ± 0.01	Treatment	0.56
(meter/s)	PWS	0.36 ± 0.05	0.37 0.06	0.37 ± 0.01	Time	0.053
	PWS150	0.37 ± 0.06	0.39 ± 0.04	0.39 ± 0.01	T x T	0.81
	Overall	0.37 ± 0.06	0.38 ± 0.06			

Values are means ± standard deviations. Bench press Tendo unit variables were analyzed by MANOVA with repeated measures. Greenhouse-Geisser treatment (T), time (T), and treatment x time (T x T) interaction p-levels are reported with univariate treatment p-levels. MANOVA analysis revealed overall Wilks' Lambda treatment ($p = 0.83$), time ($p = 0.27$), and treatment x time ($p = 0.96$).

CI -17.5, 35.2). After 7 days of supplementation, percent change in peak power was -1.6%, 1.1%, and -1.2% for PLA, PWS, and PWS150, respectively; mean power changed by 4.5%, 0.8%, and 1.2% for PLA, PWS, and PWS150, respectively; total work increased by 2.0%, 0.9%, and 3.7% for PLA, PWS, and PWS150, respectively; minimum power changed by -6.3%, 11.7%, and 11.2% for PLA, PWS, and PWS150, respectively; and mean power changed by -0.3%, -1.0%, and -0.7% for PLA, PWS, and PWS150, respectively. The results from exercise performance tests analyses provide evidence which accepts the directional hypothesis 5 which states that PWS supplementation will affect exercise performance.

Clinical Chemistry Panels

Table 4.12 presents whole blood markers assessment. MANOVA analysis revealed overall Wilks' Lambda treatment x time ($p = 0.09$). Univariate analysis revealed no significant treatment by time interaction for any whole blood marker. After 7 days of supplementation, percent change in MCV was -0.02%, 0.37%, and -0.16% for PLA,

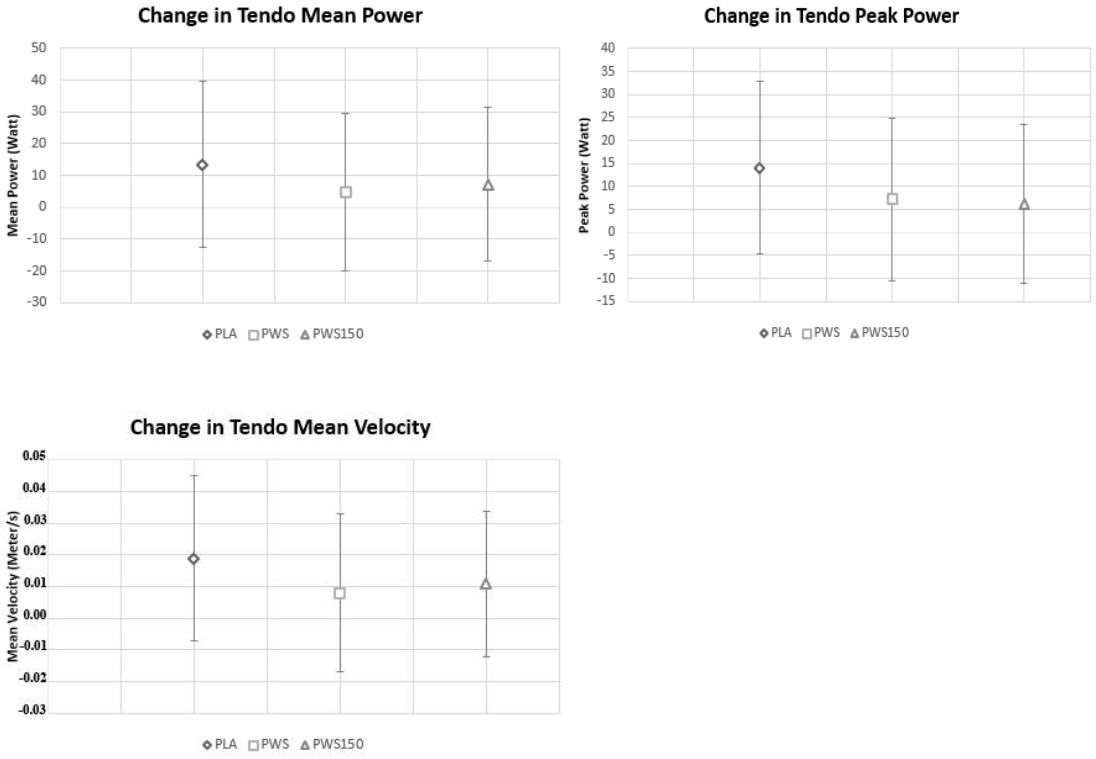

Figure 4.9 Changes from Day 0 (95% CI) in Bench Press Mean Power, Peak Power, and Mean Velocity.

Table 4.11 Anaerobic Sprint Capacity

Variable	Treatment	Day 0	Day 7			
		Mean ± SD	Mean ± SD	Mean ± SE		p-level
Mean Power (Watt)	PLA	551 ± 114	560 ± 110	550 ± 18	Treatment	0.83
	PWS	566 ± 125	562 ± 109	558 ± 18	Time	0.15
	PWS150	567 ± 118	593 ± 159	580 ± 22	T x T	0.23
	Overall	562 ± 109	573 ± 128			
Peak Power (Watt/kg)	PLA	1,523 ± 382	1,466 ± 451	1,547 ± 70	Treatment	0.65
	PWS	1,610 ± 492	1,595 ± 491	1,614 ± 77	Time	0.27
	PWS150	1,646 ± 411	1,611 ± 535	1,628 ± 76	T x T	0.87
	Overall	1,597 ± 427	1,561 ± 490			
Total Work (Joules)	PLA	16,687 ± 3,467	16,644 ± 3,290	16,531 ± 548	Treatment	0.87
	PWS	16,515 ± 3,536	17,096 ± 3,329	16,612 ± 553	Time	0.50
	PWS150	17,329 ± 3,650	16,644 ± 3290	17,237 ± 575	T x T	0.16
	Overall	16,859 ± 3,507	16,977 ± 3,335			
Minimum Power (Watt)	PLA	237 ± 79	221 ± 77	217 ± 14	Treatment	0.53
	PWS	219 ± 78	239 ± 70	227 ± 12	Time	0.22
	PWS150	235 ± 88	189 ± 117	212 ± 17	T x T	0.09
	Overall	230 ± 81	216 ± 92			
Rate of Fatigue (%)	PLA	85.9 ± 7.9	85.4 ± 8.0	87.6 ± 1.3	Treatment	0.69
	PWS	86.4 ± 8.5	83.3 ± 5.5	85.8 ± 1.3	Time	0.50
	PWS150	86.5 ± 10.1	87.1 ± 11.4	86.8 ± 1.7	T x T	0.55
	Overall	86.3 ± 8.8	85.3 ± 8.7			

Values are means ± standard deviations. Anaerobic sprint capacity data were analyzed by MANOVA with repeated measures. Greenhouse-Geisser treatment (T), time (T), and treatment x time (T x T) interaction p-levels are reported with univariate treatment p-levels. MANOVA analysis revealed overall Wilks' Lambda treatment ($p = 0.89$), time ($p = 0.29$), and treatment x time ($p = 0.44$).

PWS, and PWS150, respectively; MCH changed by 4.6%, 5.4%, and -0.31% for PLA, PWS, and PWS150, respectively; MCHC changed by 4.8%, 5.1%, and -0.01% for PLA, PWS, and PWS150, respectively; RBCDW changed by 2.5%, 1.4%, and -1.4% for PLA, PWS, and PWS150, respectively; platelet count changed by -8.0%, -7.1%, and 1.1% for PLA, PWS, and PWS150, respectively; WBC changed by -1.6%, 3.7%, and -0.95% for PLA, PWS, and PWS150, respectively; RBC changed by -4.2%, -5.7%, and 2.9% for PLA, PWS, and PWS150, respectively; hematocrit changed by -5.8%, -3.6%, and 2.1% for PLA, PWS, and PWS150, respectively; and hemoglobin changed by -1.5%, -1.2%, and 1.6% for PLA, PWS, and PWS150, respectively.

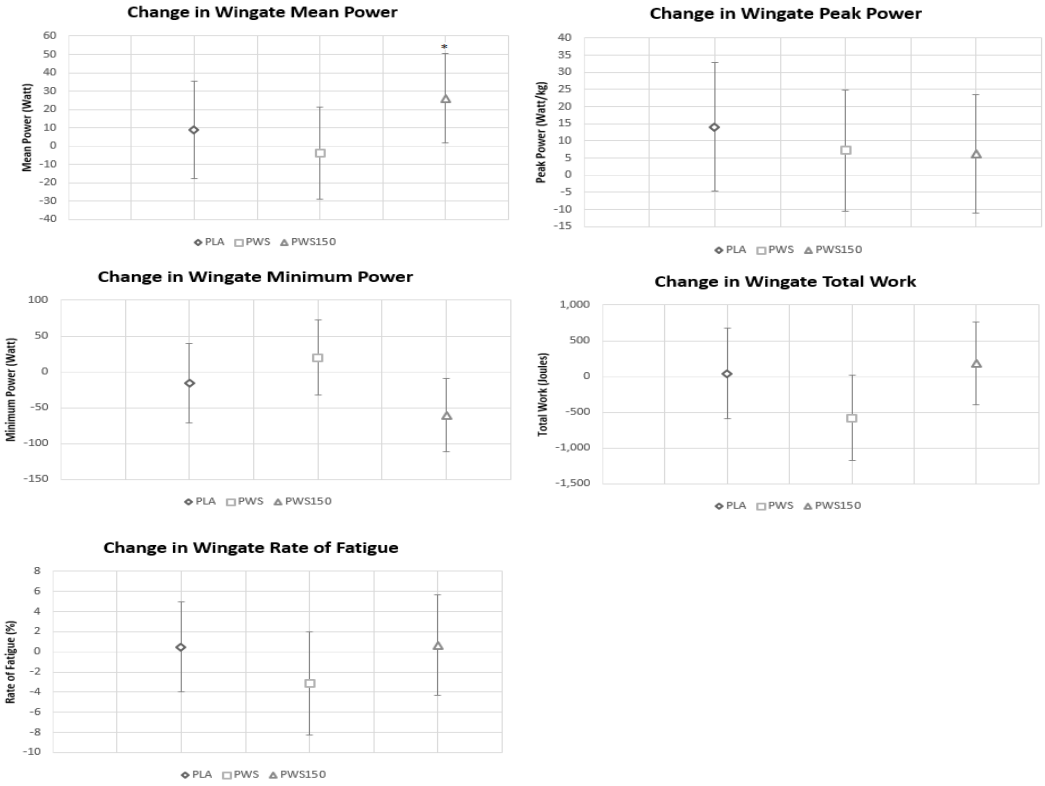

Figure 4.10 Changes from Day 0 (95% CI) in Wingate Mean Power, Peak Power, Minimum Power, Total Work, and Rate of Fatigue. * Represents $p < 0.05$ difference from day 0.

Blood Chemistry

General markers of health are presented in Table 4.13. MANOVA analysis revealed overall Wilks' Lambda treatment x time ($p = 0.64$). Although a significant time effect was detected for serum Cr ($p = 0.02$), univariate analysis did not show any significant treatment x time interaction or mean changes for any health marker. After 7 days of supplementation, percent change in ALP was -5.0%, 2.1%, and -0.95% for PLA, PWS, and PWS150, respectively; ALT changed by 13.9%, -1.4%, and -1.3% for PLA, PWS, and PWS150, respectively; AST changed by 0.97%, 1.6%, and 9.5% for PLA,

PWS, and PWS150, respectively; CK changed by 14.9%, 1.7%, and 11.1% for PLA, PWS, and PWS150, respectively; LDH changed by 4.7%, 1.3%, and 0.61% for PLA, PWS, and PWS150, respectively; BUN changed by -3.4%, 2.4%, and 1.6% for PLA, PWS, and PWS150, respectively; creatinine ratio changed by 0.51%, -0.52%, and 1.5% for PLA, PWS, and PWS150, respectively; BUN:creatinine ratio changed by -3.1%, 3.5%, and 0.92% for PLA, PWS, and PWS150, respectively; and Cr changed by 14.3%, 13.7%, and 13.9% for PLA, PWS, and PWS150, respectively. Findings for plasma lipid and glucose levels are presented in Table 4.14. MANOVA analysis revealed overall Wilks' Lambda treatment x time ($p = 0.34$). Although a tendency was observed towards a significant interaction for TC/HDL ratio between PLA and PWS ($p = 0.09$, partial $\eta^2 = 0.08$ – medium effect), univariate analysis revealed no significant time x treatment interaction for any plasma lipids and glucose. After 7 days of supplementation, percent change in TC was -5.2%, 2.4%, and -0.51% for PLA, PWS, and PWS150, respectively; HDL changed by 0.21%, 0.89%, and 4.6% for PLA, PWS, and PWS150, respectively; TC:HDL ratio changed by -4.4%, 2.8%, and 1.1% for PLA, PWS, and PWS150, respectively; LDL changed by 0.71%, 9.2%, and -0.28% for PLA, PWS, and PWS150, respectively; TG changed by -5.2%, 10.9%, and -4.7% for PLA, PWS, and PWS150, respectively; and glucose changed by -6.1%, 5.5%, and 0.86% for PLA, PWS, and PWS150, respectively. Table 4.15 indicates Chi squared categorical analysis. We did not observe any significant effects when examining changes for blood parameters outside of normal clinical boundaries. This is not to say that some participant's blood parameters did not exceed normal clinical limits at follow-up. However, an examination of our data

Table 4.12 Whole Blood Markers

Marker	Treatment	Day 0	Day 7			
		Mean ± SD	Mean ± SD	Mean ± SE		p-level
MCV	PLA	93.2 ± 3.7	88.8 ± 21.6	91.0 ± 2.5	Treatment	0.57
(fL)	PWS	93.0 ± 3.4	93.4 ± 3.7	93.2 ± 0.5	Time	0.34
	PWS150	93.4 ± 3.5	93.2 ± 3.5	93.3 ± 0.5	T x T	0.35
	Overall	93.2 ± 3.5	91.8 ± 12.8			
MCH	PLA	31.0 ± 1.8	31.0 ± 8.4	31.0 ± 0.9	Treatment	0.54
(pg/cell)	PWS	31.1 ± 2.5	32.8 ± 3.5	31.9 ± 0.5	Time	0.49
	PWS150	32.2 ± 2.9	32.0 ± 2.4	32.1 ± 0.4	T x T	0.48
	Overall	31.4 ± 2.4	31.9 ± 5.4			
MCHC	PLA	33.3 ± 1.1	33.0 ± 9.0	33.1 ± 1.0	Treatment	0.43
(g/dl)	PWS	33.4 ± 1.8	35.1 ± 3.8	34.3 ± 0.5	Time	0.58
	PWS150	34.4 ± 2.8	34.3 ± 2.4	34.4 ± 0.4	T x T	0.53
	Overall	33.7 ± 2.0	34.1 ± 5.7			
RBCDW	PLA	12.8 ± 0.5	12.4 ± 3.0	12.6 ± 0.3	Treatment	0.47
(%)	PWS	12.9 ± 0.6	13.0 ± 0.7	13.0 ± 0.1	Time	0.60
	PWS150	13.0 ± 0.6	12.8 ± 0.7	12.9 ± 0.1	T x T	0.64
	Overall	12.9 ± 0.6	12.8 ± 1.8			
Platelet Count	PLA	239 ± 51	198 ± 85	218 ± 11	Treatment	0.98
($x10^3/\mu l$)	PWS	232 ± 59	211 ± 65	221 ± 10	Time	0.01
	PWS150	221 ± 51	220 ± 49	221 ± 8	T x T	0.17
	Overall	231 ± 53	210 ± 68*			
WBC	PLA	6.5 ± 1.8	6.3 ± 1.7	6.4 ± 0.2	Treatment	0.77
($x10^3/\mu l$)	PWS	5.9 ± 1.5	6.1 ± 1.5	6.0 ± 0.2	Time	0.60
	PWS150	6.3 ± 1.8	6.1 ± 1.7	6.2 ± 0.2	T x T	0.57
	Overall	6.5 ± 1.8	6.1 ± 1.6			
RBC	PLA	4.8 ± 0.4	4.3 ± 1.2	4.6 ± 0.1	Treatment	0.71
($x10^6/\mu l$)	PWS	4.8 ± 0.4	4.5 ± 0.5	4.6 ± 0.1	Time	0.053
	PWS150	4.6 ± 0.4	4.7 ± 0.4	4.7 ± 0.1	T x T	0.10
	Overall	4.8 ± 0.4	4.5 ± 0.8			
Hematocrit	PLA	45.4 ± 4.1	39.9 ± 12.9	42.7 ± 1.6	Treatment	0.56
(%)	PWS	44.2 ± 4.4	42.5 ± 5.9	43.3 ± 0.8	Time	0.07
	PWS150	44.1 ± 3.8	44.7 ± 4.2	44.4 ± 0.6	T x T	0.12
	Overall	44.6 ± 4.1	42.4 ± 8.6			
Hemoglobin	PLA	15.1 ± 1.4	14.0 ± 3.6	14.6 ± 0.4	Treatment	0.53
(g/dl)	PWS	15.0 ± 1.1	14.7 ± 1.2	14.9 ± 0.1	Time	0.23
	PWS150	15.0 ± 1.1	15.2 ± 1.4	15.1 ± 0.2	T x T	0.18
	Overall	15.0 ± 1.2	14.7 ± 2.3			

Values are means ± standard deviations. Whole blood markers were analyzed by MANOVA with repeated measures. Greenhouse-Geisser treatment (T), time (T), and treatment x time (T x T) interaction p-levels are reported with univariate treatment p-levels. MANOVA analysis revealed overall Wilks' Lambda treatment ($p = 1.0$), time ($p = 0.43$), and treatment x time ($p = 0.09$). * Represents $p < 0.05$ difference from day 0.

shows these perturbations to be distributed equally across all treatments, inclusive of the PLA. The results from general health markers analyses provide evidence which accepts the directional hypothesis 6 which states that there will be no significant differences in health markers.

Short Sleep and Caffeine Questionnaires

Tables 4.16, 4.17, and 4.18 present stimulant effects before and after 7 days of supplement ingestion. MANOVA analysis of SQQ revealed overall Wilks' Lambda treatment x time ($p = 0.26$). MANOVA analysis did not indicate any significant interactions among treatments regarding sleep quality results. The results from SQQ provide evidence which accepts the directional hypothesis 7 which states that there will be no significant differences in sleep quality inventory.

MANOVA analysis of CQ revealed overall Wilks' Lambda treatment x time ($p = 0.33$). The results from CQ indicated that there was a significant decrease in response to "*Heavy feelings in arms and legs*" in PWS150 compared to PWS and PLA treatments ($p = 0.02$). The overall multivariate test did not show any significant difference among treatments. Therefore, results from CQ provide evidence which accepts the directional hypothesis 8 which states that there will be no significant differences in caffeine tolerance inventory.

Table 4.13 General Health Markers

	Marker	Treatment	Day 0	Day 7			
			Mean ± SD	Mean ± SD	Mean ± SE		p-level
Liver Enzymes	ALP	PLA	85.6 ± 18.6	80.5 ± 19.3	83.0 ± 3.0	Treatment	0.61
	(U/L)	PWS	88.5 ± 21.3	89.2 ± 22.5	88.9 ± 3.5	Time	0.25
		PWS150	89.0 ± 22.0	86.5 ± 18.7	87.7 ± 3.5	T x T	0.45
		Overall	87.6 ± 20.2	85.4 ± 20.3			
	ALT	PLA	28.4 ± 11.5	32.6 ± 21.5	30.5 ± 2.7	Treatment	0.89
	(U/L)	PWS	29.1 ± 11.8	28.1 ± 10.9	28.6 ± 1.8	Time	0.81
		PWS150	30.9 ± 13.6	28.9 ± 10.6	29.9 ± 2.1	T x T	0.29
		Overall	29.4 ± 12.1	29.9 ± 15.3			
	AST	PLA	30.0 ± 9.5	30.0 ± 12.7	30.0 ± 1.8	Treatment	0.85
	(U/L)	PWS	29.8 ± 9.3	28.8 ± 9.0	29.3 ± 1.4	Time	0.64
		PWS150	26.8 ± 7.6	29.7 ± 11.8	28.3 ± 1.7	T x T	0.46
		Overall	29.0 ± 8.9	29.5 ± 11.1			
Muscle Enzymes	CK	PLA	327 ± 267	328 ± 227	327 ± 41	Treatment	0.74
	(U/L)	PWS	298 ± 237	260 ± 176	279 ± 33	Time	0.60
		PWS150	251 ± 148	322 ± 205	286 ± 31	T x T	0.12
		Overall	293 ± 223	301 ± 201			
	LDH	PLA	178 ± 36	184 ± 56	181 ± 7	Treatment	0.76
	(U/L)	PWS	174 ± 29	175 ± 27	175 ± 4	Time	0.74
		PWS150	175 ± 35	173 ± 32	174 ± 5	T x T	0.83
		Overall	176 ± 33	178 ± 40			
Kidney Enzymes & Ratio	BUN	PLA	18.5 ± 5.7	17.0 ± 3.5	17.8 ± 0.7	Treatment	0.90
	(mg/dl)	PWS	17.2 ± 4.5	17.1 ± 4.5	17.2 ± 0.7	Time	0.31
		PWS150	17.7 ± 5.4	17.5 ± 4.8	17.6 ± 0.9	T x T	0.59
		Overall	17.8 ± 5.1	17.2 ± 4.2			
	Creatinine	PLA	1.34 ± 0.32	1.32 ± 0.28	1.33 ± 0.05	Treatment	0.83
	(mg/dl)	PWS	1.34 ± 0.24	1.32 ± 0.22	1.33 ± 0.04	Time	0.76
		PWS150	1.28 ± 0.19	1.29 ± 0.25	1.29 ± 0.04	T x T	0.90
		Overall	1.32 ± 0.25	1.31 ± 0.25*			
	BUN:Creatinine	PLA	14.1 ± 4.3	13.3 ± 3.7	13.7 ± 0.6	Treatment	0.87
	Ratio	PWS	13.2 ± 4.1	13.3 ± 4.3	13.2 ± 0.6	Time	0.39
		PWS150	14.0 ± 4.5	13.7 ± 3.6	13.8 ± 0.7	T x T	0.65
		Overall	13.8 ± 4.2	13.4 ± 3.9			
	Creatine	PLA	191.7 ± 69.9	205.7 ± 82.1	198.7 ± 12.2	Treatment	0.70
	(μM)	PWS	191.6 ± 80.3	218.1 ± 98.4	204.8 ± 14.5	Time	0.02
		PWS150	211.1 ± 86.8	235.1 ± 158.4	223.1 ± 20.5	T x T	0.85
		Overall	198.2 ± 78.4	219.6 ± 116.2*			

Values are means ± standard deviations. Health markers were analyzed by MANOVA with repeated measures. Greenhouse-Geisser treatment (T), time (T), and treatment x time (T x T) interaction p-levels are reported with univariate treatment p-levels. MANOVA analysis revealed overall Wilks' Lambda treatment ($p = 0.97$), time ($p = 0.91$), and treatment x time ($p = 0.64$). * Represents $p < 0.05$ difference from day 0.

Table 4.14 Blood Lipids and Glucose

Marker	Treatment	Day 0	Day 7			
		Mean ± SD	Mean ± SD	Mean ± SE		p-level
TC	PLA	187.2 ± 46.2	172.3 ± 38.8	179.8 ± 6.9	Treatment	0.81
(mg/dl)	PWS	185.3 ± 32.6	188.2 ± 39.3	186.8 ± 5.7	Time	0.26
	PWS150	186.6 ± 39.0	182.9 ± 36.6	184.8 ± 6.1	T x T	0.28
	Overall	186.4 ± 38.9	181.2 ± 38.2			
HDL	PLA	68.7 ± 21.9	67.3 ± 20.1	68.0 ± 3.3	Treatment	0.99
(mg/dl)	PWS	67.5 ± 17.3	67.7 ± 17.4	67.6 ± 2.7	Time	0.87
	PWS150	67.4 ± 18.5	69.4 ± 19.4	68.4 ± 3.0	T x T	0.68
	Overall	67.9 ± 19.0	68.1 ± 18.7			
TC:HDL	PLA	2.77 ± 0.48	2.64 ± 0.48	2.71 ± 0.08	Treatment	0.72
Ratio	PWS	2.80 ± 0.52	2.86 ± 0.49	2.84 ± 0.08	Time	0.64
	PWS150	2.77 ± 0.46	2.78 ± 0.50	2.78 ± 0.08	T x T	0.09
	Overall	2.78 ± 0.48	2.76 ± 0.49			
LDL	PLA	130.5 ± 53.1	127.6 ± 58.8	129.0 ± 8.9	Treatment	0.42
(mg/dl)	PWS	135.6 ± 55.2	146.7 ± 63.5	141.1 ± 9.5	Time	0.52
	PWS150	153.5 ± 60.0	154.0 ± 68.5	153.8 ± 10.3	T x T	0.43
	Overall	139.8 ± 59.1	142.8 ± 63.6			
TG	PLA	96.1 ± 32.5	89.5 ± 35.9	92.8 ± 5.5	Treatment	0.39
(mg/dl)	PWS	102.7 ± 40.6	106.9 ± 45.0	104.8 ± 6.8	Time	0.22
	PWS150	118.7 ± 63.9	96.4 ± 35.7	107.6 ± 8.4	T x T	0.28
	Overall	105.8 ± 47.7	97.6 ± 39.1			
Glucose	PLA	111.2 ± 26.7	101.1 ± 14.9	106.2 ± 3.5	Treatment	0.81
(mg/dl)	PWS	108.1 ± 22.8	112.1 ± 18.9	110.1 ± 3.3	Time	0.46
	PWS150	108.7 ± 21.4	108.7 ± 23.6	108.7 ± 3.6	T x T	0.11
	Overall	109.4 ± 23.3	107.3 ± 19.6			

Values are means ± standard deviations. Health markers were analyzed by MANOVA with repeated measures. Greenhouse-Geisser treatment (T), time (T), and treatment x time (T x T) interaction p-levels are reported with univariate treatment p-levels. MANOVA analysis revealed overall Wilks' Lambda treatment ($p = 0.99$), time ($p = 0.41$), and treatment x time ($p = 0.32$).

Table 4.15 Prevalence of Blood Chemistry Changes Exceeding Normal Clinical Bounds

	Marker	Category	PLA	PWS	PWS150	p-level
Lipids & Glucose	**Cholesterol**	No Change	10	11	9	0.87
		Normal at Day 0, High at Day 7	2	3	2	
		High at Day 0, High at Day 7	2	3	4	
		High at Day 0, Normal at Day 7	5	2	4	
	HDL-C	No Change	19	19	18	0.36
		Normal at Day 0, High at Day 7	0	0	0	
		High at Day 0, High at Day 7	0	0	0	
		High at Day 0, Normal at Day 7	0	0	1	
	LDL-C	No Change	9	9	8	0.41
		Normal at Day 0, High at Day 7	1	3	0	
		High at Day 0, High at Day 7	5	6	8	
		High at Day 0, Normal at Day 7	4	1	3	
	Triglycerides	No Change	18	14	14	0.21
		Normal at Day 0, High at Day 7	0	3	2	
		High at Day 0, High at Day 7	1	0	0	
		High at Day 0, Normal at Day 7	0	2	3	
	Glucose	No Change	9	8	10	0.19
		Normal at Day 0, High at Day 7	2	5	2	
		High at Day 0, High at Day 7	6	5	3	
		High at Day 0, Normal at Day 7	5	0	2	
Muscle	**LDH**	No Change	0	0	3	0.10
		Normal at Day 0, High at Day 7	18	19	15	
		High at Day 0, High at Day 7	1	0	0	
		High at Day 0, Normal at Day 7	0	0	1	
	Creatine Kinase	No Change	0	0	3	0.41
		Normal at Day 0, High at Day 7	4	7	6	
		High at Day 0, High at Day 7	2	1	1	
		High at Day 0, Normal at Day 7	11	9	8	
Kidney	**Creatinine**	No Change	0	0	3	0.18
		Normal at Day 0, High at Day 7	9	10	11	
		High at Day 0, High at Day 7	3	1	0	
		High at Day 0, Normal at Day 7	4	3	3	
	BUN	No Change	0	0	3	0.13
		Normal at Day 0, High at Day 7	17	18	13	
		High at Day 0, High at Day 7	0	1	1	
		High at Day 0, Normal at Day 7	2	0	2	
Liver	**ALP**	No Change	0	0	3	0.23
		Normal at Day 0, High at Day 7	18	17	14	
		High at Day 0, High at Day 7	0	1	1	
		High at Day 0, Normal at Day 7	0	0	1	
	ALT	No Change	0	0	3	0.052
		Normal at Day 0, High at Day 7	17	19	15	
		High at Day 0, High at Day 7	2	0	0	
		High at Day 0, Normal at Day 7	0	0	1	
	AST	No Change	0	0	3	0.12
		Normal at Day 0, High at Day 7	18	18	14	
		High at Day 0, High at Day 7	1	1	2	
		High at Day 0, Normal at Day 7	0	0	0	

Data are frequency of occurrence. Significance is by chi-square analysis.

Table 4.16 Sleep Effects Before and After 7 Days of Supplement Ingestion (1)

	Treatment		Time (wks) Day 0	Day 2	Day 4	Day 7			
			Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SE		p-level
Hours of Sleep during past 48hrs (hrs)	PLA		6.71 ± 1.15	7.26 ± 1.36	7.26 ± 1.32	6.86 ± 1.49	7.03 ± 0.15	Treatment	0.99
	PWS		7.04 ± 1.77	7.01 ± 1.39	7.02 ± 1.47	6.88 ± 1.17	7.07 ± 0.17	Time	0.81
	PWS150		6.83 ± 1.73	7.20 ± 1.51	6.93 ± 1.64	7.04 ± 2.02	7.06 ± 0.20	T x T	0.72
	Overall		7.04 ± 1.59	7.16 ± 1.40	7.07 ± 1.46	6.93 ± 1.57†			
Time to Fall Asleep (min)	PLA		17.2 ± 12.6	16.8 ± 16.0	14.8 ± 8.6	22.1 ± 21.2	17.7 ± 1.7	Treatment	0.45
	PWS		20.7 ± 15.9	23.0 ± 17.1	21.4 ± 16.9	24.4 ± 22.1	22.4 ± 2.0	Time	0.06
	PWS150		17.2 ± 12.6	20.0 ± 15.7	15.0 ± 7.6	19.1 ± 13.0	17.8 ± 1.3	T x T	0.78
	Overall		18.4 ± 12.9	19.9 ± 16.2	17.1 ± 12.0	21.8 ± 19.0			
Hours of Sleep	PLA		6.71 ± 1.35	7.53 ± 3.08	7.34 ± 2.24	6.73 ± 2.95	7.08 ± 0.28	Treatment	0.78
	PWS		6.70 ± 1.69	7.10 ± 2.24	6.66 ± 2.11	7.13 ± 3.10	6.90 ± 0.27	Time	0.32
	PWS150		7.71 ± 3.48	7.30 ± 2.65	7.89 ± 3.64	6.78 ± 2.26	7.42 ± 0.35	T x T	0.16
	Overall		7.04 ± 2.37	7.31 ± 2.64	7.30 ± 2.75	6.88 ± 2.75			
Sleep Quality	PLA	(1) Very Good	1.94 ± 0.70	1.78 ± 0.53	1.84 ± 0.68	2.05 ± 0.70	1.91 ± 0.08	Treatment	0.35
	PWS	(2) Fairly Good	2.00 ± 0.66	2.05 ± 0.84	2.15 ± 0.76	2.31 ± 0.82	2.13 ± 0.09	Time	0.04
	PWS150	(3) Fairly Bad	1.78 ± 0.53	2.05 ± 0.62	1.73 ± 0.65	2.00 ± 0.57	1.89 ± 0.07	T x T	0.26
	Overall	(4) Very Bad	1.91 ± 0.63	1.96 ± 0.68	1.91 ± 0.71	2.12 ± 0.70*†			
Enthusiasm	PLA	(1) No Problem	1.73 ± 0.80	1.73 ± 0.65	1.52 ± 0.51	1.73 ± 0.56	1.68 ± 0.07	Treatment	0.57
	PWS	(2) Slight Problem	1.63 ± 0.68	1.52 ± 0.51	1.52 ± 0.61	1.52 ± 0.69	1.55 ± 0.07	Time	0.27
	PWS150	(3) Somewhat of a problem	1.84 ± 0.76	1.57 ± 0.60	1.68 ± 0.74	1.78 ± 0.71	1.72 ± 0.08	T x T	0.74
	Overall	(4) Big Problem	1.73 ± 0.74	1.61 ± 0.59	1.57 ± 0.62	1.68 ± 0.65			
Bed Partner	PLA	(1) No Roommate	1.89 ± 1.28	1.94 ± 1.22	2.05 ± 1.31	2.15 ± 1.38	2.01 ± 0.15	Treatment	0.92
	PWS	(2) Roommate in other Room	2.10 ± 1.19	2.00 ± 1.20	1.94 ± 1.12	1.84 ± 1.11	1.97 ± 0.13	Time	0.79
	PWS150	(3) Partner in Same room	1.73 ± 1.14	1.89 ± 1.24	1.94 ± 1.22	1.89 ± 1.24	1.87 ± 0.14	T x T	0.19
	Overall	(4) Partner in Bed	1.91 ± 1.19	1.94 ± 1.20	1.98 ± 1.20	1.96 ± 1.23			
Snoring	PLA	(1) Loud snoring	0.47 ± 1.12	0.31 ± 0.94	0.31 ± 0.94	0.15 ± 0.68	0.32 ± 0.11	Treatment	0.71
	PWS	(2) Long pauses between breaths	0.68 ± 1.24	0.15 ± 0.68	0.36 ± 0.95	0.47 ± 1.12	0.46 ± 0.12	Time	0.07
	PWS150	(3) Twitching/ Jerking	0.31 ± 0.94	0.31 ± 0.94	0.15 ± 0.68	0.31 ± 0.94	0.24 ± 0.09	T x T	0.55
	Overall	(4) Disorientation	0.49 ± 1.10	0.26 ± 0.85*	0.28 ± 0.86*	0.31 ± 0.92			

Values are means ± standard deviations. Sleep quality data were analyzed by MANOVA with repeated measures. Greenhouse-Geisser treatment (T), time (T), and treatment x time (T x T) interaction p-levels are reported with univariate treatment p-levels. MANOVA analysis revealed overall Wilks' Lambda treatment ($p = 0.86$), time ($p = 0.11$), and treatment x time ($p = 0.56$).Time is expressed according to the 24 clock ± SD as hr:min. * represents $p < 0.05$ difference from Day 0.† represents $p < 0.05$ difference from Day 4.

Table 4.17 Sleep Effects Before and After 7 Days of Supplement Ingestion (2)

Sleep Characteristics		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	*Cochran's Q*
Cannot get to sleep within 30 minutes	No	17	16	17	15	18	14	16	16	15	15	18	17	*0.46*
	Yes	2	3	2	4	1	5	3	3	4	4	1	2	
Wake up in the middle of the night or early morning	No	10	10	11	11	10	9	13	9	11	10	10	9	*0.68*
	Yes	9	9	8	8	9	10	6	10	8	9	9	10	
Have to get up to use the bathroom	No	14	13	15	15	12	14	14	14	14	15	15	15	*0.57*
	Yes	5	6	4	4	7	5	5	5	5	4	4	4	
Cannot breathe comfortably	No	18	19	18	19	19	19	19	19	19	19	19	19	*0.53*
	Yes	1	0	1	0	0	0	0	0	0	0	0	0	
Cough or snore loudly	No	17	17	18	18	18	18	18	18	18	19	19	19	*0.82*
	Yes	2	2	1	1	1	1	1	1	1	0	0	0	
Feel too cold	No	19	19	19	19	18	19	19	19	17	18	19	18	*0.34*
	Yes	0	0	0	0	1	0	0	0	2	1	0	1	
Feel too hot	No	17	17	16	15	19	18	18	18	18	18	18	18	*0.46*
	Yes	2	2	3	4	0	1	1	1	1	1	1	1	
Had bad dreams	No	19	19	19	19	19	19	19	18	19	18	18	18	*0.44*
	Yes	0	0	0	0	0	0	0	1	0	1	1	1	
Have pain	No	17	16	17	15	16	17	18	18	17	18	18	19	*0.56*
	Yes	2	3	2	4	3	2	1	1	2	1	1	0	

The presented values are frequencies. Sleep quality nominal data were analyzed by non-parametric Cochran's Q test.

Table 4.18 Stimulant Effects Before and After 7 Days of Supplement Ingestion (Caffeine)
0 = Not at all, 1 = A little, 2 = Moderately, 3 = Quite a bit 4 = Extremely

	Treatment	Time (wks)						
		Day 0	Day 2	Day 4	Day 7			
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SE		p-level
Drowsy /sleepy	PLA	1.31 ± 1.10	0.73 ± 0.80	0.73 ± 0.87	1.10 ± 1.14	0.97 ± 0.12	Treatment	0.87
	PWS	1.15 ± 1.11	0.84 ± 1.01	0.78 ± 0.71	0.94 ± 0.91	0.93 ± 0.11	Time	0.09
	PWS150	0.84 ± 0.95	0.84 ± 1.01	0.78 ± 0.91	0.94 ± 1.07	0.86 ± 0.11	T x T	0.74
	Overall	1.10 ± 1.06	0.80 ± 0.97	0.77 ± 0.82*	1.00 ± 1.03			
Self-confidence	PLA	2.73 ± 0.87	2.42 ± 0.83	2.42 ± 0.76	2.42 ± 0.96	2.50 ± 0.10	Treatment	0.96
	PWS	2.63 ± 0.76	2.57 ± 0.90	2.57 ± 0.83	2.31 ± 1.00	2.51 ± 0.10	Time	0.03
	PWS150	2.42 ± 0.83	2.52 ± 0.96	2.52 ± 0.90	2.37 ± 1.02	2.45 ± 0.10	T x T	0.37
	Overall	2.59 ± 0.82	2.50 ± 0.88	2.50 ± 0.82	2.33 ± 1.00*			
Yawning	PLA	0.94 ± 1.02	0.31 ± 0.58	0.42 ± 0.50	0.73 ± 0.87	0.61 ± 0.09	Treatment	0.70
	PWS	0.84 ± 0.76	0.42 ± 0.76	0.47 ± 0.61	1.00 ± 1.00	0.68 ± 0.09	Time	< 0.001
	PWS150	1.15 ± 0.89	0.57 ± 0.83	0.52 ± 0.77	0.84 ± 1.11	0.78 ± 0.11	T x T	0.73
	Overall	0.98 ± 0.89	0.43 ± 0.73*	0.47 ± 0.62*	0.85 ± 0.98§†			
Alert	PLA	2.15 ± 1.01	2.26 ± 1.09	2.31 ± 1.00	2.00 ± 1.15	2.18 ± 0.12	Treatment	0.90
	PWS	2.52 ± 0.90	2.31 ± 1.05	1.89 ± 0.87	2.26 ± 0.87	2.25 ± 0.11	Time	0.14
	PWS150	2.52 ± 0.69	2.26 ± 0.87	2.26 ± 0.73	2.10 ± 0.93	2.29 ± 0.09	T x T	0.25
	Overall	2.40 ± 0.88	2.28 ± 0.99	2.15 ± 0.88	2.12 ± 0.98*			
Tired /Fatigued	PLA	1.36 ± 1.11	0.73 ± 0.80	0.84 ± 0.95	1.26 ± 0.93	1.05 ± 0.12	Treatment	0.92
	PWS	1.15 ± 1.11	0.89 ± 1.00	1.26 ± 0.87	1.15 ± 1.06	1.12 ± 0.11	Time	0.10
	PWS150	1.10 ± 0.80	0.94 ± 1.07	1.00 ± 1.10	1.05 ± 1.12	1.03 ± 0.09	T x T	0.59
	Overall	1.21 ± 1.01	0.85 ± 0.98*	1.03 ± 0.98	1.15 ± 1.03			
Content	PLA	2.15 ± 0.95	1.94 ± 1.02	1.94 ± 1.12	1.89 ± 0.99	1.99 ± 0.12	Treatment	0.68
	PWS	2.47 ± 0.84	2.26 ± 0.87	2.21 ± 1.03	1.94 ± 1.07	2.22 ± 0.11	Time	0.07
	PWS150	2.00 ± 0.88	2.21 ± 0.91	2.15 ± 0.89	2.00 ± 0.94	2.09 ± 0.10	T x T	0.35
	Overall	2.21 ± 0.90	2.14 ± 0.93	2.10 ± 1.01	1.94 ± 0.98*			
Difficulty Concentrating	PLA	1.05 ± 0.91	0.52 ± 0.77	0.63 ± 0.49	0.89 ± 0.65	0.78 ± 0.09	Treatment	0.90
	PWS	0.57 ± 0.69	0.73 ± 0.87	0.84 ± 0.50	0.68 ± 0.67	0.71 ± 0.08	Time	0.41
	PWS150	0.78 ± 0.91	0.68 ± 0.58	0.57 ± 0.76	0.78 ± 0.85	0.71 ± 0.09	T x T	0.15
	Overall	0.80 ± 0.85	0.64 ± 0.74	0.68 ± 0.60	0.78 ± 0.72			
Irritable	PLA	0.31 ± 0.47	0.26 0.56	0.15 ± 0.37	0.21 ± 0.41	0.24 ± 0.05	Treatment	0.93
	PWS	0.36 ± 0.59	0.15 ± 0.37	0.21 ± 0.53	0.15 ± 0.37	0.22 ± 0.05	Time	0.48
	PWS150	0.26 ± 0.65	0.15 ± 0.37	0.31 ± 0.67	0.31 ± 0.58	0.26 ± 0.07	T x T	0.73
	Overall	0.31 ± 0.57	0.19 ± 0.44	0.22 ± 0.53	0.22 ± 0.46			
Heavy feelings in arms and legs	PLA	0.63 ± 1.01	0.42 ± 0.60 [a]	0.47 ± 0.84 [a,b]	0.63 ± 0.95 [a]	0.54 ± 0.10	Treatment	0.42
	PWS	0.52 ± 0.84	0.36 ± 0.59 [a]	0.21 ± 0.41	0.78 ± 0.91 [a]	0.47 ± 0.08	Time	0.08
	PWS150	0.26 ± 0.56	0.68 ± 1.15 [b,c]	0.10 ± 0.31 [c]	0.15 ± 0.50 [b,c]	0.30 ± 0.08	T x T	0.02
	Overall	0.47 ± 0.82	0.49 ± 0.82	0.26 ± 0.58*§	0.52 ± 0.84†			
Depressed Mood	PLA	0.05 ± 0.22	0.001 ± 0.001	0.10 ± 0.31	0.001 ± 0.001	0.04 ± 0.02	Treatment	0.74
	PWS	0.10 ± 0.31	0.05 ± 0.22	0.05 ± 0.22	0.10 ± 0.45	0.08 ± 0.04	Time	0.59
	PWS150	0.05 ± 0.22	0.10 ± 0.45	0.15 ± 0.50	0.05 ± 0.22	0.09 ± 0.04	T x T	0.66
	Overall	0.07 ± 0.25	0.05 ± 0.29	0.10 ± 0.36	0.05 ± 0.29			

Table 4.18 Continued

	Treatment	Time (wks) Day 0	Day 2	Day 4	Day 7			
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SE		p-level
Grouchy	PLA	0.15 ± 0.37	0.10 ± 0.31	0.10 ± 0.31	0.26 ± 0.73	0.04 ± 0.02	Treatment	0.94
	PWS	0.10 ± 0.45	0.15 ± 0.68	0.15 ± 0.37	0.05 ± 0.22	0.08 ± 0.04	Time	0.69
	PWS150	0.10 ± 0.31	0.05 ± 0.22	0.21 ± 0.53	0.15 ± 0.50	0.09 ± 0.04	T x T	0.38
	Overall	0.12 ± 0.38	0.10 ± 0.45	0.15 ± 0.41	0.15 ± 0.52			
Urge to do work related activity	PLA	1.78 ± 1.35	1.78 ± 1.22	1.73 ± 1.09	1.42 ± 1.01	1.68 ± 0.13	Treatment	0.89
	PWS	1.73 ± 1.14	2.05 ± 0.62	1.47 ± 0.69	1.84 ± 1.01	1.78 ± 0.10	Time	0.62
	PWS150	1.63 ± 1.16	1.52 ± 1.21	1.78 ± 1.03	1.63 ± 1.11	1.64 ± 0.13	T x T	0.13
	Overall	1.71 ± 1.20	1.78 ± 1.06	1.66 ± 0.95	1.63 ± 1.04			
Flu-like feelings	PLA	0.001 ± 0.001	0.001 ± 0.001	0.05 ± 0.22	0.001 ± 0.001	0.01 ± 0.02	Treatment	0.37
	PWS	0.001 ± 0.001	0.001 ± 0.001	0.001 ± 0.001	0.001 ± 0.001	0.001 ± 0.001	Time	0.32
	PWS150	0.001 ± 0.001	0.001 ± 0.001	0.001 ± 0.001	0.001 ± 0.001	0.001 ± 0.001	T x T	0.37
	Overall	0.001 ± 0.001	0.001 ± 0.001	0.01 ± 0.13	0.001 ± 0.001			
Headache	PLA	0.21 ± 0.41	0.15 ± 0.37	0.47 ± 0.90	0.31 ± 0.67	0.29 ± 0.07	Treatment	0.39
	PWS	0.15 ± 0.50	0.10 ± 0.45	0.15 ± 0.37	0.21 ± 0.53	0.16 ± 0.05	Time	0.16
	PWS150	0.21 ± 0.53	0.001 ± 0.001	0.21 ± 0.63	0.21 ± 0.53	0.16 ± 0.06	T x T	0.81
	Overall	0.19 ± 0.47	0.08 ± 0.34	0.28 ± 0.67§	0.24 ± 0.57§			
Talkative	PLA	1.31 ± 1.29	1.36 ± 1.38	1.52 ± 1.30	1.26 ± 1.14	1.37 ± 0.14	Treatment	0.98
	PWS	1.31 ± 1.20	1.50 ± 1.47	1.05 ± 1.12	1.42 ± 1.21	1.32 ± 0.14	Time	0.60
	PWS150	1.42 ± 1.07	1.37 ± 1.42	1.47 ± 1.34	1.05 ± 1.07	1.34 ± 0.14	T x T	0.25
	Overall	1.35 ± 1.17	1.42 ± 1.32	1.35 ± 1.26	1.24 ± 1.13			
Sluggish	PLA	0.57 ± 0.69	0.42 ± 0.50	0.31 ± 0.58	0.52 ± 0.77	0.46 ± 0.07	Treatment	0.60
	PWS	0.42 ± 0.69	0.31 ± 0.67	0.21 ± 0.41	0.31 ± 0.47	0.32 ± 0.07	Time	0.10
	PWS150	0.42 ± 0.60	0.36 ± 0.59	0.26 ± 0.56	0.52 ± 0.69	0.39 ± 0.07	T x T	0.96
	Overall	0.47 ± 0.65	0.36 ± 0.58	0.26 ± 0.51*	0.45 ± 0.65†			
Upset stomach	PLA	0.10 ± 0.31	0.10 ± 0.31	0.21 ± 0.71	0.21 ± 0.41	0.16 ± 0.05	Treatment	0.52
	PWS	0.26 ± 0.73	0.001 ± 0.001	0.15 ± 0.50	0.15 ± 0.50	0.14 ± 0.06	Time	0.25
	PWS150	0.10 ± 0.31	0.001 ± 0.001	0.05 ± 0.22	0.10 ± 0.31	0.07 ± 0.03	T x T	0.79
	Overall	0.15 ± 0.49	0.03 ± 0.18	0.14 ± 0.51	0.15 ± 0.41§			
Clearheaded	PLA	1.84 ± 1.16	1.63 ± 1.11	1.63 ± 1.11	1.47 ± 1.02	1.64 ± 0.13	Treatment	0.95
	PWS	1.73 ± 0.80	1.84 ± 1.06	1.73 ± 1.24	1.63 ± 1.11	1.74 ± 0.12	Time	0.08
	PWS150	1.68 ± 1.15	1.89 ± 1.28	1.68 ± 1.10	1.42 ± 1.07	1.67 ± 0.13	T x T	0.76
	Overall	1.75 ± 1.03	1.78 ± 1.14	1.68 ± 1.13	1.50 ± 1.05§			
Desire to socialize	PLA	1.57 ± 1.26	1.73 ± 1.28	1.63 ± 1.25	1.26 ± 1.04	1.55 ± 0.14	Treatment	0.97
	PWS	1.47 ± 1.12	1.68 ± 1.33	1.31 ± 1.36	1.47 ± 1.21	1.50 ± 0.13	Time	0.03
	PWS150	1.47 ± 1.17	1.57 ± 1.07	1.63 ± 1.34	1.21 ± 1.18	1.47 ± 0.14	T x T	0.61
	Overall	1.50 ± 1.16	1.66 ± 1.21	1.54 ± 1.19	1.31 ± 1.13§			
Energetic	PLA	1.73 ± 1.14	1.89 ± 1.14	1.89 ± 1.24	1.31 ± 1.00	1.71 ± 0.13	Treatment	0.99
	PWS	1.78 ± 1.18	1.89 ± 1.19	1.47 ± 1.02	1.63 ± 1.16	1.70 ± 0.13	Time	0.06
	PWS150	1.78 ± 0.97	1.63 ± 1.01	1.94 ± 1.26	1.47 ± 0.90	1.71 ± 0.12	T x T	0.24
	Overall	1.77 ± 1.08	1.80 ± 1.10	1.77 ± 1.18	1.47 ± 1.01*§†			

Table 4.18 Continued

	Treatment	Time (wks) Day 0	Day 2	Day 4	Day 7			
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SE		p-level
Nausea/ vomiting	PLA	0.05 ± 0.22	0.001 ± 0.001	0.21 ± 0.71	0.10 ± 0.31	0.04 ± 0.02	Treatment	0.50
	PWS	0.15 ± 0.37	0.001 ± 0.001	0.001 ± 0.001	0.10 ± 0.31	0.07 ± 0.03	Time	0.84
	PWS150	0.001 ± 0.001	0.10 ± 0.45	0.001 ± 0.001	0.001 ± 0.001	0.03 ± 0.05	T x T	0.11
	Overall	0.07 ± 0.25	0.03 ± 0.26	0.07 ± 0.41	0.07 ± 0.25			
Muscle pain/stiffness /aches	PLA	0.57 ± 0.83	0.63 ± 0.95	0.42 ± 0.60	0.47 ± 0.84	0.53 ± 0.09	Treatment	0.73
	PWS	0.57 ± 0.76	0.89 ± 0.80	0.42 ± 0.60	0.68 ± 0.88	0.64 ± 0.09	Time	0.06
	PWS150	0.57 ± 0.69	0.68 ± 1.10	0.42 ± 0.76	0.31 ± 0.82	0.50 ± 0.10	T x T	0.82
	Overall	0.57 ± 0.75	0.73 ± 0.95	0.42 ± 0.65§	0.49 ± 0.84			
Discouraged	PLA	0.001 ± 0.001	0.10 ± 0.31	0.10 ± 0.31	0.10 ± 0.31	0.08 ± 0.03	Treatment	0.80
	PWS	0.05 ± 0.22	0.001 ± 0.001	0.10 ± 0.31	0.15 ± 0.37	0.08 ± 0.03	Time	0.12
	PWS150	0.05 ± 0.22	0.10 ± 0.31	0.15 ± 0.50	0.15 ± 0.37	0.12 ± 0.04	T x T	0.82
	Overall	0.03 ± 0.18	0.07 ± 0.25	0.12 ± 0.38	0.14 ± .35*			
Dizziness	PLA	0.26 ± 0.73	0.001 ± 0.001	0.05 ± 0.22	0.10 ± 0.31	0.11 ± 0.05	Treatment	0.91
	PWS	0.15 ± 0.37	0.05 ± 0.22	0.001 ± 0.001	0.10 ± 0.45	0.08 ± 0.04	Time	0.03
	PWS150	0.15 ± 0.37	0.001 ± 0.001	0.15 ± 0.50	0.001 ± 0.001	0.08 ± 0.04	T x T	0.46
	Overall	0.19 ± 0.51	0.01 ± 0.13*	0.07 ± 0.31	0.07 ± 0.31			
Desire to work out	PLA	2.36 ± 1.21	2.26 ± 1.24	1.94 ± 1.31	1.78 ± 1.13	2.09 ± 0.14	Treatment	0.93
	PWS	2.21 ± 1.27	2.10 ± 1.04	1.78 ± 1.27	2.10 ± 1.48	2.05 ± 0.14	Time	0.07
	PWS150	2.10 ± 0.99	2.05 ± 1.07	1.94 ± 1.43	1.78 1.27	1.97 ± 0.14	T x T	0.77
	Overall	2.22 ± 1.14	2.14 ±1.10	1.89 ± 1.31	1.89 ± 1.29*			
Queasy	PLA	0.05 ± 0.22	0.001 ± 0.001	0.26 ± 0.65	0.001 ± 0.001	0.08 ± 0.04	Treatment	0.96
	PWS	0.10 ± 0.31	0.001 ± 0.001	0.15 ± 0.50	0.10 ± 0.31	0.09 ± 0.04	Time	0.053
	PWS150	0.05 ± 0.22	0.10 ± 0.45	0.21 ± 0.53	0.001 ± 0.001	0.09 ± 0.04	T x T	0.70
	Overall	0.07 ± 0.25	0.03 ± 0.26	0.21 ± 0.55§	0.03 ± 0.18†			
Nauseous	PLA	0.05 ± 0.22	0.001 ± 0.001	0.15 ± 0.50	0.001 ± 0.001	0.05 ± 0.03	Treatment	0.84
	PWS	0.15 ± 0.37	0.001 ± 0.001	0.001 ± 0.001	0.10 ± 0.31	0.07 ± 0.03	Time	0.62
	PWS150	0.05 ± 0.22	0.10 ± 0.45	0.001 ± 0.001	0.001 ± 0.001	0.04 ± 0.03	T x T	0.09
	Overall	0.08 ± 0.28	0.03 ± 0.26	0.05 ± 0.29	0.03 ± 0.18			
Vomiting	PLA	0.001 ± 0.001	0.001 ± 0.001	0.05 ± 0.22	0.05 ± 0.22	0.03 ± 0.02	Treatment	0.13
	PWS	0.001 ± 0.001	0.001 ± 0.001	0.001 ± 0.001	0.001 ± 0.001	0.001 ± 0.001	Time	0.50
	PWS150	0.001 ± 0.001	0.001 ± 0.001	0.001 ± 0.001	0.001 ± 0.001	0.001 ± 0.001	T x T	0.60
	Overall	0.001 ± 0.001	0.001 ± 0.001	0.01 ± 0.13	0.01 ± 0.13			
Headachy	PLA	0.21 ± 0.53	0.15 ± 0.37	0.42 ± 0.90	0.26 ± 0.56	0.26 ± 0.07	Treatment	0.63
	PWS	0.26 ± 0.45	0.10 ± 0.31	0.15 ± 0.37	0.15 ± 0.50	0.17 ± 0.05	Time	0.14
	PWS150	0.21 ± 0.53	0.001 ± 0.001	0.15 ± 0.50	0.26 ± 0.56	0.16 ± 0.05	T x T	0.52
	Overall	0.22 ± 0.50	0.08 ± 0.28*	0.24 ± 0.63	0.22 ± 0.53§			
Anxious	PLA	0.73 ± 1.19	0.21 ± 0.71	0.31 ± 0.94	0.42 ± 0.96	0.42 ± 0.11	Treatment	0.99
	PWS	0.47 ± 1.20	0.36 ± 0.95	0.36 ± 0.95	0.47 ± 0.84	0.42 ± 0.11	Time	0.42
	PWS150	0.21 ± 0.53	0.36 ± 0.59	0.57 ± 1.07	0.47 ± 0.84	0.41 ± 0.09	T x T	0.11
	Overall	0.47 ± 1.00	0.31 ± 0.75	0.42 ± 0.98	0.45 ± 0.86			

Table 4.18 Continued

	Treatment	Time (wks) Day 0	Day 2	Day 4	Day 7			
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SE		p-level
Nervous	PLA	0.10 ± 0.31	0.05 ± 0.22	0.10 ± 0.31	0.15 ± 0.50	0.11 ± 0.04	Treatment	0.87
	PWS	0.10 ± 0.45	0.05 ± 0.22	0.21 ± 0.71	0.10 ± 0.31	0.12 ± 0.05	Time	0.70
	PWS150	0.10 ± 0.31	0.10 ± 0.31	0.10 ± 0.45	0.001 ± 0.001	0.08 ± 0.04	T x T	0.78
	Overall	0.10 ± 0.36	0.07 ± 0.25	0.14 ± 0.51	0.08 ± 0.34			
Jittery	PLA	0.78 ± 1.18	0.26 ± 0.65	0.15 ± 0.50	0.21 ± 0.41	0.36 ± 0.09	Treatment	0.79
	PWS	0.63 ± 1.21	0.05 ± 0.22	0.001 ± 0.001	0.47 ± 1.02	0.29 ± 0.10	Time	<0.001
	PWS150	0.63 ± 1.30	0.10 ± 0.31	0.05 ± 0.22	0.21 ± 0.53	0.25 ± 0.09	T x T	0.68
	Overall	0.68 ± 1.21	0.14 ± 0.44*	0.07 ± 0.31*	0.29 ± 0.70*†			
Craving for caffeine	PLA	0.36 ± 1.01	0.47 ± 1.07	0.68 ± 1.20	0.63 ± 1.16	0.54 ± 0.13	Treatment	0.90
	PWS	0.31 ± 0.74	0.47 ± 0.90	0.52 ± 0.96	0.52 ± 0.96	0.46 ± 0.10	Time	0.13
	PWS150	0.63 ± 1.21	0.57 ± 1.16	0.63 ± 1.11	0.57 ± 1.12	0.61 ± 0.13	T x T	0.60
	Overall	0.43 ± 1.00	0.50 ± 1.03	0.61 ± 1.08*	0.57 ± 1.06			
Craving for coffee	PLA	0.36 ± 0.95	0.36 ± 0.95	0.31 ± 1.00	0.42 ± 1.07	0.37 ± 0.11	Treatment	0.82
	PWS	0.15 ± 0.50	0.42 ± 0.83	0.42 ± 0.90	0.52 ± 0.96	0.38 ± 0.09	Time	0.11
	PWS150	0.52 ± 1.07	0.57 ± 1.16	0.47 ± 1.02	0.57 ± 1.16	0.54 ± 0.12	T x T	0.32
	Overall	0.35 ± 0.87	0.45 ± 0.98	0.40 ± 0.96	0.50 ± 1.05			

Values are means ± standard deviations. Caffeine questionnaire data were analyzed by MANOVA with repeated measures. Greenhouse-Geisser treatment (T), time (T), and treatment x time (T x T) interaction p-levels are reported with univariate treatment p-levels. MANOVA analysis revealed overall Wilks' Lambda treatment ($p = 0.99$), time ($p < 0.001$), and treatment x time ($p = 0.33$). [a] denotes a significant difference from PWS150. [b] denotes a significant difference from PWS. [c] denotes a significant difference from PLA. * represents $p < 0.05$ difference from Day 0. § represents $p < 0.05$ difference from Day 2.† represents $p < 0.05$ difference from Day 4.

CHAPTER V

DISCUSSION AND CONCLUSIONS

Discussion

A variety of PWS's which contain several ingredients are broadly marketed to athletes and recreational exercisers with claims of improved performance or exercise effectiveness. The primary aim of this study was to examine a PWS at two doses on performance readiness, exercise performance and potential alterations in thermogenic and hemodynamic function and blood chemistries denoting hepatorenal and muscle enzyme function in healthy individuals. Furthermore, this study sought out to determine whether PWS150 at ~150% the PWS dose was more efficacious than the PWS to improve readiness to perform, cognitive function, and exercise performance. Our study adds to the known literature on PWS' by examining measured and perceived readiness to perform exercise. This latter point is important as number of multi-ingredient PWS' are available in the market, few studies attempt to match "exercise preparedness" and exercise performance.

In the current study, we observed significant increases in readiness, denoted by improved cognitive function examined by the Stroop Word-Color Test. Overall, we found that the PWS150 significantly improved all Stroop tested domains, as well as the sum of all scores combined. The changes denoted for the PLA and PWS treatments are less clear as the PLA demonstrated an improvement in two domains, but not the

summated score, while the PWS showed improvement in one domain, yet, demonstrated significant improvement for the summated score. Given the results of the Stroop Test we accept our hypothesis that the PWS150 formula improved performance readiness via improved cognitive function. While significant improvements were observed in PWS150 compared to PWS and PLA, other researchers reported similar improvements. Hoffman et al. [217] examined the acute effect of a high energy drink (120ml) containing caffeine, β-alanine, vitamin C, and herbal compounds on reaction time, subjective feelings of energy, fatigue, alertness and focus. Subjective feelings of energy (3.5 ± 0.5 vs. 3.1 ± 0.5) and focus (3.8 ± 0.5 vs. 3.3 ± 0.7) were significantly higher during PWS supplementation compared to PLA, respectively. In addition, the authors observed a tendency towards an increase in average alertness ($p = 0.06$) in PWS compared to PLA. Our results in cognitive function are also in agreement with Hogervorst et al. [10] that revealed an additive effect of a dietary supplement on the activating and cognition enhancing effects of exercise. Caffeine supplementation prior to (150mg/L) and during (6ml/kg) exercise improved memory, concentration, and complex reaction times in athletes after exercise. Conversely, Judelson et al. [218] did not observe any effects of 3 or 6mg/kg/day of caffeine supplementation for 5 days on cognitive performance, psychomotor skills, and mood.

In the current study, we did not observe a significant increase in readiness to perform using VAS. Therefore, we reject the hypothesis that PWS is capable of enhancing perceptions of readiness to perform. Our results in readiness to perform are in agreement with Gonzalez et al. [219] who reported no significant change in VAS

determined feeling of energy when ingesting a PWS containing 2.1g of taurine, glucuronolactone and caffeine, 7.9g of leucine, isoleucine, valine, arginine and glutamine, 5g of di-creatine citrate, and 2.5g of β-alanine. Similarly, Joy et al. [220] reported that no differences were present in psychometric scores of perceived recovery, soreness, or readiness to train by consuming 48g of rice or whey protein isolate on training days, 3 days/week, for eight weeks as a part of a daily undulating periodized resistance-training program. On the contrary, Hoffman et al. [217] observed a significantly greater feeling of energy and focus compared to PLA after ingesting a supplement containing various amino acids. Research by Kedia et al. [221] indicated significant improvements in VAS scores for energy ($p < 0.024$) and concentration ($p < 0.041$) along with consistently higher levels of focus accompanied by less fatigue when a blend of PWS (5.3g/day) containing caffeine anhydrous, Cr monohydrate, vitamin C, and B vitamins was consumed.

In the current study, we did not see any significant changes in REE components including $\dot{V}O_2$, $\dot{V}CO_2$, and RER. Based on the literature, caffeine levels has shown to peak 30-120 min after oral intake [222]. We conducted the post-ingestion REE measurement immediately after ingesting the assigned supplement. That may explain why we did not observe significant thermogenic changes after supplement ingestion. Judice and colleagues [223] investigated the impact of a moderate dose of caffeine (5 mg/kg/day) on REE in a 4-day period and the acute effects on REE in physically active males. The findings revealed no acute or long-term effects of caffeine on REE among participants. Conversely, some other studies have indicated that ingesting a caffeine-

containing supplement increases REE components. For example, Astrup et al. [224] showed that the highest and the lowest caffeine dose (400 and 100mg) raised REE above PLA but not the intermediate dose (200 mg of caffeine). Ryan et al. [225] reported that ingestion of a thermogenic supplement containing caffeine, capsaicin, bioperine, and niacin increased energy expenditure and oxygen uptake values. Miles-Chan et al. [226] reported that consumption of a sugar free energy drink containing 120 mg of caffeine increased REE by about 4% and that the changes observed were due to the caffeine in the drinks rather than other nutrients (i.e., taurine and glucuronolactone).

In the present study, we observed a significant increase in post-bench press SBP in PWS treatment ~1-hr after ingesting the supplement and a significant decrease in PLA, while observing no significant differences in PWS150. The increase in SBP in PWS and the decrease in PLA were well-within normal resting BP changes (180-200 max). Since no changes in HR, SBP or DBP were observed after leg press and Wingate tests, the changes were most likely transient and unrelated to supplementation. In addition, no participant had hypotensive response (SBP < 90mmHG, DBP < 60mmHG) to either dose of PWS studied. Therefore, we reject the hypothesis that two formulae are capable of changing HR and BP responses. Some studies have shown the effects of dietary supplements on HR and BP [227-229]. The elevation of HR and BP following an ingestion of caffeine-containing supplements seems plausible in light of several related physiological mechanisms. Caffeine affects neurotransmission at both central and peripheral sites, primarily due to its antagonistic action at adenosine receptors [230]. Bloomer et al. [231] reported that acute geranamine (50mg) supplementation resulted in

an increase in SBP (~20%) and DBP (~17%), without an increase in HR. The largest increase was observed at 60 min post-ingestion. On the other hand, Dong et al. [228] observed that L-arginine intervention significantly lowered SBP by 5.4 mmHG (95% CI −8.5 to −2.3, p = 0.001) and DBP by 2.6 mmHG (95% CI −3.7 to −1.5, p = 0.001), compared to PLA. Our findings for HR are in agreement with Larsen et al. [227] who reported no significant change in HR after nitrate supplementation (6.2mg/kg/day) for three days. Similarly, Galvan et al. [232] did not observe any significant changes for HR, SBP, and DBP in resistance-trained males after acute ingestion of either Cr monohydrate or Cr nitrate (12g/day). Daniels et al. [86] found out that DBP and HR were unaffected by acute caffeine ingestion (6mg/kg) during dynamic leg exercise. Conversely, Larsen et al. [233] and Webb et al. [121] both observed a decrease in DBP of 3.7 and 8.1 mmHG after ~430mg (70 kg body weight at 6.2mg/kg/day^{-1}) and 1,400mg nitrate supplementation, respectively.

We observed no significant alterations in whole blood makers and general health markers. However, plasma Cr concentrations significantly increased over time among all treatments (p = 0.02). In addition, no significant treatment x time interactions were observed for any blood lipid marker. Therefore, we accept our hypothesis that two formulae do not adversely alter clinical markers within the context of study over a 7-day period. Our results are in agreement with Galvan et al. [232] that did not find significant changes in TC, HDL, TC/HDL ratio, LDL, and TG after acute and chronic dose-dependent Cr nitrate supplementation and exercise performance. Earnest et al. [234] also observed no significant changes in HDL after the Cr supplementation period.

In the present study, there was no significant difference over time in total lifting volume, peak power, average power, and average velocity during the bench press test after 7 days of supplementation. Similar results in bench press performance were observed by Green et al. [84]. Physically active men and women performed three sets of bench press to failure at 80% of 1RM with a dosage of 6mg/kg of caffeine. No significant difference was shown for bench press between caffeine and PLA treatments. Others have also reported similar results in bench press power. Astorino et al. [35] had participants perform one set of repetitions to failure for bench press at 60% 1RM with 6mg/kg of caffeine. No significant difference was found for bench press with caffeine compared to PLA. Martinez et al. [235] did not also observe the significant acute effects of a caffeine-containing PWS in upper body power or strength. On the contrary, Forbes et al. [236] observed the significant acute effect of a caffeine-containing energy drink (2mg/kg) on upper body muscle endurance. In addition, Goldstein et al. [237] reported that acute caffeine ingestion ($6mg/kg^{-1}$) significantly increased 1RM in the bench press but not bench press repetitions to failure at 60% 1RM in resistance trained women.

We observed no significant difference among treatments in leg press lifting volume. Similarly, Outlaw et al. [238] found no significant treatment × time interaction in leg press performance following eight days of supplementation with either a PWS containing Cr monohydrate-β-alanine blend (8.4g), BCAAs (4.8g), and caffeine (275mg) or PLA in 30 min before a resistance training workout and after completing baseline testing. Walter et al. [239] indicated that the acute ingestion of a supplement containing caffeine (200mg), capsaicin (33.3mg), bioperine (5mg), and niacin (20mg) did not alter

leg press performance. Conversely, Green et al. [84] reported that caffeine consumption (6mg/kg) resulted in a greater number of repetitions during leg press to failure at 10 repetition maximum.

We did not observe significant Treatment x Time interaction among treatments in overall peak power, mean power, total work, rate of fatigue, and minimum power during anaerobic sprint capacity test; however, we found a significant change in Wingate mean power at day 7 only in PWS150. In addition, some percent change improvements were seen in anaerobic total work and minimum power. Similar improvements were reported by others. Jagim et al. [240], reported a 3% improvement in total work during a 30-sec cycle ergometer test after 28 days of Cr and two doses of a buffered form of Cr supplementation. Ahmun et al. [241] observed a 4.9%, 4.4%, and 0.5% increase in peak power, minimum power, and fatigue index respectively, on a cycle ergometer after acute Cr supplementation. Koçak and Karli [242] revealed that the average and peak power mean scores obtained from anaerobic sprint post-test (8.12 W/kg and 10.5 W/kg) were significantly higher than pretest (7.23 W/kg and 8.99 W/kg) for the Cr supplement group. On the contrary, Williams et al. [243] found that 300mg of ephedra and caffeine did not positively enhance Wingate test performance. Research by Greer et al. [244] reported that ingestion of caffeine ($6mg/kg^{-1}$) did not significantly enhance performance during 4 repeated Wingate tests.

The lack of difference in strength and most anaerobic sprint capacity variables can be explained by several reasons. Basically, low-doses of the supplement may explain the lack of difference between the two doses of PWS. For example, the Cr nitrate doses

contained in PWS and PWS150 supplements were 2.0g and 3.0g respectively. These doses for Cr are lower than the typical 5-7g/day which has been demonstrated by several studies to be effective at inducing ergogenic aids in conjunction with resistance training [93, 245, 246]. In the Willoughby and Rosene study [246], 6g/day^{-1} of Cr for 12 weeks with performing resistance training trice weekly resulted in preferential increases in muscle strength and mass. A normal intra-personal variation in performance could have also influence the exercise performance before and after the supplementation period. Another reason could be the differences in length of time of the current protocol and other studies like that of Lowery et al. [94] which may at least partially explain our insignificant exercise performance results. In that study, the length of training was eight weeks and they found significant group-by-time interaction in which the PWS supplementation resulted in a significant increase in strength of the bench press (18.4% vs. 9.6%) compared to PLA.

Summary and Conclusion

A strength of our study is that we used a cross-over design that was adjusted for gender. We believe that this latter point is important as it is not untypical for similar trial to use mixed gender cohorts without such adjustments, assuming that men and women will respond similarly to supplementation schema. We also recruited participants with at least six months of strength training experience, inclusive of some of the performance parameters we examined, thus minimizing training and familiarity effects. A potential limitation was the length of the study. In a recent chronic study from our group (Jung et al., in press), some improvements were observed in cognitive function and 1RM strength

performance. Therefore, conducting more chronic trials would likely provide a more robust assessment of the potential for alterations in blood chemistries, improved performance readiness or exercise performance. Thus, while the results of our study demonstrated an increase in cognitive performance using a higher dosed formula (i.e. PWS150) unmatched by perceived readiness, exercise performance, hematology or adverse alterations in pre- and post-exercise hemodynamic responses to exercise.

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APPENDIX A

Informed Consent

TEXAS A&M UNIVERSITY HUMAN SUBJECTS PROTECTION PROGRAM
CONSENT FORM

Project Title: **Pharmacokinetic, Hemodynamic and Ergogenic Assessment of a Pre-Workout Dietary Supplement**

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

Why Is This Study Being Done?
The purpose of this study is to examine the acute effects of a pre-workout dietary supplement at a standard dose and one that is delivered at 150% of the standard dose on energy metabolism, cardiovascular hemodynamics, blood metabolites and mental focus.

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

How Many People Will Be Asked To Be In This Study?
Approximately 15 people (participants) will be invited to participate in this study locally.

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

What Will I Be Asked To Do In This Study?
You will be asked to not exercise for 48 hours nor eat or drink calorie containing foods or drinks 12 hours before each exercise testing session/visit. In addition you will be asked to refrain from ingesting caffeine and over the counter medication with known stimulant use for 48 hours prior to all the testing sessions/visits. Your participation in this study will last up to approximately six weeks since you will receive all three treatments and include thirteen total visits (visit 1 ~ 1 hour/visits 2,5,6,9,10,13 ~ 2 hours/visits 3,4,7,8,11,12 ~ 15 minutes). These visits are detailed below and in Table 1.

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Table 1 – Protocol Overview

T1 (Familiarization)	T2 (Day 1)	T3 (Day 3)	T4 (Day 5)	T5 (Day 7)
Phone Screening	Fasting Blood Sample	Stroop C&W Test	Stroop C&W Test	Fasting Blood Sample
Familiarization	Resting HR/BP/ECG – 5 min.; start REE (0 min.)	Ready to Perform VAS	Ready to Perform VAS	Resting HR/BP/ECG – 5 min.; start REE (0 min.)
Physical Exam	Ingest supplement in a randomized and counterbalanced manner (30 min.)	Sleep Quality	Sleep Quality	Ingest supplement in a randomized and counterbalanced manner (30 min.)
Body Weight	Stop REE (60 min.)	Caffeine Questionnaire	Caffeine Questionnaire	Stop REE (60 min.)
Body Composition	Stroop C&W Test			Stroop C&W Test
1 Repetition Maximum Determination on the Bench Press and Leg Press	Ready to Perform VAS			Ready to Perform VAS
Anaerobic Sprint Practice Test	Sleep Quality			Sleep Quality
Schedule Testing	Caffeine Questionnaire			Caffeine Questionnaire
Refrain from exercise, caffeine and use of over-the-counter stimulants for 48-hours prior to each testing session and fast for 12 hours prior to the T2 and T5 testing session	Resting HR/BP/ECG/BIA – 5 min.			Resting HR/BP/ECG/BIA – 5 min.
	Bench Press Warm-Up			Bench Press Warm-Up
	Bench Press (3 sets of 10 reps @ 70% of 1RM with 2-min rest btw. sets. Total reps to failure on last set)			Bench Press (3 sets of 10 reps @ 70% of 1RM with 2-min rest btw. sets. Total reps to failure on last set)
	Resting HR/BP – 1 min. after testing			Resting HR/BP – 1 min. after testing
	5 min. rest			5 min. rest
	Leg Press Warm-up			Leg Press Warm-up
	Leg Press (3 sets of 10 reps @ 70% of 1RM with 2-min rest btw. sets. Total reps to failure on last set)			Leg Press (3 sets of 10 reps @ 70% of 1RM with 2-min rest btw. sets. Total reps to failure on last set)
	Resting HR/BP – 1 min. after testing			Resting HR/BP – 1 min. after testing
	5 min. rest			5 min. rest
	Wingate AC Test			Wingate AC Test
	Resting HR/BP – 1 min. after testing			Resting HR/BP – 1 min. after testing
	Supplement for six additional days			4 – Day Food Record - One week washout

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Visit 1 – Familiarization (T1)
This visit will last about one hour. During this visit we will explain the details of the study, ask you to sign human subject consent forms, a radiation exposure questionnaire and personal and medical history information forms. We will then complete a general physical on you that may include measurement of blood (5 ml or about 1 teaspoon according to standard procedures) to determine if you can participate in the study. Next we will measure your height, weight and body composition one time. We will then ask you to perform a warm-up and one repetition maximum test on the bench press and leg press with a two minute recovery between attempts. Next we will familiarize you to the sprint bike test. Next you will be given a food log and asked to record all calorie containing food and drinks for a total of four days (including one weekend day) and turn in at your third visit. Finally we will schedule you for your remaining testing sessions.

Visit 2 & 5 – (T2 and T5)
These visits will last about two hours. We will first ask you to donate approximately 20 ml (about four teaspoons) of fasting blood from a vein in your arm according to standard procedures. We then measure a 12 lead electrocardiograph (ECG), heart rate (HR) and blood pressure (BP) for approximately five minutes to measure standard ECG signaling and potential adverse changes. Next we start the resting energy expenditure (REE) test. We then divide you into a group and ask you to ingest your assigned pre-workout dietary supplement after 30 minutes on the REE at a standard dose or one that is delivered at 150% of the standard dose. The third supplement group will be the dextrose placebo. We then ask you to continue to lie down and continue the REE through 60 minutes. We next ask you to complete a stroop color and word test, readiness to perform VAS, sleep quality and caffeine questionnaire. Next we will measure ECG/HR/BP and body water (BIA) for approximately five minutes to measure standard ECG signaling and potential adverse changes a second time. We will then ask you to warm-up and perform three sets of 10 repetitions at 70% of one repetition maximum on the bench press (completing as many repetitions as possible during the final set) followed by measuring HR and BP one minute after the final set. Following a five minute recovery we will ask you to perform three sets of 10 repetitions at 70% of one repetition maximum on the leg press (completing as many repetitions as possible during the final set) followed by measuring HR and BP one minute after the final set. Following a five minute recovery, we will ask you to perform a Wingate 30 second anaerobic capacity test on a cycle ergometer followed by measuring HR and BP one minute after testing. You will receive all three treatments throughout the duration of the study following a one week wash out between supplemental weeks. Table 2 shows the ingredient list for the two pre-workout dietary supplements (per two servings or 12 g.). We will ask you to ingest two scoops (about 12 g.) of your supplement mixed with eight ounces of water prior to a workout on the days you are training and approximately noon on non-training days on days two through six. You will be asked to return on day seven to repeat the same tests completed on day one. We will ask you to return for these same tests twice while taking the second supplement and twice while taking the third supplement (following a one week wash out between supplement groups). In the event of an emergency during an exercise test proper emergency response protocols (calling 9-911 for serious injury or a medical emergency, calling Biosafety/EHS for cleanup assistance or spill team response, calling UPD for incidents in public areas, retrieving the AED located in the lab, performing CPR or other First Aid techniques, etc.) will be followed by the Exercise & Sport Nutrition Laboratory (ESNL) staff depending on the severity of the emergency.

Visit 3 & 4 – (T3 and T4)
These visits will last about 15 minutes. We will ask you to complete a stroop color and word test, readiness to perform VAS, sleep quality and caffeine questionnaire. We will ask you to return for these same tests twice while taking the second supplement and twice while taking the third supplement (following a one week wash out between supplement groups).

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You may be removed from the study by the investigator for these reasons:

- You do not show up for your scheduled testing sessions/visits and the investigators are unable to contact you to reschedule
- You do not follow your assigned supplemental protocol

Table 2: Study Treatment Ingredients

Active Ingredients	One Dose	Unit	150% Dose
Beta Alanine	2.4	g	3.6
Arginine AKG 2:1	1.5	g	2.3
Creatine Nitrate	1.5	g	2.3
Ascorbic Acid Vit C	375.0	mg	562.5
N-Acetyl Tyrosine	225.0	mg	337.5
Caffeine Anhydrous	200.0	mg	300.0
Mucuna Pruriens 15%L-Dopa	75.0	mg	112.5
Niacinamide USP	45.0	mg	67.5
Purenergy™	25.0	mg	37.5
TeaCor™ Tetramethyluric acid	10.0	mg	15.0
Pyridoxal 5-Phosphate (68% Vitamin B6)	750.0	mcg	1125.0
Folic Acid USP	375.0	mcg	562.5
Methylcobalamin (Vitamin B12)	52.5	mcg	78.8

Are There Any Risks To Me?

The things that you will be doing are greater than risks that you would come across in everyday life. Although the researchers have tried to avoid risks, you may feel that some questions/procedures that are asked of you will be stressful or upsetting. You do not have to answer anything you do not want to. You will be exposed to a low level of radiation once during the body composition test, which is similar to the amount of natural background radiation you would receive in one month while living in College Station Texas. In addition, a very low level of electrical current will be passed through your body using a bioelectrical impedance analyzer (BIA). This analyzer is commercially available and has been used in the health care/fitness industry as a means to assess body composition and body water for over 20 years. The use of the body composition scanner and bioelectrical impedance analyzer have been shown to be safe methods of assessing body composition and total body water and are approved by the FDA. You may donate approximately five ml (about one teaspoon) of fasting blood once during the initial familiarization/screening visit and then approximately 20 ml (about four teaspoons) of blood six times throughout the study using standard procedures. These procedures may cause a small amount of pain when the needle is inserted into the vein as well as some bleeding and bruising. You may also experience some dizziness and/or faint if you are unaccustomed to having blood drawn. The exercise tests that will be performed may cause symptoms of fatigue, shortness of breath and/or muscular fatigue/discomfort. The exercise tests may also cause short-term muscle soreness and moderate fatigue for several days following the tests. You may also experience muscle strains/pulls during the exercise testing and/or training program. However, exercise sessions will be conducted by trained personnel and monitored to ensure you follow appropriate exercise guidelines. ***If you are a competing athlete you may test positive for Performance-enhancing drugs (PED) given that caffeine is on the NCAA banned drug list.***

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

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

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

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

Will I Be Paid To Be In This Study?
You will receive a total of $200 ($20 for the Familiarization, $25 for each exercise testing session and $5 for each questionnaire only testing session) in one check at the end of the study. Payment will occur after finishing all thirteen sessions and after all study materials (questionnaires, etc.) have been turned in to the study staff. You will be paid on a prorated basis if you are unable to complete the entire study.

Will Information From This Study Be Kept Private?
The records of this study will be kept private. No identifiers linking you to this study will be included in any sort of report that might be published. Research records will be stored securely and only Exercise & Sport Nutrition Laboratory staff will have access to the records.

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

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

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

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

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

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

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

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

_____ _____
Participant's Signature Date

_____ _____
Printed Name Date

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

_____ _____
Signature of Presenter Date

_____ _____
Printed Name Date

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APPENDIX B

Familiarization Session Face Sheet

Texas A&M University: Exercise & Sport Nutrition Laboratory
Trial: Pharmacokinetic, Hemodynamic and Ergogenic Assessment of a Pre-Workout Dietary Supplement

Familiarization

Demographics ESNL Staff Initials: _____

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

Informed Consent: _____
Radiation Consent: _____

General Screening: _____
Height: _____
Weight: _____
DXA: _____

Exercise Measures: Strength/Anaerobic Testing: ESNL Staff Initials: ____

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

1 RM: _____
70% 1RM: _____

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

1 RM: _____
70% 1RM: _____

Wingate Practice: _____

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

Updated 2/18/2015

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APPENDIX C

Testing Sessions Face Sheet

Texas A&M University: Exercise & Sport Nutrition Laboratory
Trial: Pharmacokinetic, Hemodynamic and Ergogenic Assessment of a Pre-Workout Dietary Supplement

Demographics: ESNL Staff Initials: _____

Name: _____ Testing Session: _____
Date: _____ Group: _____

Time: _____am Last Meal: _____
Hrs. Fasted: _____hr. Last Wkout: _____hr.

Resting Measures: ESNL Staff Initials: _____

Lab: _____ (2) SST/(1) EDTA

ECG(~ 5 min.): _____ #1/#2
HR: _____ bpm, BP: _____/_____ mmHg Arrhythmias: _____

Start REE: _____ #1/#2 (0 min.), Ingest supplement: _____ (30 min.), Stop REE: _____ (60 min.)

Stroop Color and Word: _____ VAS: _____ Sleep: _____ Caffeine: _____

ECG(~ 5 min.): _____ #1/#2
HR: _____ bpm, BP: _____/_____ mmHg Arrhythmias: _____
BIA: _____ FFM: _____ kg, FM: _____ kg, TBW: _____ L, ICW: _____ L, ECW: _____ L

Exercise Measures: Strength/Anaerobic Testing: ESNL Staff Initials: _____

Bench Press: Hand Position: _____

1 RM: _____ 70% 1RM: _____
Set 1-70% 1RM_____x10: **Set 2**-70% 1RM _____x10: **Set 3**- 70% 1RM _____x_____(max #)

1 minute after: HR: _____ bpm, BP: _____/_____ mmHg

Leg Press: Foot Position: _____ Sled Position: _____

1 RM: _____ 70% 1RM: _____
Set 1-70% 1RM_____x10: **Set 2**-70% 1RM _____x10: **Set 3**- 70% 1RM _____x_____(max #)

1 minute after: HR: _____ bpm, BP: _____/_____ mmHg

Wingate: _____

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

1 minute after: HR: _____ bpm, BP: _____/_____ mmHg

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APPENDIX D

Daily Food-Log Sheets

Instructions:

1) Record everything that you eat for 3 weekdays AND 1 weekend day
2) Precisely record the food item (brand if applicable), preparation method, and TOTAL quantity consumed
3) Break down mixed dishes or recipes by listing their component parts
4) For dairy and meat products, indicate fat level (i.e. low fat, extra lean, 2%, etc.)

FOOD ITEM	PREPARATION METHOD (i.e. baked, fried, grilled, etc.)	QUANTITY							
		gm	mL	cups	T or tsp.	oz.	Pieces	Sm, Med, Lg	Other
MEAL 1:									
MEAL 2:									
MEAL 3:									
MEAL 4:									

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APPENDIX E

Stroop Word-Color Test Sheets

Page 1

RED	BLUE	GREEN	RED	BLUE
GREEN	GREEN	RED	BLUE	GREEN
BLUE	RED	BLUE	GREEN	RED
GREEN	BLUE	RED	RED	BLUE
RED	RED	GREEN	BLUE	GREEN
BLUE	GREEN	BLUE	GREEN	RED
RED	BLUE	GREEN	BLUE	GREEN
BLUE	GREEN	RED	GREEN	RED
GREEN	RED	BLUE	RED	BLUE
BLUE	GREEN	GREEN	BLUE	GREEN
GREEN	RED	BLUE	RED	RED
RED	BLUE	RED	GREEN	BLUE
GREEN	RED	BLUE	RED	GREEN
BLUE	BLUE	RED	GREEN	RED
RED	GREEN	GREEN	BLUE	BLUE
BLUE	BLUE	RED	GREEN	RED
RED	GREEN	BLUE	RED	GREEN
GREEN	RED	GREEN	BLUE	BLUE
RED	BLUE	RED	GREEN	RED
GREEN	RED	GREEN	BLUE	GREEN

Page 2

XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX

Page 3

RED	BLUE	GREEN	RED	BLUE
GREEN	GREEN	RED	BLUE	GREEN
BLUE	RED	BLUE	GREEN	RED
GREEN	BLUE	RED	RED	BLUE
RED	RED	GREEN	BLUE	GREEN
BLUE	GREEN	BLUE	GREEN	RED
RED	BLUE	GREEN	BLUE	GREEN
BLUE	GREEN	RED	GREEN	RED
GREEN	RED	BLUE	RED	BLUE
BLUE	GREEN	GREEN	BLUE	GREEN
GREEN	RED	BLUE	RED	RED
RED	BLUE	RED	GREEN	BLUE
GREEN	RED	BLUE	RED	GREEN
BLUE	BLUE	RED	GREEN	RED
RED	GREEN	GREEN	BLUE	BLUE
BLUE	BLUE	RED	GREEN	RED
RED	GREEN	BLUE	RED	GREEN
GREEN	RED	GREEN	BLUE	BLUE
RED	BLUE	RED	GREEN	RED
GREEN	RED	GREEN	BLUE	GREEN

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APPENDIX F

Readiness to Perform (VAS)

Texas A&M University: Exercise & Sport Nutrition Laboratory
Trial: Pharmacokinetic, Hemodynamic and Ergogenic Assessment of a Pre-Workout Dietary Supplement - Readiness to Perform VAS

Name: _____ Testing Session: _____
Date: _____ Group: _____

INSTRUCTIONS

Circle the number or dot between numbers that best indicates how you currently feel:

I am looking forward to today's workout:

Strongly disagree 1 . . . . . 2 . . . . . Disagree . . . . . 3 . . . . . Neutral . . . . . 4 . . . . . Agree . . . . . 5 Strongly agree

I am optimistic about my future performance:

Strongly disagree 1 . . . . . 2 . . . . . Disagree . . . . . 3 . . . . . Neutral . . . . . 4 . . . . . Agree . . . . . 5 Strongly agree

I feel vigorous and energetic:

Strongly disagree 1 . . . . . 2 . . . . . Disagree . . . . . 3 . . . . . Neutral . . . . . 4 . . . . . Agree . . . . . 5 Strongly agree

My appetite is great:

Strongly disagree 1 . . . . . 2 . . . . . Disagree . . . . . 3 . . . . . Neutral . . . . . 4 . . . . . Agree . . . . . 5 Strongly agree

I have little muscle soreness:

Strongly disagree 1 . . . . . 2 . . . . . Disagree . . . . . 3 . . . . . Neutral . . . . . 4 . . . . . Agree . . . . . 5 Strongly agree

APPENDIX G

Sleep Quality Questionnaire

Participant's Initials ID#: _____

Date: _____

Time: _____

SLEEP QUALITY INDEX INSTRUCTIONS

The following questions relate to your usual sleep habits during the past 48 hrs

Please answer *all* questions.

1. During the past 48 hrs, what time have you usually gone to bed at night?

BED TIME _____

2. During the past 48 hrs, how long (in minutes) has it usually taken you to fall asleep each night?

NUMBER OF MINUTES _____

3. During the past 48 hrs, what time have you usually gotten up in the morning?

GETTING UP TIME _____

4. During the past 48 hrs, how many hours of actual sleep did you get at night? (This may be different from the number of hours you spent in bed.)

HOURS OF SLEEP PER NIGHT _____

FOR EACH OF THE REMAINING QUESTIONS.

5. During the past 48 hrs, have you had trouble sleeping because you **(Check All That Apply)**

__ Cannot get to sleep within 30 minutes

__ Wake up in the middle of the night or early morning

__ Have to get up to use the bathroom

__ Cannot breathe comfortably

__ Cough or snore loudly

__ Feel too cold

__ Feel too hot

__ Had bad dreams

__ Have pain

__ Other reason(s), please describe

6. During the past 48 hrs, how would you rate your sleep quality overall?

__ Very good

__ Fairly good

__ Fairly bad

__ Very bad _____

7. During the past 48 hrs, how much of a problem has it been for you to keep up enough enthusiasm to get things done?

__ No problem at all

__ Only a very slight problem

__ Somewhat of a problem

__ A very big problem

8. Do you have a bed partner or room mate?

__ No bed partner or room mate

__ Partner/room mate in other room

__ Partner in same room, but not same bed

__ Partner in same bed

9. If you have a roommate or bed partner, ask them how often in the past 48 hrs you have had . . .

__ Loud snoring

__ Long pauses between breaths while asleep

__ Legs twitching or jerking while you sleep

__ Episodes of disorientation or confusion during sleep

10. Other restlessness while you sleep; please describe

APPENDIX H

Caffeine Tolerance Questionnaire

Caffeine Questionnaire

Below is a list of feelings/experiences people have. Circle the number that best describes how you are feeling/what you are experiencing **RIGHT NOW**.

	Not at all	A little	Moderately	Quite a bit	Extremely
1. Drowsy/sleepy	0	1	2	3	4
2. Self-confidence	0	1	2	3	4
3. Yawning	0	1	2	3	4
4. Alert	0	1	2	3	4
5. Tired/Fatigued	0	1	2	3	4
6. Content	0	1	2	3	4
7. Difficulty Concentrating	0	1	2	3	4
8. Irritable	0	1	2	3	4
9. Heavy feelings in arms and legs	0	1	2	3	4
10. Depressed Mood	0	1	2	3	4
11. Grouchy	0	1	2	3	4
12. Urge to do work related activity	0	1	2	3	4
13. Flu-like feelings	0	1	2	3	4
14. Headache	0	1	2	3	4
15. Talkative	0	1	2	3	4
16. Sluggish	0	1	2	3	4
17. Upset stomach	0	1	2	3	4
18. Clearheaded	0	1	2	3	4
19. Desire to socialize	0	1	2	3	4
20. Energetic	0	1	2	3	4
21. Nausea/vomiting	0	1	2	3	4
22. Muscle pain/stiffness/aches	0	1	2	3	4
23. Discouraged	0	1	2	3	4
24. Dizziness	0	1	2	3	4
25. Desire to work out	0	1	2	3	4
Additional items for consideration:					
Queasy	0	1	2	3	4
Nauseous	0	1	2	3	4
Vomiting	0	1	2	3	4
Headachy	0	1	2	3	4
*Anxious	0	1	2	3	4
*Nervous	0	1	2	3	4
*Jittery	0	1	2	3	4
*Craving for caffeine	0	1	2	3	4
*Craving for coffee	0	1	2	3	4

* These symptoms have not been empirically validated as caffeine withdrawal symptoms

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