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SAFETY AND EFFICACY OF A MULTI-INGREDIENT THERMOGENIC PRE-WORKOUT SUPPLEMENT DURING LOW-INTENSITY RUNNING IN FEMALES

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SAFETY AND EFFICACY OF A THERMOGENIC PRE-WORKOUT SUPPLEMENT DURING LOW-INTENSITY RUNNING IN FEMALES

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We recommend acceptance of this thesis in partial fulfillment of the candidate's requirements for the degree of Master of Science in Human Performance.

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ABSTRACT

Erickson, J.R. Safety and efficacy of a multi-ingredient thermogenic pre-workout supplement during low-intensity running in females. MS in Exercise & Sports Science-Human Performance, May 2017, 58 pp. (C. Camic)

The purpose of this study was to examine the acute effects of a thermogenic pre-workout supplement on oxygen uptake ($\dot{V}O_2$), pulmonary ventilation ($\dot{V}_E$), carbon dioxide output ($\dot{V}CO_2$), respiratory exchange rate (RER), energy expenditure (EE), heart rate (HR), systolic blood pressure (SBP), and diastolic blood pressure (DBP). Twelve female subjects (mean ± SD: age, 25.3 ± 9.4 years; weight, 61.2 ± 6.8 kg) defined as aerobically-trained volunteered for this randomized, double-blinded, placebo-controlled, crossover study. Subjects performed an incremental running test to exhaustion on the treadmill to determine ventilatory threshold. On three separate laboratory visits, each subject consumed a standardized meal following overnight fasting and were randomly assigned to ingest the supplement (1 or 2 servings) or placebo and ran at a constant velocity at 90% VT for 30 minutes on the treadmill. The findings of the separate one-way ANOVAs with repeated measures indicated that there were significant ($P<0.05$) mean differences in DBP between the 1-dose (61 ± 7 to 72 ± 6 mmHg) and 2-dose (66 ± 5 to 75 ± 5 mmHg) conditions, resting SBP (collapsed across time) (107 ± 11 mmHg) compared to the 1-dose (102 ± 11 mmHg) and placebo (104 ± 12 mmHg) conditions.

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INTRODUCTION

Pre-workout supplementation is the nutritional strategy that typically involves consuming a mixture of bioactive compounds and dietary ingredients prior to a bout of exercise for ergogenic purposes. For example, pre-workout supplements (PWS) often contain a multitude of substances at varying quantities that include thermogenic agents, endurance boosters, strength and power enhancers, as well as numerous vitamins and minerals. The purported effects associated with PWS consist of elevated metabolic rate, increased measures of anaerobic and aerobic performance, and improvements in body composition (Vogel et al., 2015; Joy et al., 2015 Bergstrom et al., 2014). Due to the relatively brief period of time that PWS have been available, however, there are limited data concerning the influence of these multi-ingredient products on their ergogenic potential and variables related to general health (e.g. resting heart rate and blood pressure).

Thermogenic agents are compounds that increase basal metabolic rate through activation of the sympathetic nervous system (Stohs et al., 2016). For example, caffeine is one of the most commonly used thermogenic agents that stimulates the release of catecholamines (e.g. epinephrine) and renin, thereby increasing heart rate, blood pressure, lipolysis, and plasma free fatty acid concentration (Bergstrom et al., 2014; Robertson et al. 1978; Daniels et al. 1998). It has been demonstrated that acute caffeine ingestion (50 mg and 6 $mg \cdot kg^{-1}$ of body weight) increases resting thermogenesis (72 ± 25 kJ per 4 hours) (Belza et al, 2009), resting metabolic rate (6% above the basal rate baseline)

(Belza et al, 2009), fat oxidation (10.4-29.7 g) (Schubert et al., 2014) and energy expenditure during exercise (4-9%) (Ahrens et al. 2007; Wallman et al., 2010). In addition, caffeine supplementation (3-6 $mg \cdot kg^{-1}$ of body weight) has been shown to increase systolic (17%) (Daniels et al. 1998) and diastolic (6%) (McClaran et al. 2007) blood pressures, oxygen uptake ($\dot{V}O_2$) (5-11%) (Wallman et al., 2010; Ahrens et al. 2007), and time to exhaustion (30%) (Petro et al., 1998) during submaximal cycle ergometry, treadmill walking, and at rest. Collectively, the findings of these investigations (Schubert et al., 2014; Wallman et al., 2010; Ahrens et al. 2007; Petro et al., 1998; Belza et al., 2009; McCalaran et al., 2007; Daniels et al., 1998) indicated that acute caffeine supplementation may influence metabolic, cardiovascular, and performance variables at rest as well as during exercise. Furthermore, it has been suggested (Belza et al., 2009) that the combination of caffeine with other thermogenic agents (i.e. capsicum, tyrosine, *Mucuna pruriens*, green coffee bean extract, *Coleus forskohlii*, and L-carnitine) may provide a synergistic effect on each of these factors.

In addition to caffeine are many other supplements that are purported to increase fat mobilization and oxidation. Capsicum as well as capsinoids, an analogue of capsicum with low pungency, have been studied for their possible effect on appetite suppression to aid in weight loss along with their influence on fatness and energy metabolism (i.e. increased resting fat oxidation) in humans (Lejeune et al., 2003; Snitker et al. 2009). It has been demonstrated that chronic capsinoid supplementation (6 $mg \cdot d^{-1}$ for 12 weeks) with a moderate caloric deficit (300-500 kcal) decreases abdominal adiposity ($-1.11 \pm 1.83\%$ vs. $-0.18 \pm 1.94\%$) and increases fat oxidation (least-squares mean difference: 21.0 mg·min) (Snitker et al. 2009) compared to placebo. Furthermore, capsaicin

supplementation (135 $mg \cdot d^{-1}$for 12 weeks) resulted in higher fat oxidation (4.2 ± 1.1 vs. 3.5 ± 0.9 $g \cdot h^{-1}$) and resting energy expenditure (0.7 ± 0.5 vs. 0.2 ± 0.5 $MJ \cdot d^{-1}$) after a 4-week very low energy diet (Lejeune et al., 2003). Another common ingredient found in many PWS, tyrosine, is a nonessential amino acid and norepinephrine precursor that may enhance the synthesis and release of catecholamines through the sympathetic nervous system (Belza, et al., 2009). *Mucuna pruriens*, a tropical legume that contains L-DOPA, has been studied for its potential to regulate blood glucose and manage hyperglycemia by the inhibition of the enzyme alpha-glucosidase that is responsible for carbohydrate digestion and glucose absorption in the digestive tract (Kavintha et al., 2014; Gluati et al., 2012). The consumption of green coffee bean extract (GCE), found in green or raw coffee, may also aid in weight loss by modifying glucose tolerance and hormone secretion (i.e. Glucose-dependent insulinotropic polypeptide) (Onakpoya et al., 2010; Johnston et al., 2003). In addition, GCE may also inhibit glucose-6-phosphatase activity, an enzyme that aids in glucose homeostasis from the liver (Onakpoya et al., 2010). Chlorogenic acid is a biologically active phenol derived from GCE. Thom (2007) found that chlorogenic acid enriched coffee supplementation (90-100 $mg \cdot g^{-1} \cdot d^{-1}$ for 12 weeks) significantly increased body fat loss (3.6 ± 0.3% vs. 0.7 ± 0.4%) in moderately overweight subjects. *Coleus forskohlii*, a plant from the Lamiaceae (mint) family, is found to contain high amounts of forskolin that has been shown to increase lipolysis and the release of free fatty acids through the elevation of cyclic adenosine monophosphate (cAMP) and the activation of hormone sensitive lipase through the phosphorylation of protein kinase (Loftus, et al., 2015). In addition, the primary metabolic functions of L-carnitine consist of transporting long- and medium-chain fatty acids into the

mitochondria for beta-oxidation and to maintain energy balance by buffering short-chain acyl groups (i.e. acetyl-CoA) (Broad et al., 2008). The proposed benefit of L-carnitine supplementation is increased fat oxidation, though it may also enhance the breakdown of branched-chain amino acids through buffering branched-chain keto acids (Broad et al., 2008).

Caffeine and these additional thermogenic agents (capsicum, tyrosine, *Mucuna pruriens*, green coffee bean extract, *Coleus forskohlii*, and L-carnitine) are combined in C4 Ripped© (Cellucor, Bryan, TX), a common PWS that appears to be marketed toward females. The reported effects of C4 Ripped© include increased lipolysis, transport, and utilization of fatty acids. To our knowledge, no research has been conducted on the safety of this product (i.e. resting heart rate, blood pressure) or the ergogenic properties of these combined ingredients when administered on an acute basis. In addition, there are limited data regarding the influence of PWS on metabolic, cardiovascular, psychological, and performance variables in the female population. Therefore, the purpose of this study is to examine the acute effects of C4 Ripped© on resting heart rate and blood pressure, substrate utilization (i.e. fat and carbohydrate oxidation), energy expenditure, and subjective measures of focus, energy, and effort during moderate-intensity treadmill running.

MATERIALS AND METHODS

Experimental Approach to the Problem

This study utilized a randomized, double-blind, placebo-controlled, within-subjects crossover design. Each subject was required to visit the laboratory on five occasions with 72-96 hours between sessions. During the first laboratory visit, each subject performed a submaximal incremental test on a treadmill to familiarize the subjects with the testing procedures. For the second laboratory visit, each subject performed an incremental test to exhaustion on a treadmill to determine their ventilatory threshold (VT). On the third laboratory visit, each subject consumed a standardized meal following overnight fasting (8 hours) and sat quietly for 30 minutes. The subjects were then randomly assigned to ingest the supplement (1 or 2 servings) or placebo and sat quietly for another 30 minutes. The ingredients of the supplement are provided in Table 1. The placebo was Crystal Light® and was controlled for similar appearance and taste. At the 15-minute and 30-minute ingestion periods, resting heart rate and blood pressure were recorded. Subjects then performed a 30-minute constant-velocity treadmill run at 90% of their VT. The subjects then returned to the laboratory for their fourth and fifth visits to ingest the remaining substances (1 supplement serving, 2 supplement servings, or placebo) and underwent the same testing procedures (including time of day) as the third visit. Each subject recorded 2-day food logs prior to each laboratory visit.

Subjects

Twelve female subjects (mean ± SD: age= 25.3 ± 9.4 years; body weight= 61.2 ± 6.8 kg) defined as aerobically-trained (habitual physical activity 3-5 days per week at 80-91% heart rate max) that complete at least 20 running miles per week were recruited to participate in this investigation. In addition, the subjects did not report or exhibit any of the following that could significantly affect the outcome of the study: (i) history of medical or surgical events, including cardiovascular disease, metabolic, renal, hepatic, or musculoskeletal disorders; (ii) use of any medication; (iii) use of nutritional supplements; (iv) habitual use of caffeine; or (v) participation in another clinical trial or investigation of another investigational product within 30 days prior to screening/enrollment. All subjects were encouraged to maintain current dietary and exercise habits for the duration of the study and were asked to avoid eating and drinking anything other than water 8 hours prior to laboratory visits 3-5. In addition, the subjects were asked to abstain from caffeine for at least two weeks prior to the beginning of the study. The study was approved by the University of Wisconsin- La Crosse Institutional Review Board for the Protection of Human Subjects, and all participants completed a health history questionnaire and signed a written informed consent document prior to testing.

Determination of $\dot{V}O_{2\ max}$ and VT

On the second laboratory visit, all subjects were fitted with a heart rate monitor (Polar Electro Inc., Lake Success, NY) and rested for 30 minutes to determine resting heart rate and blood pressure. Blood pressure was determined by using an automated inflatable sphygmomanometer (Omron Intelli Sense Model BP760). Following the rest period, the subjects were informed of the $\dot{V}O_{2\ max}$ testing protocol. This protocol consisted

of 2-minute stages beginning with a speed of 8 kilometers per hour (kph) and increasing by 1 kph each stage until the subject reached volitional exhaustion. Oxygen uptake ($\dot{V}O_2$), pulmonary ventilation ($\dot{V}_E$), carbon dioxide output ($\dot{V}CO_2$), respiratory exchange rate (RER), and heart rate (HR) were recorded as 30-second averages throughout the test. Gas exchange was monitored using an AEI Moxus metabolic cart (AEI Technologies, Pittsburgh, PA). The gas analyzers were calibrated against room air and with a certified standard mixture of oxygen (14.00%) and carbon dioxide (6.88%). The flow volume of the metabolic cart was calibrated with a 3-liter calibration syringe. The test was considered maximal if the subject met at least three of the following four criteria: a) RER was $\geq$ 1.1; b) heart rate was $\geq$ 90% age-predicted max; c) $\dot{V}O_2$ plateaued $\leq$ to 150 $mL \cdot min^{-1}$ over the last 30 seconds of the test; d) rate of perceived exertion was $\geq$ 18. The subjects were verbally encouraged to exert themselves to maximal effort. The VT was determined by using the Ventilation Curve method ($\dot{V}_E$ vs. $\dot{V}O_2$) (Beaver et al., 1986) via noninvasive gas exchange and was defined as the $\dot{V}O_2$ value that corresponds to the breakpoint in the minute ventilation ($\dot{V}_E$) versus $\dot{V}O_2$ relationship. Running velocities from the incremental test were plotted against $\dot{V}O_2$ values and the regression equation derived was used to determine the running velocity that corresponds to 90% VT.

Standardized Meal and Supplementation Protocol

On visits three, four, and five, the subjects consumed a standardized meal consisting of (24% carbohydrates, 43% protein, 36% fat, and 150 kcals) 60 minutes prior to exercise. In a double-blind manner, subjects were assigned to consume either one or two servings of C4 Ripped® or a placebo with 6 ounces of water 30 minutes after the

consumption of the standardized meal and 30 minutes prior to the exercise protocol, as recommended by the manufacturer (Cellucor© Bryan, TX).

Heart Rate and Blood Pressure

For visits 3-5, resting heart rate was recorded with a heart rate monitor (Polar Electro Inc., Lake Success, NY) and resting blood pressure was measured with an automated inflatable sphygmomanometer (Omron Intelli Sense Model BP760) before ingesting the conditions, 15 minutes after ingesting the conditions, and 30 minutes after ingesting the conditions.

Constant Velocity Runs

On laboratory visits three, four, and five, the subjects were fitted with a heart rate monitor (Polar Electro Inc., Lake Success, NY) and rested for 60 minutes to determine resting heart rate and blood pressure. During the rest period, the subjects were informed of the constant velocity run protocol. This protocol consisted of the subjects running at a constant velocity of 90% VT for 30 minutes. $\dot{V}O_2$, $\dot{V}_E$, $\dot{V}CO_2$, RER, and HR were recorded as 5-minute averages throughout the test. The same metabolic cart calibration methods as the $\dot{V}O_{2\ max}$ test were used. RPE was taken every 2 minutes.

Estimation of Substrate Utilization

Substrate utilization was estimated by the gas exchange values recorded at 5-minute intervals during the constant velocity run. Energy expenditure of non-protein RER was determined from gas exchange values by using the equations provided in Table 2 (Jeukendrup et al., 2004).

STATISTICAL METHODS

Separate one-way ANOVAs with repeated measures were used to compare mean resting values for HR, systolic blood pressure, and diastolic blood pressure values among conditions (1 supplement serving, 2 supplement servings, placebo). In addition, separate two-way ANOVAs with repeated measures were used to compare fat and carbohydrate oxidation, RER, $\dot{V}O_2$, $\dot{V}_E$, $\dot{V}CO_2$, and HR values among the conditions (1 supplement serving, 2 supplement servings, placebo) at the common time points (5, 10, 15, 20, 25, 30 minutes) of the 30-minute run at 90% VT. When appropriate, follow-up tests included one-way ANOVAs with repeated measures and paired-sample *t*-tests with Bonferroni corrections (0.05 / 3 = 0.0167). An alpha of $p \leq 0.05$ was considered statistically significant for all interaction effects and follow-up ANOVAs. In addition, separate one-way ANOVAs with repeated measures were used to compare the total caloric (kilocalories) and macronutrient (grams of protein, carbohydrate, and fat) intakes among the conditions and visits (3-5).

RESULTS

Macronutrient Data

The results of the one-way repeated measures ANOVAs indicated there were no significant differences among conditions for total kilocalories ($p = 0.127$), fat ($p = 0.236$), carbohydrate ($p = 0.408$), or protein ($p = 0.235$) consumed (Table 2).

Heart Rate

The two-way repeated measures ANOVA indicated there were was no significant condition x time interaction ($p = 0.316$; partial $\eta^2 = 0.094$) or main effect for condition ($p = 0.299$; partial $\eta^2 = 0.104$), but there was a main effect for time ($p < 0.001$; partial $\eta^2 = 0.984$) for heart rate at rest and during exercise (Table 3).

Blood Pressure

The two-way repeated measures ANOVA for systolic blood pressure indicated there was no significant condition x time interaction ($p = 0.290$; partial $\eta^2 = 0.102$) or main effect for time ($p = 0.124$; partial $\eta^2 = 0.158$), but there was a main effect for condition ($p = 0.002$; partial $\eta^2 = 0.445$) (Table 3). The marginal mean averaged across time for systolic blood pressure was significantly greater in the 2-dose group (110 ± 10 mmHg) compared to the 1-dose (106 ± 10 mmHg) and placebo groups (104 ± 10 mmHg). For diastolic blood pressure, there was a significant condition x time interaction ($p = 0.024$; partial $\eta^2 = 0.193$) (Table 3). The follow-up one-way repeated measures ANOVAs and paired sample *t*-tests indicated that the changes (i.e. delta scores) in diastolic blood pressure from PRE to 15-minutes post ingestion ($p = 0.160$) and PRE to post-exercise ($p = 0.093$)

were not significantly different among conditions. From PRE to 30-minutes post ingestion, however, the 2-dose (9 ± 4 mmHg) and 1-dose (11± 9 mmHg) groups exhibited significantly greater increases in diastolic blood pressure compared to placebo (3 ± 4 mmHg).

Respiratory Exchange Ratio

The two-way repeated measures ANOVA for RER indicated there were was no significant condition x time interaction ($p = 0.154$; partial $\eta^2 = 0.119$) or main effect for condition ($p = 0.759$; partial $\eta^2 = 0.025$), but there was a main effect for time ($p < 0.001$; partial $\eta^2 = 0.401$) (Table 4).

Oxygen Consumption

The two-way repeated measures ANOVA for $\dot{V}O_2$ indicated there were was no significant condition x time interaction ($p = 0.154$; partial $\eta^2 = 0.119$) or main effect for condition ($p = 0.907$; partial $\eta^2 = 0.041$), but there was a main effect for time ($p < 0.001$; partial $\eta^2 = 0.872$) (Table 4).

Carbon Dioxide Production

The two-way repeated measures ANOVA for $\dot{V}CO_2$ indicated there were was no significant condition x time interaction ($p = 0.377$; partial $\eta^2 = 0.090$) or main effect for condition ($p = 0.227$; partial $\eta^2 = 0.126$), but there was a main effect for time ($p < 0.001$; partial $\eta^2 = 0.815$) (Table 4).

Minute Ventilation

The two-way repeated measures ANOVA for $\dot{V}_E$ indicated there were was no significant condition x time interaction ($p = 0.583$; partial $\eta^2 = 0.072$), but there were main effects for condition ($p < 0.001$; partial $\eta^2 = 0.652$) and time ($p < 0.001$; partial $\eta^2 =$

0.800) (Figure 1). The follow-up one-way repeated measures ANOVAs and paired sample *t*-tests indicated that there were significant mean differences among conditions at all time points (Figure 1).

Fat Oxidation

The two-way repeated measures ANOVA for fat oxidation indicated there were was no significant condition x time interaction ($p = 0.367$; partial $\eta^2 = 0.091$) or main effect for condition ($p = 0.690$; partial $\eta^2 = 0.033$), but there was a main effect for time ($p = 0.001$; partial $\eta^2 = 0.323$) (Table 5).

Carbohydrate Oxidation

The two-way repeated measures ANOVA for carbohydrate oxidation indicated there were was no significant condition x time interaction ($p = 0.237$; partial $\eta^2 = 0.106$) or main effect for condition ($p = 0.652$; partial $\eta^2 = 0.038$), but there was a main effect for time ($p < 0.001$; partial $\eta^2 = 0.601$) (Table 5).

Energy Expenditure

The two-way repeated measures ANOVA for EE indicated there were was no significant condition x time interaction ($p = 0.949$; partial $\eta^2 = 0.034$) or main effect for condition ($p = 0.138$; partial $\eta^2 = 0.164$), but there was a main effect for time ($p < 0.001$; partial $\eta^2 = 0.871$) (Table 5).

Table 1. Ingredients in C4 Ripped ® in one serving.

	Ingredients	Quantity (mg)
	Caffeine Anhydrous	150
C4 Ripped Blend	L-Carnitine Tartrate, Green Coffee bean extract (standardized for Chlorogenic Acids), Capsimax® Cayenne (Capsicum annum) fruit extract, *Coleus forskohlii* root extract	1000
Explosive Energy Blend	N-Acetyl-L-Tyrosine, Velvet Bean (*Mucuna pruriens*) seed extract (standardized for L-Dopa)	371
	Beta-alanine	1600
	Arginine	1000

Table 2. Total kilocalories and macronutrients values (mean ± SD) consumed for all conditions (n = 12).

	Placebo	1-Dose	2-Dose
Kcals	1604 ± 423	1833 ± 569	1636 ± 446
Carbohydrates (g)	189 ± 47	204 ± 88	180 ± 65
Protein (g)	68 ± 17	83 ± 33	80 ± 29
Fat (g)	67 ± 29	77 ± 28	68 ± 31

Note: There were no significant ($P > 0.05$) differences among conditions for Kcals or macronutrients.

Table 3. Heart rate and blood pressure values (mean ± SD) during rest and exercise among conditions (n = 12).

	Heart Rate (bpm)			Systolic Blood Pressure (mmHg)			Diastolic Blood Pressure (mmHg)		
	Placebo	1-Dose	2-Dose	Placebo	1-Dose	2-Dose*	Placebo	1-Dose	2-Dose
Baseline	67 ± 12	68 ± 12	65 ± 11	104 ± 12	102 ± 11	107 ± 11	64 ± 7	61 ± 7	66 ± 5
Post 15	66 ± 12	64 ± 9	64 ± 8	101 ± 9	104 ± 11	111 ± 12	67 ± 6	67 ± 7	74 ± 6
Post 30	69 ± 10	64 ± 12	66 ± 14	105 ± 10	110 ± 9	112 ± 10	67 ± 6	72 ± 6†	75 ± 5†
EXERCISE (minutes)									
5	150 ± 10	147 ± 12	144 ± 16						
10	159 ± 15	159 ± 16	157 ± 17						
15	164 ± 17	164 ± 17	162 ± 19						
20	167 ± 17	167 ± 18	165 ± 19						
25	169 ± 18	171 ± 18	169 ± 20						
30	172 ± 18	174 ± 19	170 ± 20						
5-MIN POST EXERCISE				105 ± 9	107 ± 9	110 ± 6	65 ± 8	69 ± 4	70 ± 6

*Significant ($P < 0.05$) main effect for condition collapsed across time (2-Dose > Placebo and 1-Dose). Significantly ($P < 0.05$) greater increase in diastolic blood pressure from baseline to post-30 minute ingestion compared to placebo.

Table 4. Measures of gas exchange values (mean ± SD) during exercise among conditions (n = 12).

	RER			$\dot{V}CO_2$			$\dot{V}O_2$		
	Placebo	1-Dose	2-Dose	Placebo	1-Dose	2-Dose	Placebo	1-Dose	2-Dose
Minutes									
5	0.91 ± 0.05	0.89 ± 0.06	0.89 ± 0.05	1852 ± 183	1818 ± 173	1847 ± 188	2045 ± 198	2041 ± 225	2071 ± 229
10	0.93 ± 0.03	0.92 ± 0.04	0.93 ± 0.04	2046 ± 176	2043 ± 202	2078 ± 195	2204 ± 205	2222 ± 251	2254 ± 233
15	0.93 ± 0.02	0.92 ± 0.04	0.92 ± 0.04	2062 ± 168	2066 ± 199	2111 ± 202	2236 ± 206	2245 ± 240	2285 ± 242
20	0.93 ± 0.03	0.93 ± 0.04	0.93 ± 0.04	2077 ± 161	2087 ± 178	2126 ± 207	2243 ± 198	2265 ± 236	2303 ± 236
25	0.92 ± 0.03	0.92 ± 0.04	0.92 ± 0.04	2080 ± 168	2080 ± 179	2127 ± 205	2267 ± 198	2276 ± 235	2316 ± 224
30	0.91 ± 0.03	0.90 ± 0.04	0.91 ± 0.04	2076 ± 170	2055 ± 195	2116 ± 231	2279 ± 200	2286 ± 238	2327 ± 232

Note: There were no significant ($P > 0.05$) differences among conditions for RER, $\dot{V}CO_2$, or $\dot{V}O_2$.

Table 5. Substrate utilization and energy expenditure values (mean ± SD) during exercise among conditions (n = 12).

	Fat Oxidation ($g \cdot min^{-1}$)			**Carbohydrate Oxidation ($g \cdot min^{-1}$)**			**Energy Expenditure ($kcals \cdot min^{-1}$)**		
	Placebo	1-Dose	2-Dose	Placebo	1-Dose	2-Dose	Placebo	1-Dose	2-Dose
Minutes									
5	0.32 ± 0.18	0.37 ± 0.23	0.37 ± 0.22	1.89 ± 0.47	1.75 ± 0.49	1.79 ± 0.50	8.12 ± 0.80	8.13 ± 0.93	8.24 ± 0.94
10	0.26 ± 0.11	0.29 ± 0.16	0.29 ± 0.16	2.27 ± 0.26	2.20 ± 0.34	2.26 ± 0.36	8.73 ± 0.83	8.81 ± 1.02	8.93 ± 0.95
15	0.29 ± 0.10	0.29 ± 0.19	0.28 ± 0.17	2.24 ± 0.20	2.23 ± 0.42	2.31 ± 0.38	8.86 ± 0.83	8.90 ± 0.98	9.05 ± 0.98
20	0.27 ± 0.11	0.29 ± 0.18	0.29 ± 0.16	2.29 ± 0.22	2.26 ± 0.35	2.31 ± 0.40	8.89 ± 0.80	8.98 ± 0.97	9.13 ± 0.95
25	0.30 ± 0.12	0.32 ± 0.19	0.31 ± 0.17	2.22 ± 0.28	2.20 ± 0.38	2.28 ± 0.43	8.99 ± 0.80	9.03 ± 0.96	9.19 ± 0.90
30	0.33 ± 0.13	0.38 ± 0.19	0.34 ± 0.18	2.16 ± 0.31	2.05 ± 0.42	2.19 ± 0.52	9.05 ± 0.81	9.09 ± 0.97	9.24 ± 0.93

Note: There were no significant ($P > 0.05$) differences among conditions for fat oxidation, carbohydrate oxidation, or energy expenditure.

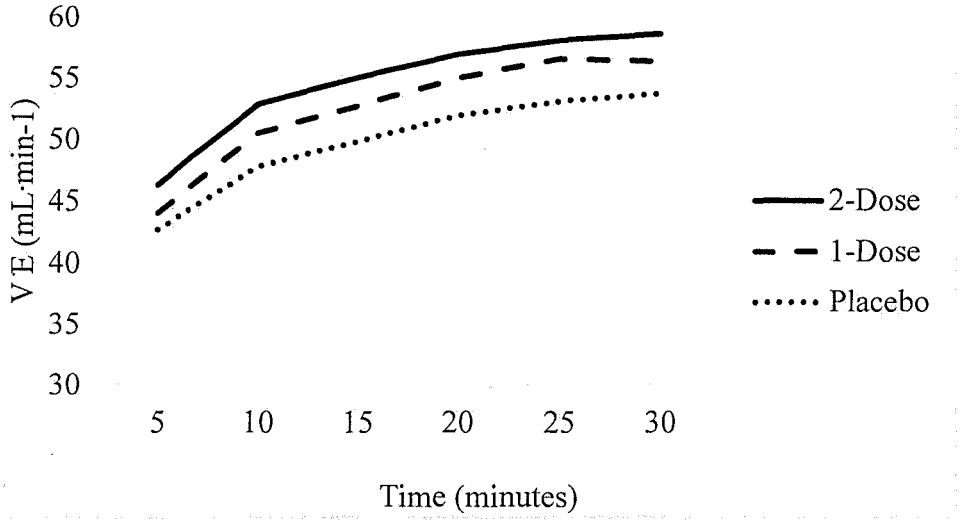

Figure 1. 5-minute ventilation averages among conditions during 30 minutes of treadmill running.

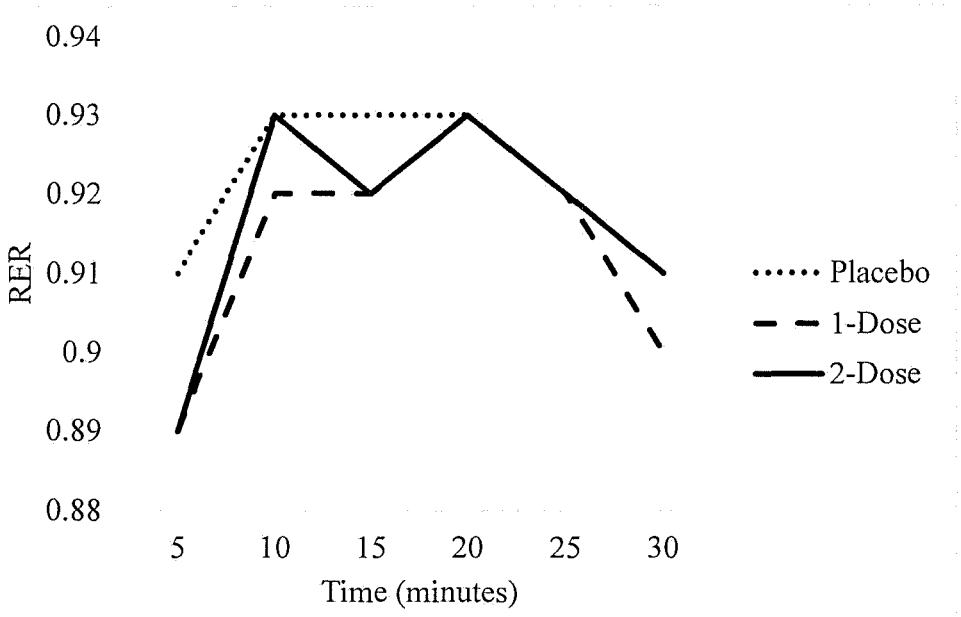

Figure 2. 5-minute RER averages among conditions during 30 minutes of treadmill running.

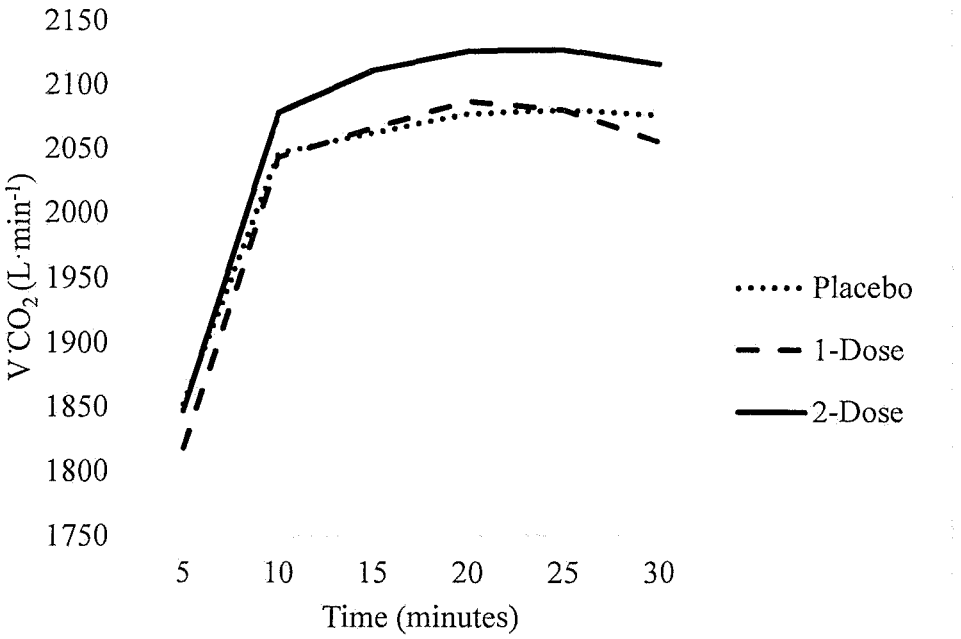

Figure 3. 5-minute $\dot{V}CO_2$ averages among conditions during 30 minutes of treadmill running.

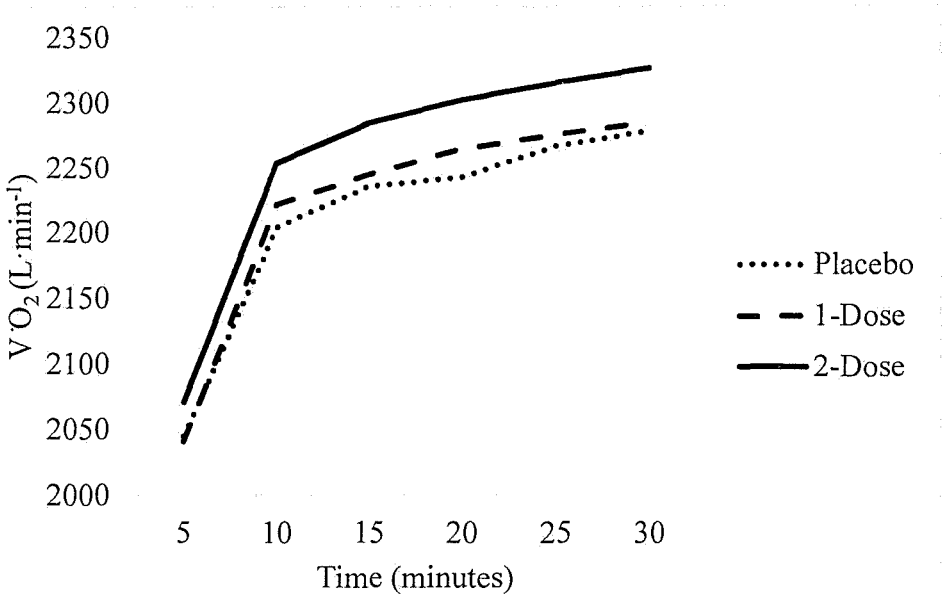

Figure 4. 5-minute $\dot{V}O_2$ averages among conditions during 30 minutes of treadmill running.

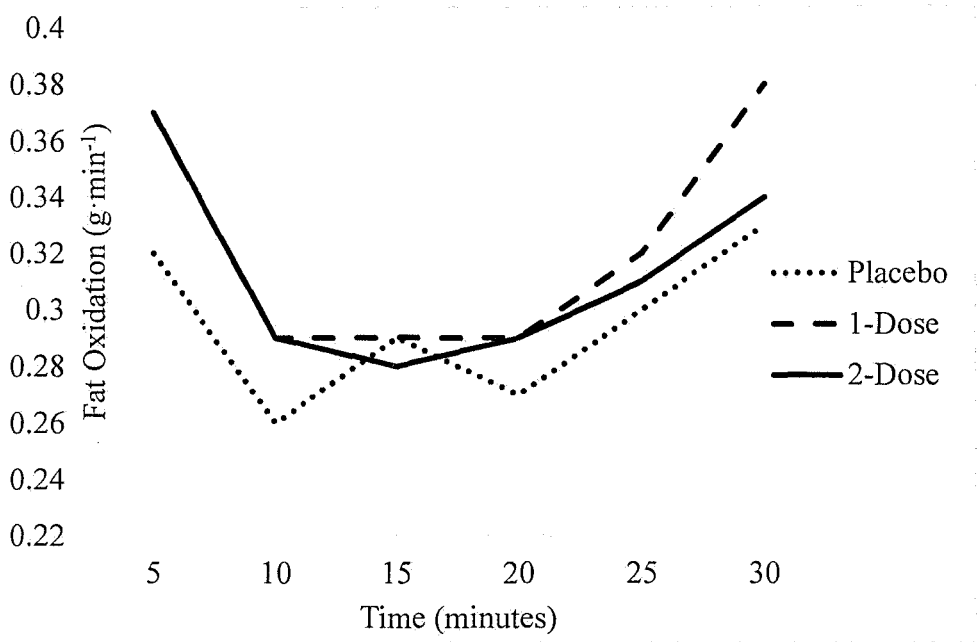

Figure 5. 5-minute fat oxidation averages among conditions during 30 minutes of treadmill running.

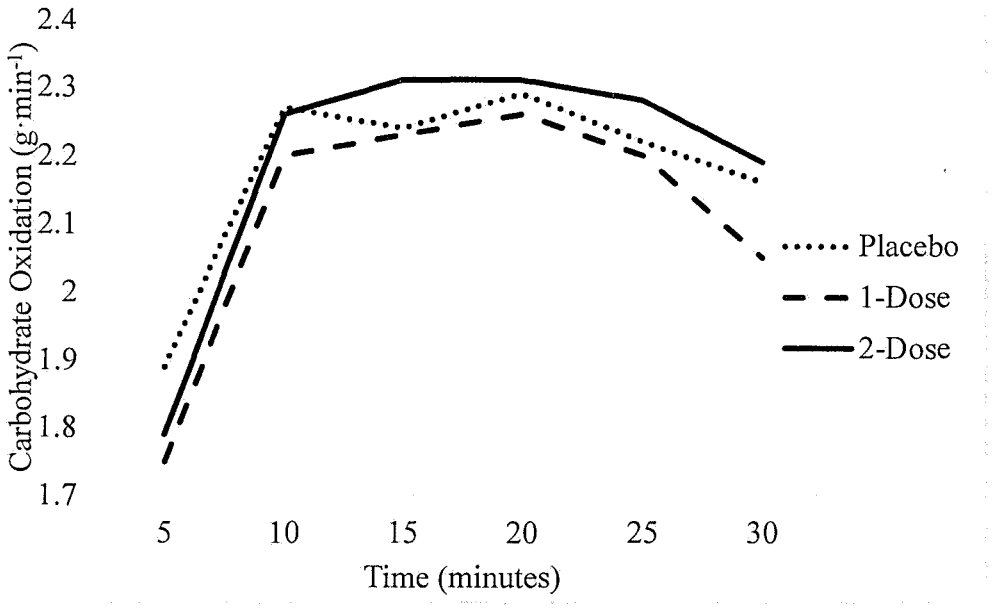

Figure 6. 5-minute carbohydrate oxidation averages among conditions during 30 minutes of treadmill running.

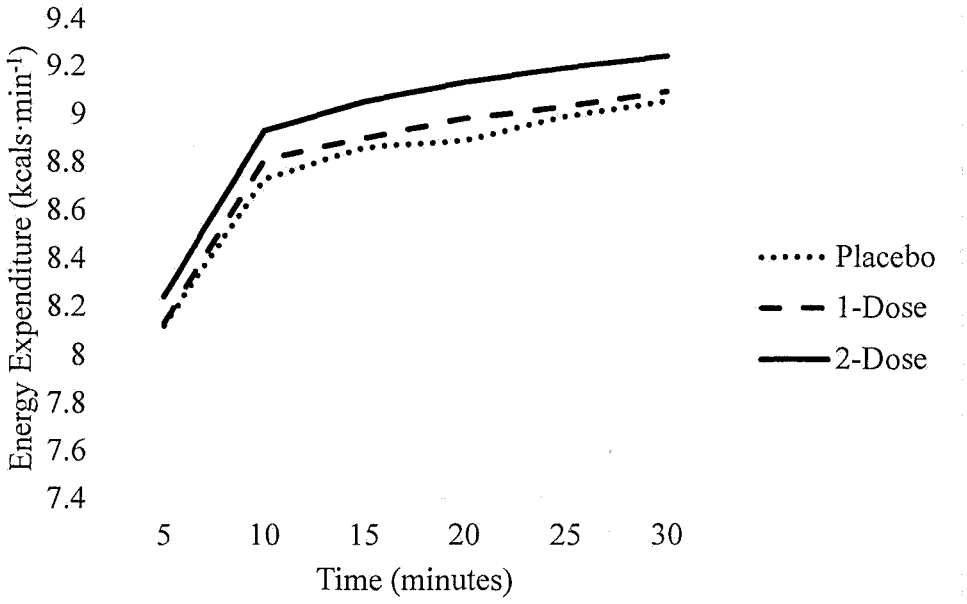

Figure 7. 5-minute average energy expenditure values among conditions during 30 minutes of treadmill running.

DISCUSSION

Resting and Exercise Heart Rate & Blood Pressure

In the present investigation, the acute ingestion of a single or double recommended dosage of the thermogenic supplement had no significant effect on resting or exercise HR values during 30 minutes of moderate intensity running (Table 3). Previous studies that have examined the influence of caffeine and caffeine-based pre-workout supplements have reported conflicting findings (Bergstrom et al., 2013; Daniels et al., 1998; Hoffman et al., 2009; Jung et al., 2009; McClaran et al., 2007). For example, Hoffman et al. (2009) found increases in resting HR two hours (70.4 ± 9.4 to 74.3 ± 12.6 bpm) and 3 hours (70.4 ± 9.4 to 72.3 ± 9.1 bpm) post ingestion of a caffeine-based (317 mg) weight loss supplement. In contrast, Daniels et al. (1998) reported that consuming caffeine (6 $mg \cdot kg^{-1}$ body weight) did not change HR at rest or during 55 minutes of cycle ergometry at 65% $\dot{V}O_{2max}$. McClaran et al. (2007), however, found decreases in HR (4-7 bpm) during submaximal cycle ergometry after caffeine administration (3 $mg \cdot kg^{-1}$). Similar to our findings, Bergstrom et al. (2013) found that HR did not significantly increase at rest or during 60 minutes of treadmill walking or during exercise after administering a thermogenic supplement containing caffeine (200 mg), capsicum (100 mg), and *Mucuna pruriens* seed extract (500 mg).

The present findings also indicated that the 2-dose condition led to significantly ($p < 0.05$) greater increases in resting SBP (collapsed across time) compared to the 1-dose and placebo conditions (Table 3). In addition, there were significantly greater increases (p

< 0.05) in DBP from baseline to 30-minutes post supplementation for the 1-dose (61 ± 7 to 72 ± 6 mmHg) and 2-dose (66 ± 5 to 75 ± 5 mmHg) conditions, but not the placebo (64 ± 7 to 67 ± 6 mmHg). Previous findings (Bergstrom et al., 2013; Daniels et al., 1998; Hoffman et al., 2009; McClaran et al., 2007) of the effect of caffeine and caffeine-based PWS on SBP and DBP have reported inconsistent results. Specifically, Daniels et al. (1998) demonstrated that consuming caffeine (6 $mg \cdot kg^{-1}$ body weight) increased resting SBP (17%) 20 and 40 minutes post-ingestion, but did not increase DBP. In addition, McClaran et al. (2007) found increases in resting SBP (116 ± 13 to 123 ± 10 mm Hg) after 30 minutes post-caffeine ingestion (3 $mg \cdot kg^{-1}$). Furthermore, Hoffman et al. (2009) reported increases in SBP 1 hour (113 ± 10 to 116 ± 8 mmHg), 2 hours (113 ± 10 to 121 ± 7 mmHg), and 3 hours (113 ± 10 to 119 ± 9 mmHg) post supplementation of a caffeine-containing (317 mg) weight loss supplement. Our findings were also inconsistent with Jung et al. (2009) who found no significant differences in SBP or DBP after administering a caffeine-containing (284 mg) PWS. Bergstrom et al. (2013) also found no changes in SBP after administering a caffeine-containing (200 mg) thermogenic supplement at rest or during exercise, but found significant increases in DBP 15, 30, and 60 minutes post-exercise. Collectively, the findings of these studies (Bergstrom et al., 2013; Daniels et al. 1998; Hoffman et al., 2009; Jung et al., 2009; McClaran et al. 2007) indicated that the effects of caffeine-based supplements on heart rate and blood pressure are conflicting, and may be due to differences in supplementation protocol (i.e. dosage, administration timing, blend of ingredients), conditions examined (i.e. at rest or intensity of exercise), or prior history with caffeine.

A possible explanation for the changes in HR and blood pressure found in the present investigation may be due to the physiological mechanisms associated with caffeine and other ingredients including increases in total peripheral resistance (TPR), the blocking of adenosine receptors, and augmented catecholamine release. Total Peripheral Resistance is the force required to maintain blood flow from the aorta to venous exit (Plowman & Smith, 2014). The physiological effects of caffeine consist of increased TPR through blood vessel vasoconstriction by blocking adenosine receptors that function to dilate coronary arteries to allow for increased blood flow during exercise (Higgins, 2013). The blocking of adenosine receptors causes adenosine to increase within the body which stimulates the release of catecholamines (i.e. norepinephrine) (Higgins, 2013). Catecholamines act on beta receptors and promote the release of renin that results in an increase in blood pressure (Bergstrom et al., 2014; Daniels et al., 1998; Higgins, 2013; Robertson et al., 1978). Other possible reasons for variance among studies may be due to individual tolerances to caffeine as well as caffeine wash-out period differences (48 hours, 2 weeks, 6 weeks, or no prior caffeine use).

In summary, our findings as well as the aforementioned studies (Bergstrom et al., 2013; Daniels et al. 1998; Hoffman et al., 2009; Jung et al., 2009; McClaran et al. 2007) had varying conclusions of the influence of caffeine and caffeine-containing supplements on HR, SBP, and DBP. Each of the studies had different caffeine and supplement restrictions prior to and throughout the duration of the study. These differences as well as variance among individual subjects and study protocols may have resulted in the inconsistencies among studies.

Metabolic Data

Our findings showed no changes in EE, fat or carbohydrate oxidation among the supplement conditions and placebo at any time point during the 30-minute run at 90% VT. Many researchers (Ahrens et al. 2007; Belza et al., 2009; Bergstrom et al., 2013; Hoffman et al., 2009; Schubert et al., 2014; Wallman et al., 2010) that have examined the effects of caffeine and caffeine-containing supplements on EE and substrate utilization reported various findings. For example, Belza et al. (2009) demonstrated that caffeine supplementation (50 mg) provided a positive thermogenic response (72 ± 25 kJ per 4 hours) versus the placebo condition, and increased resting metabolic rate (6% above the basal rate baseline). Hoffman et al. (2009) investigated the effects of a caffeine-containing supplement on resting EE and found increases in EE 1-3 hours post-supplementation compared to the placebo group (hour 1: 0.96 ± 0.27 vs. 1.25 ± 0.39 $kcal \cdot min^{-1}$, hour 2: 1.03 ± 0.35 vs. 1.29 ± 0.34 $kcal \cdot min^{-1}$, hour 3: 1.05 ± 0.37 vs. 1.31 ± 0.28 $kcal \cdot min^{-1}$). Similar to our findings, Ahrens et al. (2007) reported that a caffeine dose (3 $mg \cdot kg^{-1}$ of body weight) had no effect on EE in women at rest or during exercise. Wallman et al. (2010), however, found that there was a significant increase in EE (304 ± 296 vs. 322 ± 315 kJ) at the 15 minute mark of 15-minute cycling bout at 65% of age-predicted maximal heart rate after caffeine supplementation (6 $mg \cdot kg^{-1}$ of body weight) in sedentary females. Bergstrom et al. (2013) reported an increase in post exercise EE (5-7%) after supplementing with a PWS containing 200 mg of caffeine. Furthermore, Schubert et al. (2014) examined the influence of caffeine (6 $mg \cdot kg^{-1}$ of body weight) ingestion 90 minutes prior to exercise on energy expenditure as well as during an hour of cycling at 65% $\dot{V}O_2$ max. The authors (Schubert et al., 2014) reported that the caffeine

supplement resulted in significantly greater resting EE 30 minutes post-supplementation (369 ± 62 vs. 420 ± 99 kJ), during exercise (414 ± 75 vs. 3390 ± 673 kJ), and 2 hours post exercise (438 ± 88 vs. 517 ± 90 kJ) compared to a control. Regarding substrate utilization, Schubert et al. (2014) found that fat oxidation increased 60 minutes prior to exercise (4.1 ± 1.0 vs. 5.4 ± 1.4 g), during exercise (24.3 ± 7.2 vs. 30.4 ± 9.6 g), 1 hour post exercise (6.5 ± 2.0 vs. 7.4 ± 2.0 g) and 2 hours post exercise (4.5 ± 1.1 vs. 6.2 ± 2.3 g) compared to a control.

Capsicum and Coleus forskohlii have been investigated in chronic studies (Henderson et al., 2005; Inoue et al., 2007; Lejeune et al., 2003; Loftus et al., 2015; Snitker et al., 2009) and have exhibited positive effects on energy expenditure and fat oxidation; however, further research must be conducted on the acute effects of these ingredients on metabolic variables, heart rate, and blood pressure.

A potential reason for differences among studies may be due to the duration of exercise we used (30 minutes) compared to the other study protocols. With longer durations of exercise at lower intensities, there may have been more time to mobilize more fatty acids to utilize as a fuel source.

Incidences of increased EE and fat oxidation in these studies (Belza et al., 2009; Bergstrom et al., 2013; Hoffman et al., 2009; Schubert et al., 2014; Wallman et al., 2010) were likely due to catecholamine release (e.g. norepinephrine via caffeine stimulation (Bergstrom et al., 2014; Daniels et al., 1998; Robertson et al., 1978). Catecholamines act on beta receptors to promote the release of the renin, thereby increasing lipolysis and free fatty acid concentration (Bergstrom et al., 2014; Daniels et al., 1998; Robertson et al., 1978).

The present findings also indicated that there were no differences in the responses for $\dot{V}O_2$, $\dot{V}CO_2$, or RER among the conditions at any time point. The lack of changes in $\dot{V}O_2$, $\dot{V}CO_2$, and RER support why there were no changes in EE, fat and carbohydrate oxidation. The effect of caffeine and caffeine-containing supplements on $\dot{V}O_2$ $\dot{V}CO_2$, and RER have been investigated (Ahrens et al., 2007; Bergstrom et al., 2013; Jung et al., 2017; McClaran et al., 2007; Wallman et al., 2010) and show various findings. For example, Jung et al. (2017) found that supplementing with a caffeine-containing weight loss supplement increased resting $\dot{V}O_2$ (684 ± 376 vs. 1,034 ± 584 ml/min), $\dot{V}CO_2$ (634 ± 262 vs. 1,372 ± 604 ml/min), and RER (1.48 ± 0.67 vs. 2.79 ± 0.89) toward the end of a 30-minute rest protocol. During exercise, other studies (Ahrens et al., 2007; Bergstrom et al., 2013) demonstrated that caffeine supplementation (6 $mg \cdot kg^{-1}$ of body weight and 200 mg, respectively) increased $\dot{V}O_2$ (4%) in women during 60 minutes of treadmill walking with no change in RER in either protocol. Furthermore, Wallman et al. (2010) found that there was a significant increase in $\dot{V}O_2$ (17.78 ± 3.93 vs. 15.85 ± 3.64 $ml \cdot kg^{-1} \cdot min^{-1}$) at the 15 minute mark of a 15-minute cycling bout at 65% of age-predicted maximal heart rate after caffeine supplementation (6 $mg \cdot kg^{-1}$ of body weight) in sedentary females, but there was no change in RER at any time point (Wallman et al., 2010). In contrast, McClaran et al. (2007) saw a decrease in RER (0.89 ± 0.08 vs. 0.84 ± 0.09) and no change in $\dot{V}O_2$ after administering caffeine (3.0 $mg \cdot kg^{-1}$ body weight) to subjects during submaximal cycle ergometry. The findings of these investigations and our findings indicated that caffeine and caffeine-containing supplements at various dosages may or may not impact RER and could enhance performance during submaximal exercise and at rest depending on the supplementation protocol.

CONCLUSIONS

In summary, we found that a thermogenic PWS containing caffeine, beta alanine, arginine, L-carnitine, green coffee bean extract, *Capsicum annuum*, *Coleus forskohlii*, tyrosine, and *Mucuna pruriens* had no effect on postsupplementation HR, EE, fat or carbohydrate oxidation, $\dot{V}O_2$, $\dot{V}CO_2$, or RER during 30 minutes of submaximal treadmill running. However, there were significantly greater increases in resting SBP and DBP for the 1 and 2-dose conditions compared to the placebo. Our findings suggest that the elevations in resting SBP with no change in HR may be a result of increased TPR. The lack of changes in metabolic data may have been due to the duration of treadmill running. We chose 30 minutes of postsupplementation rest as recommended by the manufacturer and 30 minutes of submaximal treadmill running to mimic the exercise duration and intensity of a recreational runner. Therefore, our results suggest that a small and moderate dose of caffeine combined with a thermogenic blend causes small changes in resting cardiovascular function, but does not affect metabolic rate during 30 minutes of submaximal treadmill running.

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APPENDIX A

INFORMED CONSENT

Statement of Informed Consent

Title of Project

Safety and efficacy of a multi-ingredient thermogenic pre-workout supplement during low-intensity running in college-aged females.

Purpose and Procedures

- This project is intended to examine the effects of an acute dose of a thermogenic pre-workout supplement on resting heart rate and blood pressure, as well as gas exchange variables, energy expenditure, substrate utilization, and psychological measures in females during steady state low-intensity treadmill running.
- My participation will involve five testing visits (1-1.5 hours each) to the Human Performance Laboratory (225 Mitchell Hall) which will include one maximal and four submaximal treadmill tests.
- Each visit will require me to run on a treadmill for 10-30 minutes with moderate-to-maximal effort while breathing into a mouthpiece that collects my expired gases.
- During the third, fourth, and fifth visits, I will be required to visit the laboratory in the morning in a fasted state (no prior food or beverage other than water for 8 hours). In addition, I will be asked to ingest: 1) a standardized meal (Muscle Milk drink) as well as one or two servings of the supplement or placebo 30 minutes prior to each of the 30-minute submaximal tests. One serving of the supplement (Cellucor® C4 Ripped) contains Vitamin C (250 mg), Niacin (30 mg), Vitamin B6 (500 mcg), Folic Acid (250 mcg), Vitamin B12 (35 mcg), calcium (9 mg), beta alanine (1.6 g), arginine AKG (1 g), C4® Ripped Blend [1 g; L-Carnitine Tartrate, Green Coffee bean extract (standardized for Chlorogenic Acids), Capsimax® Cayenne (*Capsicum annuum*) fruit extract, *Coleus forskohlii* root extract], and Explosive Energy Blend [371 mg; N-Acetyl-L-Tyrosine, Caffeine Anhydrous (150 mg), Velvet Bean (*Mucuna pruriens*) seed extract (standardized for L-Dopa)]. The placebo is Crystal Light®.
- The total time requirement for my participation is 6 hours over a three-week period.

Potential Risks

- According to the manufacturer (Cellucor®; https://www.cellucor.com/c4-ripped): "THIS PRODUCT IS ONLY INTENDED TO BE CONSUMED BY HEALTHY ADULTS, 18 YEARS OF AGE OR OLDER. Do not use this product if you are pregnant or nursing. Before using this product, consult a licensed, qualified, health care professional, including but not limited to, if: you are taking antidepressants such as a MAOI (Monoamine Oxidase Inhibitor) or SSRI, blood thinners, nonsteroidal anti-inflammatory drugs, pseudoephedrine, or you are taking any other dietary supplement, prescription drug or over-the-counter medication; or if, you suspect you have or have been treated for, diagnosed with or have a family history of, any medical condition, including but not limited to: high or low blood pressure, diabetes, glaucoma, anxiety, cardiovascular, psychiatric or seizure disorders, cardiac arrhythmia, stroke, heart, liver, kidney or thyroid disease, or difficulty urinating due to prostate enlargement. This product contains caffeine and should not be used by

individuals wishing to eliminate caffeine from their diet or in combination with caffeine or stimulants from other sources, including but not limited to, coffee, tea, soda, or other dietary supplements and medications. Discontinue use 2 weeks prior to surgery. Immediately discontinue use and contact a medical doctor if you experience any adverse reaction to this product." In order to reduce any risks associated with the supplement, the dose(s) as recommended by the manufacturer (Cellucor®) will be utilized. In the unlikely event that you should suffer an illness as a direct consequence of the supplement, the acute medical care required to treat the injury can be provided at the Student Health Center (1300 Badger St. La Crosse, WI 54601) from the hours of 8:00AM – 4:00PM Monday through Friday.

- Performing maximal tests may lead to muscle tears or soreness, substantial fatigue, dizziness, headache, acute elevation of blood pressure, heart attack, stroke, or sudden death. According to the American College of Sports Medicine, the absolute risk of sudden cardiac death during vigorous physical activity has been estimated at one per year for every 15,000 – 18,000 people.
- Individuals trained in CPR, Advanced Cardiac Life Support, and First Aid will be in the laboratory, and the test will be terminated if complications occur.

Rights and Confidentiality

- My participation in this study is voluntary. I can withdraw or refuse to participate or answer any questions at any time without consequence.
- The results of this study may be published in scientific literature or presented at meetings using grouped data only.
- All information will be kept confidential through the use of number codes and my data will not be linked with personally identifiable information.

Possible Benefits

- My main benefit from participation in this study will be the feedback on my level of physical fitness including knowledge of my ventilatory threshold and peak oxygen uptake.

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You may ask any questions concerning this research and have those questions answered before agreeing to participate in or during the study. Or you may call the principal investigator, Jamie Erickson or Dr. Clayton Camic. Questions regarding the protection of human subjects may be addressed to the University of Wisconsin-La Crosse Institutional Review Board for the Protection of Human Subjects: (608)785-8124 or irb@uwlax.edu.

Participant (Print Name): _____

Participant (Sign Name): _____
Date:_____

Principal Investigator: _____
Date:_____

I have observed the informed consent process for this subject and am writing my name below to signify that I believe that the subject understands the nature of the study and the risks that they are being asked to assume.

Witness: _____ Date: _____

APPENDIX B

REVIEW OF LITERATURE

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Pre-workout supplementation is the nutritional strategy that often involves consuming a mixture of bioactive compounds and dietary ingredients prior to a bout of exercise for ergogenic purposes. The use of pre-workout supplements (PWS) has become increasingly popular in competitive athletes and recreational exercisers despite adequate research on the safety and efficacy of these products. For example, PWS typically contain a multitude of ingredients at varying quantities that include thermogenic substances, endurance boosters, strength and power enhancers, as well as numerous vitamins and minerals. The purported effects associated with PWS consist of elevated metabolic rate, increased measures of anaerobic and aerobic performance, and improvements in body composition. Due to the relatively brief period of time that PWS have been available, however, there are limited data concerning the influence of these multi-ingredient products on their ergogenic potential and variables related to general health (e.g. resting heart rate and blood pressure).

Thermogenic Agents

Thermogenic agents are compounds that increase basal metabolic rate through stimulation of the sympathetic nervous system. Often, a number of these compounds are combined in a single PWS to obtain effects that include increased fat oxidation, energy expenditure, heart rate, blood flow, blood pressure, and the suppression of appetite

(Vogel et al., 2015). Powerful thermogenic agents such as ephedra and dimethylamylamine (DMAA) have been associated with several accidental deaths and subsequently recalled off the market. In particular, ephedra (i.e. ephedrine, Ma Huang) is a stimulant previously used to control weight and aid in sports performance, but has been prohibited by the Food and Drug Administration (FDA) as a supplement since 2004 due to its linkage with heart attacks and stroke ("FDA Issues", 2004). Dimethylamylamine, another stimulant that promotes weight loss and enhances performance, was banned in 2012 after 86 people fell ill or died of heart, nervous system or psychiatric disorders after using supplements containing this ingredient ("Stimulant", 2013). These instances show potential consequences of taking powerful thermogenics in regards to cardiovascular and nervous system health. The FDA does not pre-approve or test supplements before they are allowed on the market, but can issue warning letters and press criminal charges on companies who add banned substances into their products ("Dietary Supplements", 2015). Given this information, it is important to research the interactions of various thermogenic agents and other ingredients in PWS to determine the safety and efficacy of their respective dosages.

Caffeine

There are several less potent thermogenic agents such as caffeine, capsicum, tyrosine, *Mucuna pruriens*, green coffee bean extract, *Coleus forskohlii*, and L-carnitine that may influence fat oxidation, exercise performance, and energy expenditure during exercise. For example, caffeine stimulates the release of catecholamines (e.g. noradrenaline) that act on beta receptors and promotes the release of the renin, thereby increasing heart rate, blood pressure, lipolysis, and free fatty acid concentration

(Bergstrom et al., 2014; Daniels et al., 1998; Robertson et al., 1978). McClaran et al. (2007) investigated the effects of caffeine supplementation (1.5 and 3 $mg \cdot kg^{-1}$) on heart rate and blood pressure at rest as well as during submaximal and maximal cycle ergometry. Heart rate decreased during submaximal exercise compared to placebo conditions (4-7 bpm lower), but not at rest or during maximal exercise. Blood pressure significantly increased during rest after the ingestion of 3 $mg \cdot kg^{-1}$ of caffeine when compared to placebo conditions (116 ± 13 vs. 123 ± 10 mm Hg), but not during the exercise bouts. In addition, Daniels et al. (1998) analyzed the effects of caffeine (6 $mg \cdot kg^{-1}$ body weight) on heart rate and blood pressure at rest and during cycle ergometry at 65% $\dot{V}O_{2\ max}$ for 55 minutes. Caffeine ingestion increased systolic blood pressure at rest (17%) and during exercise when compared to placebo conditions although the increases during exercise were secondary to those at rest. No significant changes were seen in heart rate. In conjunction, the findings of these studies (Daniels et al. 1998; McClaran et al. 2007) indicated that the effects of caffeine on heart rate and blood pressure are conflicting, and may be due to differences in supplementation protocol (i.e. dosage, administration timing) or conditions examined (i.e. at rest or intensity of exercise).

Previous investigations (Ahrens et al. 2007; Belza et al., 2009; Petro et al., 1998; Schubert et al., 2014; Wallman et al., 2010) have also analyzed the effects of caffeine consumption on a number of performance variables. Specifically, Schubert et al. (2014) examined the influence of caffeine (6 $mg \cdot kg^{-1}$ of body weight) ingestion 90 minutes prior to exercise on energy expenditure during an hour of cycling at 65% $\dot{V}O_2$ max (Schubert et al., 2014). The authors (Schubert et al., 2014) reported that the caffeine supplement resulted in significantly greater energy expenditure and fat oxidation prior to, during, and

2 hours post-exercise compared to the placebo trial. In addition, the caffeine supplement resulted in more exercise enjoyment and perceived the exercise task to be less difficult (Schubert et al., 2014). Wallman et al. (2010) also studied the effects of caffeine consumption (6 $mg \cdot kg^{-1}$ of body weight) versus placebo on exercise performance in sedentary females during two consecutive cycling bouts (Wallman et al., 2010). First, a 15-minute cycling bout at 65% of age-predicted maximal heart rate (phase A) was completed, followed by 10 minutes of cycling as fast as the participant was able (phase B). It was found that there was a significant increase in energy expenditure (22 ± 5 vs. 20 ± 5 kJ) and $\dot{V}O_2$ (17.78 ± 3.93 vs. 15.85 ± 3.64 $ml \cdot kg^{-1} \cdot min^{-1}$) at the 15 minute mark of phase A during the caffeine condition; however, no significant changes were seen during phase B. In addition, Ahrens et al. (2007) demonstrated that caffeine supplementation (6 $mg \cdot kg^{-1}$ of body weight) increased resting $\dot{V}O_2$ (5%) and energy expenditure (4%) in women during treadmill walking at 94 $m \cdot min^{-1}$ (3.5 mph). Furthermore, Belza et al. (2009) found that caffeine supplementation (50 mg) provided a positive thermogenic response of 72 ±25 kJ per 4 hours verses the placebo condition, and increased resting metabolic rate 6% above the basal rate baseline. It has also been reported (Petro et al. 1998) that supplementation with caffeine (5 $mg \cdot kg^{-1}$ of body weight) increased time to exhaustion (46.54 ± 8.05 vs. 32.42 ± 14.81 min) during cycling at exercise intensities below the anaerobic threshold compared to placebo. Thus, the findings of these investigations (Ahrens et al. 2007; Belza et al., 2009; Petro et al., 1998; Schubert et al., 2014; Wallman et al., 2010) indicated that supplementation with caffeine at various dosages may increase energy expenditure and performance during submaximal exercise and at rest.

Capsicum

Capsicum has been studied for its possible effect on appetite suppression to aid in weight loss (Lejeune et al., 2003). In addition, capsinoids, an analogue of capsicum with low pungency, have been examined for its influence on fatness and energy metabolism in humans. For example, Snitker et al. (2009) recruited 80 subjects desiring to lose weight that were assigned to either 6 $mg \cdot d^{-1}$ of capsinoids or a placebo for 12 weeks. Dietary intervention occurred prior to the study, and the subjects were instructed to induce a moderate calorie deficit (~300-600 kcal). It was found that capsinoid supplementation decreased abdominal adiposity ($-1.11 \pm 1.83\%$) compared to the placebo group ($-0.18 \pm 1.94\%$) and increased fat oxidation (least-squares mean difference: 21.0 mg·min). In addition, Lejeune et al. (2003) administered 135 $mg \cdot d^{-1}$ of capsaicin, the pungent principle in hot red peppers, to subjects to determine its efficacy in weight maintenance and fat oxidation during and after a 4-week very-low-energy diet (VLED) to attain weight loss. Study findings concluded that capsaicin supplementation resulted in higher fat oxidation and energy expenditure after the VLED when compared to the placebo group (Lejeune et al., 2003). Furthermore, Inoue et al. (2007) administered 3 $mg \cdot kg^{-1}$ and 10 $mg \cdot kg^{-1}$ of capsinoids per day for 4 weeks and a placebo to three different groups with a body mass index (BMI) over 23 $kg \cdot m^{-2}$ to determine the effect on energy metabolism. $\dot{V}O_2$, resting energy expenditure, and fat oxidation increased compared to pre-administration values in the supplement group, but not to a greater extent than the placebo. Inoue et al. (2007) computed a meta-analysis on subjects with a BMI ≥ 25 $kg \cdot m^{-2}$ and found that $\dot{V}O_2$ significantly increased in the 10 $mg \cdot kg^{-1}$ supplement group, whereas resting energy expenditure and fat oxidation significantly increased in both the 3 and 10

$mg \cdot kg^{-1}$ capsinoid groups. It has been suggested (Hendrix et al., 2010) that the combination of capsicum and caffeine supplementation may work synergistically to further increase energy expenditure, reduce energy intake, and improve exercise performance. In particular, Hendrix et al. (2010) administered an acute dose of a supplement containing caffeine (400 mg) and capsicum extract (66.7 mg) and measured 1 repetition maximum (1RM) bench press and leg extension as well as time to exhaustion (TTE) on a cycle ergometer corresponding to 80% $\dot{V}O_2$peak. The results showed that no significant difference in 1RM bench press (82.9 ± 12.4 vs. 82.9 ± 12.3 kg), leg extension (121.7 ± 22.4 vs. 119.2 ± 17.6 kg) or TTE (692.3 ± 214.8 vs. 668.5 ± 195.7 s) occurred in the supplement trial compared to the placebo. In conjunction, these findings (Hendrix et al., 2010; Inoue et al., 2007; Lejeune et al., 2003; Snitker et al., 2009) indicated that capsicum may have positive effects on energy expenditure, fat oxidation, and $\dot{V}O_2$ but not performance variables related to muscular strength or time to exhaustion.

Tyrosine

Amino acids may benefit exercisers by increasing lean muscle mass through muscle protein synthesis, enhancing recovery and reducing muscle soreness (Joy et al., 2015). Tyrosine, a nonessential amino acid commonly found in PWS, is a noradrenaline precursor that may enhance the synthesis and release of noradrenaline in the sympathetic nervous system (Belza, et al., 2009). Belza, Toubro, and Astrup (2009) administered 400 mg pills of tyrosine to subjects to determine its effects on thermogenesis and energy intake. It was found that tyrosine increased carbohydrate oxidation by 1% and induced no thermogenic effect; however, resting metabolic rate was elevated 2% above the basal metabolic rate baseline (Belza, et al., 2009). Tyrosine has also been investigated for its

effects on prolonged endurance exercise. In a study analyzing the effects of repeated dosing (60, 30, & 0 minutes before exercise and 30, 60, & 90 minutes into the exercise bout) of tyrosine and carbohydrate ingestion on endurance exercise performance (Chinevere et al., 2002), a 5 $ml \cdot kg^{-1}$ solution with polydextrose (70 $g \cdot l^{-1}$ of carbohydrate), a 5 $ml \cdot kg^{-1}$ of body weight solution with tyrosine (25 $ml \cdot kg^{-1}$ of body weight), a 5 $ml \cdot kg^{-1}$ solution with polydextrose (70 $g \cdot l^{-1}$) and tyrosine (25 $ml \cdot kg^{-1}$ of body weight), or a placebo were given to competitive cyclists during cycle ergometry for 90 minutes at 70% $\dot{V}O_{2peak}$. Following the 90-minute time trial, subjects immediately began a time trial performance test with a workload equivalent to the amount of work completed while cycling at 70% $\dot{V}O_{2peak}$. The results showed that ingesting tyrosine with and without carbohydrates did not elicit any ergogenic benefit for cycling performance. These findings (Belza, et al., 2009; Chinevere et al., 2002) suggested that tyrosine supplementation may increase resting metabolic rate, but does not induce thermogenic effects during cycling or at rest on an acute basis.

Mucuna pruriens

Mucuna pruriens is a tropical legume used in Ayurvedic medicine to treat central nervous system diseases and geriatric disorders (Kavintha et al., 2014). It has been suggested that *Mucuna pruriens* regulates blood glucose and manages hyperglycemia by the inhibition of the enzyme alpha-glucosidase that is responsible for carbohydrate digestion and glucose absorption in the digestive tract (Gluati et al., 2012). A study conducted in diabetic rats found a significant decrease in blood glucose levels 2 hours after administering 100 and 200 $mg \cdot kg^{-1}$ of body weight of *Mucuna pruriens* seed extract (Kavnitha et al., 2014). A similar study (Majekodunmi et al., 2011) analyzed the anti-

diabetic properties of *Mucuna pruriens* seed extract by administering 5-100 $mg \cdot kg^{-1}$ of the extract to diabetic rats and found that there was a dose-dependent decrease in blood glucose ranging from 18.6-55.4%, respectively (Majekodunmi et al., 2011). This study concluded that the antidiabetic activities of *Mucuna pruriens* compare to those of the drug, glibenclamide. These findings (Kavnitha et al., 2014; Majekodunmi, et al., 2011) suggested that supplementing with *Mucuna pruriens* (5-200 $mg \cdot kg^{-1}$ of body weight) may aid in decreasing blood glucose.

Green Coffee Bean Extract

The consumption of green coffee bean extract (GCE), found in green or raw coffee, may aid in weight loss by modifying glucose tolerance and hormone secretion (Onakpoya et al., 2010). Green coffee bean extract modifies glucose levels after meals by reducing its absorption in the intestine through increasing the dispersal of the sodium electrochemical gradient (Onakpoya et al., 2010). Chlorogenic acid, a biologically active phenol derived from GCE, may result in this pattern of altered intestinal glucose uptake (Thom, 2007). Green coffee bean extract may also inhibit glucose-6-phosphatase (an enzyme that aids in glucose homeostasis) activity from the liver (Onakpoya et al., 2010). A study analyzing the effects of chlorogenic acid enriched coffee on weight loss administered two coffee substances containing 30-40 $mg \cdot g^{-1}$ and 90-100 $mg \cdot g^{-1}$ of chlorogenic acid to moderately overweight subjects every day for 12 weeks (Thom, 2007). Thom (2007) reported that weight loss for each group was statistically significant; however, fat loss from the start of the study to the end was only statistically significant in the group consuming the coffee substance containing 90-100 $mg \cdot g^{-1}$ of chlorogenic acid ($3.6 \pm 0.3\%$ vs. $0.7 \pm 0.4\%$). Though this study found statistical significance in fat loss in

moderately overweight subjects consuming this specific dosage of chlorogenic acid, a strict effective dosage has yet to be determined and there are no data concerning its effectiveness when administered acutely.

Coleus Forskohlii

Coleus forskohlii, a plant from the Lamiaceae (mint) family, is found to contain high amounts of forskolin that has been shown to increase lipolysis and the release of free fatty acids through the elevation of cyclic adenosine monophosphate (cAMP) and the activation of hormone sensitive lipase through the phosphorylation of protein kinase (Loftus, et al., 2015). Increasing lipolysis will cause the body to use fat as an energy source, which may result in fat loss (Henderson, et al., 2005). Loftus et al. (2015) investigated the effects of *coleus forskohlii* supplementation (250 mg) two times per day for 12 weeks in overweight and obese subjects undergoing a hypocaloric diet (~500 kcal/day deficit). This supplement protocol did not significantly affect anthropometric measures nor weight loss; however, reduced waist and hip circumferences occurred likely due to the hypocaloric diet. Henderson et al. (2005) administered 250 mg of 10% forskolin (CF) extract 2 times per day for 12 weeks to moderately overweight female subjects to investigate its effect on body composition management. The researchers (Henderson et al., 2005) concluded that though the CF extract group did not lose weight, it may have aided in weight maintenance in healthy overweight females. These findings (Henderson et al., 2005; Loftus et al., 2015) suggest that forskolin supplementation (250 mg 2 times per day for 12 weeks) may aid in weight maintenance in overweight and obese individuals.

L-carnitine

L-carnitine's (LC) primary metabolic functions consist of transporting long- and medium-chain fatty acids into the mitochondria for beta-oxidation and to maintain energy balance within the mitochondria by buffering short-chain acyl groups (i.e. acetyl-CoA) (Broad et al., 2008). The proposed benefit of LC supplementation is increased fat oxidation, though it may also enhance the breakdown of branched-chain amino acids through buffering branched-chain keto acids (Broad et al., 2008). Broad et al. (2008) investigated this claim by prescribing LC (2 $g \cdot d^{-1}$ for 14 days) to determine its effects on fat, protein, and carbohydrate metabolism during a 90-minute cycling bout (70% $\dot{V}O_{2max}$) after the 2-week supplementation period. This supplementation protocol did not alter fat, carbohydrate, and protein contribution to metabolism during steady-state cycling; however, ammonia accumulation was suppressed (60 min exercise: 97 ± 26 vs. 80 ± 9 $\mu mol \cdot L^{-1}$; 90 min exercise: 116 ± 47 vs. 87 ± 25 $\mu mol \cdot L^{-1}$). Thus, LC may have reduced the metabolic stress of the exercise bout or changed ammonia production or removal. In addition, Broad et al. (2005) investigated the effects of LC supplementation (2 $g \cdot d^{-1}$ for 4 weeks) on substrate metabolism during 90 minutes of steady state cycle ergometry. It was found that there were no differences in substrate use during the 90 minute cycling bout; however, plasma acyl-carnitine increased (58% of total carnitine change) significantly over the exercise period (Broad et al., 2005). Similarly, Broad et al. (2011) measured substrate metabolism after LC supplementation (3 $g \cdot d^{-1}$ for 15 days) during cycle ergometry. During the exercise trial, subjects cycled for 20 minutes at 20% of power output at $\dot{V}O_{2peak}$ followed by 20 minutes at 40%, 60%, and 80% $\dot{V}O_{2peak}$ for a total of 80 minutes at a self-selected cadence. It was found that no significant changes in

carbohydrate and fat oxidation occurred at any exercise intensity after LC supplementation compared to the placebo group (Broad et al., 2011). Overall, these findings (Broad et al., 2005; Broad et al., 2008; Broad et al., 2011) indicated that LC supplementation (2-3 $g \cdot d^{-1}$ for 2-4 weeks) does not influence substrate metabolism during steady state cycle ergometry.

Beta-alanine

Not only are pre-workout supplements of interest to strength and power athletes, but also those desiring to enhance endurance exercise. Common supplements that may improve endurance exercise consist of beta-alanine and nitrates. For example, beta-alanine has been shown to increase muscle carnosine levels (in doses of 3.2-6.4 $g \cdot d^{-1}$ for 2 to 10 weeks) that works as a buffer by limiting hydrogen ion accumulation in the body, leading to improved performance in exercise lasting approximately 1-3 minutes and contributes approximately 10% of the total buffering capacity of the working muscles (Kresta, et al., 2014). In particular, beta-alanine supplementation increases the amount of intramuscular carnosine by roughly 65%, causing the total buffering capacity to increase to 15% (Artoli, et al., 2010). Slight increases (2.5%) in time to exhaustion (TTE) during endurance exercise have been seen with beta-alanine supplementation (Artoli, et al., 2010). This may be due to the improvement of the anaerobic component at the end of a TTE graded exercise test (Artoli et al., 2010). Though these effects have been found in only one study to date, it may be assumed that beta-alanine supplementation may have a small effect on endurance performance due to a shift in anaerobic threshold (Artoli et al., 2010). Beta-alanine supplementation may also result in improved body composition as

well as improved anaerobic exercise markers such as ventilatory threshold and blood lactate levels (Kresta, et al., 2014).

The influence of beta-alanine as a supplement for delaying fatigue has been investigated during incremental cycle ergometry using the physical working capacity at the fatigue threshold (PWC_{FT}) test. Specifically, the PWC_{FT} test identifies the power output that corresponds to the onset of neuromuscular fatigue (NMF) by analyzing within-stage increases in electromyographic (EMG) amplitude (Stout et al., 2007). In particular, Stout et al. (2007) examined supplementation with beta alanine (3.2 $g \cdot d^{-1}$ on days 1-7 and 6.4 $g \cdot d^{-1}$ on days 8-28) for 28 days on $\dot{V}O_2$ max, PWC_{FT}, VT, and TTE. The authors (Stout et al., 2007) reported that VT and PWC_{FT} increased in the beta-alanine group pre- to post-supplementation (VT: 1.30 to 1.51 $L \cdot min^{-1}$; PWC_{FT}: 114 to 130 W) and TTE increased in the beta-alanine group (1118 to 1147 s). These findings show that supplementation with beta-alanine (86 $mg \cdot kg^{-1} \cdot d^{-1}$) for 28 days delays neuromuscular fatigue and VT thereby improving TTE during submaximal cycle ergometry in women. In addition, McCormack et al. (2013) found that older adults supplementing with two servings per day of either 1200 or 800 mg of beta-alanine for 12 weeks had increases in PWC_{FT} (1200 mg: 50.8 ± 22.6 to 58.8 ± 22.6 W; 800 mg: 47.7 ± 17.4 to 57.3 ± 22.9 W) during cycle ergometry. Muscular strength and endurance were also measured using a handgrip dynamometer and a 30-second sit-to-stand (STS) test. There were no significant changes in grip strength; however, STS scores increased in the 1200 mg group (13.3 ± 3.6 vs. 17.1 ± 7.5%). The authors (McCormack et al. 2013) concluded that consuming very low (800 mg) and low (1200 mg) doses of beta-alanine may increase the PWC_{FT} as well as muscular endurance in older adults. Furthermore, Stout et al. (2008) examined the

effects of beta-alanine supplementation (800 mg x 3 per day for 90 days) in elderly adults to determine its effect on neuromuscular fatigue during cycle ergometry. It was found that this supplementation protocol increased PWC_{FT} by 28.5% as a result of increased intramuscular carnosine that resulted in greater H^+ buffering capacity (Stout et al., 2008). These findings (Stout et al., 2007: McCormack et al. 2013: Stout et al., 2008) illustrated that chronic beta-alanine supplementation may delay the onset of neuromuscular fatigue in women and older adults, resulting in improved aerobic performance; however, acute doses of beta-alanine are likely ineffective due to loading requirements.

Arginine

Arginine is a semi-essential amino acid that is a key component to various metabolic pathways and compounds as it aids in ammonia removal and the synthesis of muscle protein, other amino acids, and creatine (Camic et al., 2010). In addition, arginine is a precursor to nitric oxide (NO), a potent vasodilator that potentially allows greater blood flow to the myocardium and skeletal muscle (Camic et al., 2010; Zak et al., 2015). Previous findings (Bailey et al., 2010; Camic et al., 2010Linden et al., 2011; Zak et al., 2015) have shown that arginine supplementation may have ergogenic effects such as reduced oxygen cost, delayed time to exhaustion, delayed neuromuscular fatigue, and improved VT. Specifically, Bailey et al. (2010) administered 6 $mg \cdot day^{-1}$ of arginine for 3 consecutive days to subjects during moderate and severe intensity cycling. This arginine supplementation protocol reduced the oxygen cost during moderate-intensity cycling (80% of the gas exchange threshold), increased time to exhaustion during severe-intensity cycling (707 ± 232 vs. 562 ± 145 s), and increased plasma nitrate concentration (159 ± 102 vs. 331 ± 198 $nmol \cdot L^{-1}$). In addition, Linden et al. (2011) found that the intravenous

infusion of 3.0 g of arginine during cycle ergometry increased glucose disposal for a 120 minute exercise bout, and significantly reduced plasma ammonia (60.6 ± 8.2 vs. 73.1 ± 9.1 mmol·L^{-1}) as well as lactate concentrations (7.1 ± 0.7 vs. 8.2 ± 1.1 mmol·L^{-1}) compared to the placebo condition. Furthermore, Zak et al. (2015) administered an acute dose of an arginine-based supplement (3.0 g) during an incremental test to exhaustion on a cycle ergometer to determine PWC_{FT}, VT, $\dot{V}O_{2peak}$ and blood lactate concentration to untrained individuals. When compared to placebo conditions, the supplement condition resulted in greater PWC_{FT} (192 ± 42 vs. 168 ± 53 W) and VT (2546 ± 313 vs. 2452 ± 342 mL·min^{-1}) values. In a similar study, Camic et al. (2010) administered either 1.5 g or 3 g of arginine for 28 days to determine its effects on the PWC_{FT} during an incremental test to exhaustion on a cycle ergometer. Supplementing with 1.5 and 3 g of arginine increased PWC_{FT} in both groups by 22.4% and 18.8%, respectively. The findings of both Camic et al. (2010) and Zak et al. (2015) suggested that arginine supplementation may delay neuromuscular fatigue and improve VT in untrained individuals due to reduced metabolic by-product accumulation (i.e. lactate, ammonia) as well as improve blood flow due to higher amounts of NO synthesis. Overall, the findings of these studies (Bailey et al., 2010; Camic et al., 2010; Linden et al., 2011; Zak et al., 2014) indicated that acute and chronic arginine supplementation may induce a multitude of ergogenic effects related to delayed fatigue.

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