International Journal of
EXERCISE SCIENCE

Original Research

Neither a Multi-Ingredient Pre-Workout Supplement nor Caffeine Were Effective at Improving Markers of Blood Flow or Upper-Body Resistance Exercise Performance

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ABSTRACT

International Journal of Exercise Science 13(2): 167-182, 2020. Few studies have measured the effects of multi-ingredient pre-workout supplements on blood flow or heart rate variability or have compared a multi-ingredient pre-workout supplement to a matched single ingredient. This study examined the effects of a multi-ingredient pre-workout supplement, an equivalent amount of caffeine, and placebo on markers of resistance training performance, blood flow, blood pressure, and heart rate variability. The study utilized a randomized, placebo-controlled, repeated-measures, crossover design. Twelve resistance-trained males (22.75 ± 4.51 yrs; 183.4 ± 7.37 cm; 91.05 ± 17.77 kg) completed the study. Resistance exercise performance was defined as total work performed during elbow flexion and extension on an isokinetic dynamometer. Blood flow was calculated using time-averaged mean velocity and blood vessel diameter of the right brachial artery, which were measured via Doppler ultrasound. Heart rate was recorded using an electrocardiogram. Neither a multi-ingredient pre-workout supplement nor caffeine alone improved upper-body resistance exercise performance or markers of blood flow relative to placebo. No differences in heart rate variability were observed across treatments. A multi-ingredient pre-workout supplement was not effective at improving performance or blood flow and did not alter autonomic nervous system function.

KEY WORDS: L-arginine, nitrate, blood flow, vasodilation, heart rate variability

INTRODUCTION

The ergogenic potential of L-arginine and other nitric oxide precursors in endurance exercise is well documented (4, 6, 8, 14, 15). However, L-arginine and other nitric oxide precursors (e.g., L-arginine, L-citrulline, sodium nitrate, and beet root extract) are becoming more common in supplements which are specifically marketed for anaerobic or resistance training (47). These supplements often contain multiple ingredients and are known as multi-ingredient pre-workout supplements. The multi-ingredient pre-workout supplement examined in this study had a proprietary "Nitric Oxide Blend" which contained L-arginine nitrate, a salt of the two molecules, and beet root extract high in nitrates. Both L-arginine (42) and nitrate (41) are precursors for nitric oxide, and endothelial production of nitric oxide leads to vasodilation (53). Increased vasodilation of working tissue allows for greater nutrient and oxygen delivery thus increasing the amount of work that can be performed (8). Although many studies have examined the acute effects of various multi-ingredient pre-workout supplements, (7, 10, 13, 18, 25, 31, 33, 37–39, 43, 45, 46, 50, 55, 56, 61, 62, 65, 67, 69) no study has directly measured the effects of multi-ingredient pre-workout supplements on blood flow; one group of researchers used near infrared spectroscopy to measure muscle oxygen levels and extrapolate blood flow (10). Thus, despite the inclusion of nitric oxide precursors in multi-ingredient pre-workout supplements, their effects on blood flow are unknown.

In addition to the "Nitric Oxide Blend," the multi-ingredient pre-workout supplement investigated here contained an "Energy and CNS" blend that listed caffeine among other ingredients that can affect the nervous system and potentially improve performance. Caffeine is arguably the most commonly used ergogenic aid (32, 44) and one of the most studied. The ergogenic effects of caffeine, either alone, or as a natural ingredient in beverages, or as an ingredient in a mixture are well documented (57). The strongest evidence supports caffeine's positive effects on endurance exercise (30). Caffeine's ergogenic effects are likely related to the stimulation of the central nervous system and decreased rate of perceived effort (63). The "Energy and CNS" blend also contained choline, which is key part of the neurotransmitter acetylcholine, and vinpocetine which can increase blood flow to the brain (11). The effect of this blend on performance and central nervous system activity is also unknown.

Additionally, in a recent review in the Journal of the International Society of Sports Nutrition, the authors identified a key limitation in the methodologies of studies that have investigated multi-ingredient pre-workout supplements: many studies have not compared a multi-ingredient pre-workout supplement to an equivalent dose of a single ingredient contained in the supplement (36). Indeed, only one study has examined the acute effects of a multi-ingredient pre-workout supplement to a matched dose of a single ingredient (50). Lastly, although not an all-encompassing measure of autonomic nervous system activity, heart rate variability (HRV) is considered a measure of the balance between the parasympathetic and sympathetic branches of the central nervous system (49). The effects of caffeine on HRV have been well investigated, (48) and HRV has also been examined with an energy drink (60). However, the effects of multi-ingredient pre-workout supplements on HRV have received much less attention. In this study, we compared the effects of a multi-ingredient pre-workout supplement on upper-body resistance training performance, blood flow, and HRV in young, recreationally resistance-trained males to a matched dose of caffeine or placebo.

METHODS

Participants

Fifteen recreationally active male university students were recruited in a randomized, double-blind, placebo-controlled, crossover design. All participants completed a physical activity readiness questionnaire and a health history questionnaire, which included questions regarding medical conditions, diseases, or medications that could potentially interfere with blood flow, heart rate, or performance. Habitual caffeine intake was assessed by a study-specific food frequency questionnaire created by a registered dietitian that measured the frequency of consumption of various caffeine-containing foods (e.g., coffee, tea, or energy drinks). Participants also reported having at least six months resistance training experience and maintained at least three hours of resistance training per week. Inclusion criteria included participants' report of the following: 1) caffeine consumption ≥100 mg and ≤ 500 mg per day; 2) no chronic use of other supplements within the last 30 days; 3) complete absence of any chronic diseases, including but not limited to chronic diseases that affect the cardiovascular, nervous, and/or digestive systems; 4) absence of any other health problems; 5) no nicotine use of any kind. Three participants were removed because they failed to adhere to the fasting guidelines or did not follow washout instructions required for the testing protocol. The 12 remaining participants (mean ± *SD*, age = 22.75 ± 4.51 yrs, height = 183.4 ± 7.37 cm, body mass = 91.05 ± 17.77 kg) completed the three trials in random order. Participants that used supplements prior to the study-required washout period met required daily habitual caffeine intake during the study testing window. All subjects followed study requirements according to self-reports. Written informed consent was obtained for all 15 participants and the North Dakota State University Institutional Review Board approved the study prior to its onset. This research was carried out fully in accordance to the ethical standards of the International Journal of Exercise Science (59).

Protocol

The multi-ingredient pre-workout supplement (PRE) was commercially available *Iron Pump*™ (fruit punch flavored, MusclePharm, Denver, CO). The dose used for the PRE was one scoop (6g), which was the amount recommended by the manufacturer. The supplement facts for the PRE are shown in Table 1. The estimated caffeine content in a 6g dose of PRE was 350 mg based on nuclear magnetic resonance spectroscopy testing (data not shown). The caffeine supplements (CAF) were commercially available caffeine pills (PROLAB Nutrition, Chatsworth, CA) which were ground using a mortar and pestle to match the 350mg doses found in the PRE. The placebo (PLA) consisted of 6g of cornstarch. To mirror taste, both CAF and PLA were mixed with fruit punch flavored, 5 calorie sweetened drink (Crystal Light, Northfield, IL), and all beverages were mixed and consumed in an opaque mixing bottle to mask appearance. All participants and data collectors were blinded to the beverage being consumed during each trial.

Table 1. Supplement facts of the multi-ingredient pre-workout supplement.

	Amount
Vitamin C (as Ascorbic Acid)	250 mg
Niacin (as Niacinamide)	25 mg
Vitamin B6 (as Pyridoxine Hydrochloride)	15 mg
Vitamin B12 (as Methylcobalamin)	25 mcg
Calcium (as Calcium Silicate)	19 mg
Nitric Oxide Blend	2,075 mg
L-Arginine Nitrate, L-Glycine, Agmatine Sulfate, L-Ornithine HCl, Hawthorne Berry (Crataegus Pinnatifida) Powder, Beet root (Beta Vulgaris)	

Extract High In Nitrates	
Energy and CNS Blend	2,051 mg
Choline Bitartrate, L-Tyrosine, Caffeine Anhydrous, Vinpocetine	

Participants visited the lab four times throughout the study. The first visit familiarized participants with the procedures and the Biodex System IV dynamometer (Biodex Medical Systems, Shirley, NY) as described by Astorino et al. (2010), and participants' individualized Biodex setup measurements were recorded. Additionally, participants were measured during their first visit for maximal peak isometric force at 0° degrees and 60° for both peak elbow flexion and extension using the Biodex System IV and were provided a handout with instructions to prepare them for the following three testing trials. These trials were conducted at the same time of day (07:00 - 10:00) with at least 48 hours between trials. Each trial used repeated standardized measures at baseline (Base), 30 minutes post supplementation (+30), immediately (i.e., within < 1 minute) post-exercise (PostEx), and 60 minutes post supplementation (+60). After Base measures, participants consumed one dose of blinded supplement and remained stationary for 30 minutes. The workload for isokinetic dynamometry was adjusted to 40% of maximal for concentric-concentric flexion-extension and was tested for 5 sets of 10 repetitions with a 60 second rest period between sets. Performance was defined as work (J) performed summed across all five sets. All participants received constructive coaching to encourage full effort throughout the exercise trials. Restraint straps were used to maintain physical force and limit body movements in determining performance. All PostEx ultrasound measures were timed using a stopwatch (ACCUSPLIT, Pleasanton, CA).

All ultrasound measures were collected from the right brachial artery using pulsed wave Doppler on a Phillips HD11XE diagnostic ultrasound system with a Phillips L12-5 mm broadband linear array probe with frequency ranges from 12hz to 5Mhz (Phillips, Eindhoven, Netherlands). The location of the ultrasound probe was traced in permanent ink to ensure subsequent placement accuracy during the first session. Then, each participant received a permanent marker to retrace and darken the outlining between trials. The ultrasound measures included mean blood flow velocity (VEL), cross-sectional diameter of the right brachial artery (DIA), time averaged mean velocity (TAMV), and volumetric blood flow (FLOW). FLOW was obtained using the following equation: volume flow = cross-sectional area x TAMV. Cross-sectional area was obtained by DIA^2 x 0.785, assuming the vessel is circular in cross-section (9).

Whole blood lactate in mmol/L was measured with a Nova biomedical portable analyzer (Nova Biomedical, Waltham, MA) pre- and post-exercise, using the left hand for the finger stick and utilizing a BD 30-gauge safety lancet (BD, Franklin Lakes, NJ). Resting systolic and diastolic blood pressure (BP) was measured using a sphygmomanometer and stethoscope (American Diagnostic Corp, Hauppauge, NY) and was recorded in mmHg. N-O Indicator Strips (HumanN, Austin, TX) were used to estimate salivary nitric oxide levels at Base and +60 (58).

Heart rate (HR) and HRV were collected using electrocardiography (ECG) input. ECG recordings of five minutes in duration were performed using a PC-ECG 1200S ECG system (Norav Medical, Delray Beach, FL) with a sampling frequency of 500 Hz, low pass filter for high frequency noise, 60 Hz notch filter, and a filter for ridding the signal of noise from muscle contractions. The recorded conventional ECG waveform (PQRST) data files were further processed for HRV analysis (21). Heart rate variability examines the differences in beat-to-beat intervals based on the time in between adjacent ECG R wave to R wave (RR) intervals (22,27,28). The measure provides a dynamic, sensitive meter of the balance or tone between the two branches of the autonomic nervous system, sympathetic and parasympathetic, and, thereby, the effects of various stressors. Analyses were performed by creating a tachogram, including R wave peak, RR interval measurement, artifact removal, and plotting of the normal-to-normal RR intervals (i.e. NN intervals) vs. time, using MATLAB (Mathworks, Natick, MA). The square root of the mean of the sum of the squares of differences between adjacent NN intervals (RMSSD) was selected as the preferred dependent variable given that it displayed the greatest internal consistency and reliability day-to-day (Cronbach's alpha = 0.884, interclass correlation coefficient = 0.718) as well as being the HRV measure of choice in numerous recent studies (17, 21, 24, 26, 49, 64). Further specifics of HRV calculations are discussed elsewhere (28).

Statistical Analysis

Descriptive statistics were collected for age, height, and weight. Measures from the N-O Indicator Strips were scored according to the reading of the strip; depleted was scored as one, low was scored as two, and optimal was scored as three. N-O Indicator Strip scores taken at Base were subtracted from scores taken at +60 for each subject and treatment; three paired *t*-tests were used to evaluate differences between the three treatments. ANOVA with repeated measures was used to investigate supplement by time interactions, main effects of treatment, and main effects of time. All statistics were performed using SAS Institute Inc. 9.3, 2011 (Cary, NC) and SPSS (version 24, Armonk, NY). The alpha value was set at $p < 0.05$, however, Sidak adjustments were utilized for multiple comparisons to control for type I error.

RESULTS

Salivary nitric oxide increased significantly in multi-ingredient pre-workout supplement (PRE) compared to placebo (PLA: $p < 0.0001$) and caffeine (CAF; $p < 0.0001$); however, there were no differences between PLA and CAF ($p = 0.441$). There were no supplement by time interactions effects for VEL ($p = 0.786$), DIA ($p = 0.456$), or FLOW ($p = 0.729$). Similarly, there were no main effects of supplement for blood velocity (VEL; $p = 0.123$), right brachial artery diameter (DIA; $p = 0.188$), or blood (FLOW; $p = 0.421$). There were, however, significant main effects of time for VEL ($p < 0.001$), DIA ($p < 0.0001$), and FLOW ($p < 0.0001$; Table 2).

Table 2. Vasodilation markers with different supplements across time.

	Base			30			PostEx			60		
	PRE	**CAF**	**PLA**	**PRE**	**CAF**	**PLA**	**PRE**	**CAF**	**PLA**	**PRE**	**CAF**	**PLA**
Vel (cm• s^{-1}	15.9± 7.60#	22.2± 11.6#	17.9± 5.5#	11.7± 4.4#	16.7±6 .4#	13.9± 4.9#	63.6± 10.0*†	63.9±18 .3*†	62.6± 7.1*†	18.2± 7.7#†	24.1± 1.5#†	19.9± 8.2#†
Diam (cm)	0.45± 0.05#	0.44± 0.06#	0.44± 0.6#	0.45±0 .08#	0.44± 0.6#	0.44± 0.06#	0.52± 0.64*†	0.50±0. 6*†	0.53± 0.07*†	0.47± 0.07*#†	0.46± 0.05*#†	0.47± 0.07*#†
TAMV (cm·s^{-1})	7.87± 3.71#	11.15± 6.66#	8.54± 2.47#	5.78±2 .58*#	8.43± 3.50*#	6.71± 2.75*#	30.10± 8.18*†	28.93±1 1.97*†	30.42± 12.73*†	8.10± 3.37#†	11.80± 5.92#†	11.49± 6.76#†

Vel = velocity. Diam = diameter. TAMV = time averaged mean velocity. Base = baseline. +30 = 30 minutes post supplementation. PostEx = immediately post exercise. +60 = 60 minutes post supplementation. PRE = multi-ingredient pre-workout supplement. CAF = caffeine supplement. PLA = placebo. *denotes significance from Base. # denotes significance from PostEx. †denotes significance from +30. All *p*-values are Sidak-adjusted.

Figure 1 shows the change in volumetric blood flow from baseline to post-exercise. There were no supplement by time interactions or condition effects of systolic ($p = 0.823$; $p = 0.774$) or diastolic blood pressure ($p = 0.444$; $p = 0.203$), respectively. There were significant main effects for time for systolic ($p < 0.001$) and diastolic ($p = 0.012$) blood pressure (Table 3). There were no significant supplement by time ($p = 0.815$) or supplement ($p = 0.998$) effects observed. Significant alterations in heart rate over time were initially detected ($p = 0.12$), however, post-hoc testing did not indicate further significance within the various pairwise comparisons (Table 3).

Heart rate variability (HRV) did not show any supplement by time ($p = 0.804$) or supplement ($p = 0.092$) effects. However, a significant time effect ($p = 0.025$) for HRV was detected (Table 3). There were no significant changes in total work with the different supplements ($p = 0.094$; Figure 2). There were no significant supplement by time ($p = 0.211$) or supplement effects ($p = 0.866$) for whole blood lactate. There were significant time effects ($p < 0.001$) for whole blood lactate which are shown in Table 4.

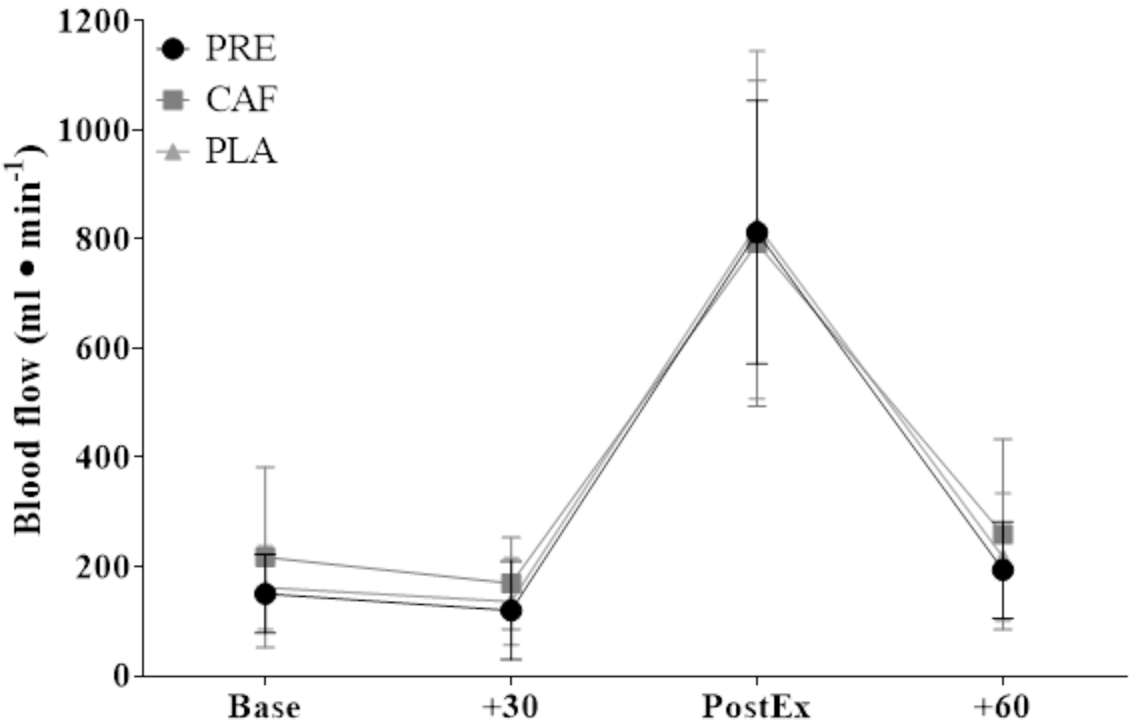

Figure 1. Mean calculated blood flow of the right brachial artery across different measurement periods. Error bars represent mean ± SD. Blood flow was calculated using TAVM • DIA² • 0.795. TAVM and DIA were measured via Doppler ultrasound. PRE = multi-ingredient preworkout supplement. CAF = caffeine supplement. PLA = placebo. Base = baseline. +30 = 30 minutes post supplementation. PostEx = immediately post exercise. +60 = 60 minutes post supplementation.

DISCUSSION

In this experiment, we report no benefit in upper-body resistance exercise performance or blood flow with a multi-ingredient pre-workout supplement or caffeine compared to placebo., There is very little indication to recommend either the multi-ingredient pre-workout supplement or caffeine to improve acute upper-body performance or blood flow in the dosage prescribed. On the other hand, the multi-ingredient preworkout supplement nor caffeine affected heart rate, blood pressure, or heart rate variability differently than placebo signifying that the supplements do not significantly affect the central nervous or cardiovascular system at least in the short term. This indicates that the multi-ingredient pre-workout supplement is acutely (one dose) safe when consumed by healthy, young, resistance-trained males.

Table 3. Blood pressure and heart rate responses to supplements across time (mean ± *SD*).

	Base			30			PostEx			60		
	PRE	CAF	PLA	PRE	CAF	PLA	PRE	CAF	PLA	PRE	CAF	PLA
Systolic (mmhg)	122± 9#	121± 8#	122± 11#	124± 9#	124± 8#	122± 11#	133± 18*†	136± 16*†	135± 15*†	124± 8*#	126± 10*#	125± 10*#
Diastolic (mmhg)	80± 9	79± 6	79± 5	81± 9*	82± 4	82± 5	82± 6	84± 4	82± 5	83± 5	84± 5	80± 5
Heart rate (bts·min-1)	90± 11	87± 9	89± 10	88±1 3	92± 12	90± 8	94± 12	94± 15	94± 9	93± 12	92± 8	92± 7
HRV (RMSSD)	57± 30	67± 38	56± 30	72± 34#	77± 34#	61± 27#	60± 27	62± 30	50± 22	55± 32	56± 30	46± 19

Base = baseline. +30 = 30 minutes post supplementation. PostEx = immediately post exercise. +60 = 60 minutes post supplementation. PRE = multi-ingredient pre-workout supplement. CAF = caffeine supplement. PLA = placebo. *denotes significance from Base. # denotes significance from PostEx. † denotes significance from +30. All *p*-values are Sidak-adjusted.

Table 4. Whole blood lactate responses to supplements across time (mean ± *SD*).

	Base			30			PostEx			60		
	PRE	CAF	PLA	PRE	CAF	PLA	PRE	CAF	PLA	PRE	CAF	PLA
Lactate (mM)	0.88± 0.27	0.76± 0.18	0.99± 0.40	0.96± 0.44	0.96± 0.45	1.07± 0.59	3.36± .0.93* †	3.25± 1.19* †	2.81± 0.76* †	1.81± 0.79* †#	2.11± 0.86* †#	1.92±0 .83*†#

Base = baseline. +30 = 30 minutes post supplementation. PostEx = immediately post exercise. +60 = 60 minutes post supplementation. PRE = multi-ingredient pre-workout supplement. CAF = caffeine supplement. PLA = placebo. * denotes significance from Base. # denotes significance from PostEx. † denotes significance from +30. All *p*-values are Sidak-adjusted.

In line with the results of others, several studies have found no improvement in upper-body resistance exercise performance with a multi-ingredient pre-workout supplement compared to placebo (10, 55, 56, 61, 65). However, others have reported benefits (13, 18, 31, 45, 46, 50). Results likely vary due to differences in supplement selection and formulation and exercise and testing protocols; few studies have examined the same multi-ingredient pre-workout supplement or have used identical protocols to measure upper-body resistance exercise performance. The equivocal nature of studies that examine multi-ingredient pre-workout supplements make it difficult to recommend multi-ingredient pre-workout supplements in general. Instead, recommendations should be based on the efficacy and safety of individual multi-ingredient pre-workout supplements. Additionally, the mixed results of these studies highlight an issue of performing multi-ingredient pre-workout supplement research: there are many multi-ingredient pre-workout supplements on the market, too many for researchers to examine every supplement. A more replicable and generalizable approach would examine known amounts of various ingredients typically found in multi-ingredient pre-workout supplements or investigate multi-ingredient pre-workout supplements that do not use proprietary blends. This approach, though, does not reflect real world use as most consumers likely buy multi-ingredient pre-workout supplements with proprietary blends or with unverified quantities of ingredients.

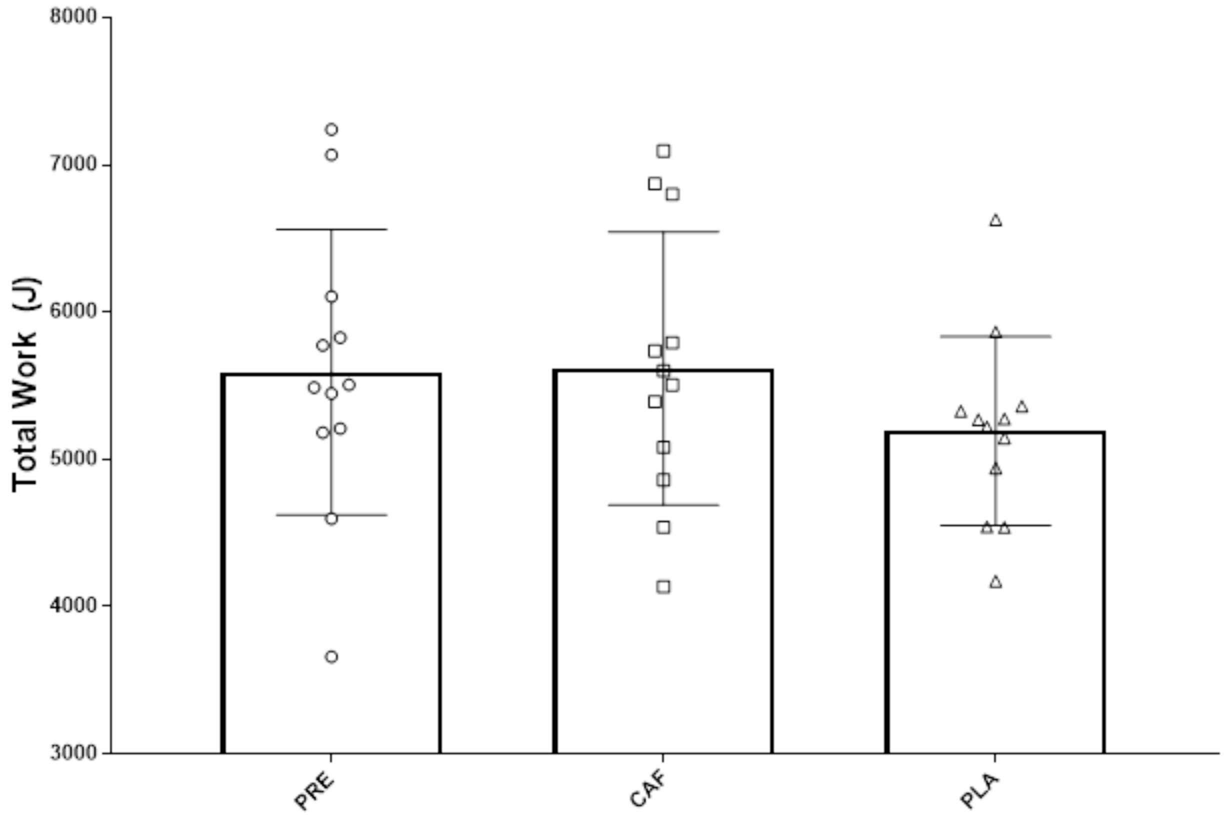

Figure 2. Performance across time for the three different supplements. Performance was accessed using total work (J) performed during elbow flexion and extension on a Biodex IV Dynamometer for 5 sets of 10 reps at 40% peak isometric force. Error bars represent mean ± SD. PRE = multi-ingredient pre-workout supplement. CAF = caffeine supplement. PLA = placebo.

This is the first study to examine a multi-ingredient pre-workout supplement and to measure blood flow via diagnostic ultrasound. It is our hope that ultrasonography will be applied by others investigating the effects of multi-ingredient pre-workout supplements because it is common for manufacturers to advertise their supplement's ability to increase blood flow and enhance muscle "pumps." Indeed, blood flow is often an overlooked measure by those investigating multi-ingredient pre-workout supplements, and ultrasound measures of blood flow are reliable (68), noninvasive (35), and relatively accessible to those working in human performance or exercise science labs.

Although we did not detect any supplement effects on blood flow, markers of salivary nitric oxide (i.e. nitrate and nitrite) were increased after the preworkout supplement compared to caffeine and placebo as indicated by the N-O Indicator Strips. Even though the strips indicated that the multi-ingredient pre-workout supplement increased salivary nitrate, no differences in blood velocity, artery diameter, time-averaged mean velocity, or blood flow were found between all three treatments. This finding is somewhat surprising given that salivary nitrate can be reduced by bacteria that reside in the oral cavity to nitrite which can be, through a series of steps, converted to nitric oxide (54). However, these observations are supported by previous reports of no correlation between these test strips and serum levels of nitrate and nitrite (58).

Unfortunately, data were collected before the publication of this article, and as such, it is unclear whether the multi-ingredient pre-workout supplement had any effect on markers of nitric oxide in serum. It is also important to note that findings regarding the efficacy of L-arginine supplementation are mixed (42) and that studies that have shown improvements in blood flow or various exercise parameters with any form of arginine supplementation have generally investigated dosages greater than those contained in the multi-ingredient pre-workout supplement. For example, several other groups of researchers have used 6g doses of arginine (6, 12, 27), and the serving size of the multi-ingredient pre-workout supplement was 6g inclusive of other ingredients.

Supplements containing nitrate, such as beet root juice and sodium nitrate, seem to be more efficacious than L-arginine, with a meta-analysis suggesting the ergogenic potential of the supplement (40). Nitrate containing supplements have been found to be successful at increasing blood markers of nitric oxide (51), improving exercise tolerance (5), efficiency (4, 52), and performance (51). The presence of nitrate in the multi-ingredient pre-workout supplement is evident by the significant results of the N-O Indicator Strips; although these strips cannot confirm serum levels of nitrate or nitrite, there were higher levels of nitrate in subjects' saliva following the multi-ingredient pre-workout supplement compared to caffeine and placebo. Thus, the multi-ingredient pre-workout supplement contained detectable, although unquantified, amounts of nitrate.

The lack of a significant increase in artery diameter, blood velocity, blood flow, time-averaged mean blood velocity, and total, could be attributed to several nonexclusive explanations. The dosage of nitrate-producing ingredients in the multi-ingredient pre-workout supplement may have been too low to elicit an ergogenic or vasodilatory response. Caffeine, due to its potential role as a vasoconstrictor during exercise (20), may have negated the vasodilatory effects of nitrate, a result that was observed in female competitive cyclists (29). Lastly, the effects of nitrate supplementation on anaerobic exercise performance have largely remained uninvestigated. As such, nitrate supplementation may not be effective for improving anaerobic exercise parameters. The results of this work support this conclusion, but studies examining the ergogenic potential of a quantified dose of nitrate on anaerobic exercise are needed.

Caffeine, unlike L-arginine and nitrate, has been extensively studied, although the efficacy of the supplement for improving resistance training is ambiguous, particularly for untrained or noncompetitive populations (30). Indeed, one group of authors who performed a systematic review of caffeine supplementation and anaerobic exercise performance deduced that caffeine supplementation enhances strength and resistance training performance primarily in trained athletes (2). The participants in this study reported participating in resistance training at least three times a week but are comparably less trained than competitive athletes. As such, the results of this study support the deduction that the efficacy of caffeine supplementation is less effective at improving anaerobic exercise in people who are not competitive athletes.

Additionally, one of the proposed mechanisms of caffeine's ergogenic effects involves caffeine's effects on perceived exertion and blood lactate levels. The correlation between blood lactate levels and perceived exertion is well established (16). Moreover, caffeine has been shown to decrease perceived exertion, (22) and in some studies increase blood lactate levels (1,19,23). Caffeine, by blunting the perceived exertion of exercise, may allow people to work at higher intensities for longer resulting in increased blood lactate levels. There were not any significant differences between treatments in our study, but the exercise protocol may not have been demanding enough for this effect to manifest. For example, lactate reached concentrations greater than 11 mM in one study where this effect was observed (23). For the current study, lactate did not go above 6 mM for any subject.

Lastly, we did not find significant difference in heart rate variability between the multi-ingredient pre-workout supplement, caffeine, and placebo indicating the supplement at the recommended dosage did not cause measured unsafe side effects. Despite our null results, heart rate variability is another measure that should be considered by researchers examining multi-ingredient pre-workout supplements. Negative cardiovascular events, namely hemorrhagic stroke, have been reported with the use of commercially available multi-ingredient pre-workout supplements (34, 70). As such, the safety of multi-ingredient pre-workout supplements should also be evaluated. Although heart rate variability alone cannot completely appraise a supplement's safety, decreased heart rate variability has been linked to mortality and risk factors for cardiovascular disease (66). Moreover, recording the measure only requires a simple electrocardiogram with accompanying software, two tools that are almost universally available to investigators in the field.

In summary, we found no benefit with a multi-ingredient pre-workout supplement or caffeine in improving blood flow or performance, although neither adversely affected the autonomic nervous system.

ACKNOWLEDGEMENTS

The authors would like to thank Ashlyn Nelson for her assistance in data collection. This study was funded by the College of Human Development and Education at North Dakota State University. None of the authors report relationships with any of the companies or manufacturers listed in this article.

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