Effects of Multi-ingredient Preworkout Supplements on Physical Performance, Cognitive Performance, Mood State, and Hormone Concentrations in Recreatinally Active Men and Women

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Abstract
Beckner, ME, Pihoker, AA, Darnell, ME, Beals, K, Lovalekar, M, Proessl, F, Flanagan, SD, Arciero, PJ, Nindl, BC, and Martin, BJ. Effects of multi-ingredient preworkout supplements on physical performance, cognitive performance, mood state, and hormone concentrations in recreationally active men and women. J Strength Cond Res XX(X): 000–000, 2020—Performance enhancement supplement research has primarily focused on the effectiveness of individual ingredients, rather than the combination. This study investigated the acute effects of 2 multi-ingredient preworkout supplements (MIPSs), with beta-alanine and caffeine (BAC) and without (NBAC), compared with placebo (PLA) on anaerobic performance, endurance capacity, mood state, cognitive function, vascular function, and anabolic hormones. Thirty exercise-trained individuals (24.4 ± 4.9 years, 15 men and 15 women) completed a fatiguing exercise protocol on 3 separate occasions, 30 minutes after ingestion of BAC, NBAC, or PLA. Outcomes were analyzed using one-way or two-way repeated-measures analysis of variance, as appropriate (alpha = 0.05). Anaerobic power was greater when supplementing with NBAC (10.7 ± 1.2 W·kg⁻¹) and BAC (10.8 ± 1.4 W·kg⁻¹) compared with PLA (10.4 ± 1.2 W·kg⁻¹) (p = 0.014 and p = 0.022, respectively). BAC improved V̇O₂peak time to exhaustion (p = 0.006), accompanied by an increase in blood lactate accumulation (p < 0.001), compared with PLA. Both NBAC and BAC demonstrated improved brachial artery diameter after workout (p = 0.041 and p = 0.005, respectively), but PLA did not. L-arginine concentrations increased from baseline to post-supplement consumption of BAC (p = 0.017). Reaction time significantly decreased after exercise for all supplements. There was no effect of supplement on mood states. Exercise-trained individuals looking to achieve modest improvements in power and endurance may benefit from consuming MIPS before exercise.

Key Words: power, endurance, lactate, caffeine, beta-alanine

Introduction
Multi-ingredient preworkout supplements (MIPSs) have become an increasingly popular ergogenic aid among athletes and fitness enthusiasts to enhance performance and augment training adaptations over time (18,21). Multi-ingredient preworkout supplements are often developed as a proprietary blend of ingredients such as beta-alanine, caffeine, citrulline, tyrosine, taurine, and creatine, among others (18), that together may provide multiple benefits including improved buffering capacity of skeletal muscle (45), increased vasodilation (12), and energy availability (8). Caffeine has been shown to be a primary active ingredient in MIPS (5,28,30) because it is absorbed quickly and generally peaks in the bloodstream within 60 minutes of ingestion, stimulating both the central and peripheral nervous system (14,18). Creatine is also among the most common ingredients in MIPS for its role in regulating oxidative metabolism through shuttling of adenosine triphosphate from the mitochondria, potentially contributing to increased aerobic capacity, increased anaerobic threshold, muscle strength and power capacity, and greater training tolerance (3,27). β-alanine is advantageous in buffering and attenuating excess proton accumulation by enhancing carnosine synthesis in skeletal muscle, which can lead to improvements in sustained performance during high-intensity activities ranging from 30 seconds to 6 minutes (9,19,37,45). However, unlike caffeine, creatine and β-alanine are most effective when concentrations are saturated, typically accomplished through a “loading phase” or daily supplementation; therefore, the acute effects are less understood (27,45). Taurine, a sulphur-containing amino acid, is also known to improve ATP turnover in the muscle cell and assist with sarcoplasmic reticulum calcium handling (46,47). A number of other amino acids including L-citrulline, L-arginine, and citrulline malate are also common in MIPS to increase nitric oxide availability leading to improvements in vasodilatory function and muscle oxygen consumption during exercise (12,13,15). Provided that many of these ingredients target different physiological mechanisms, many researchers consider the possibility of a synergistic effect when such ingredients are combined into a single formula (18).

Previous research has demonstrated the effectiveness of various MIPS formulas, often containing caffeine, beta-alanine, and...
taurine, in improving anaerobic and endurance performance. For example, Martinez et al. (30) observed greater Wingate anaerobic peak power (8.3%) and mean power (6.3%) after ingestion of MIPS compared with placebo. Likewise, Ellerbrock and Antonio (11) reported significantly greater repetitions to failure during bench press of 60% 1 repetition maximum (1RM) (19.5%) with MIPS compared with a caffeine-matched placebo. Jagim et al. (21) also identified significant improvement in bench press repetitions (7.7%) to failure in MIPS compared with placebo, as well as higher self-report ratings of alertness and lower ratings of fatigue. Self-reports of higher optimism, vigor, and energy after MIPS ingestion were observed by Jung et al. (22) in addition to improved cognitive function assessed using the Stroop Color-Word test.

Increases in exercise volume with MIPS may also augment the exercise–endocrine interaction and improve adaptations to exercise (24). Various MIPS have been shown to significantly increase insulin-like growth factor 1 (IGF-I) concentrations, an anabolic hormone, compared with placebo 30 minutes after ingestion (24) and after exercise (38). Concentrations of total and free testosterone have also been shown to significantly increase after exercise with MIPS to a greater degree than placebo (24). Notably, betaine supplementation yielded increases in IGF-I concentrations and growth hormone (GH) after ingestion, suggesting improved endocrine control in response to acute resistance and aerobic exercise (2).

In a recent review by Jagim et al. (20), beta-alanine and caffeine were identified as the 2 most prevalent ingredients among the top 100 commercially available preworkout supplements. However, these ingredients are not without potential risks or unpleasant side effects including paresthesia (i.e., tingling), elevated heart rate, restlessness, and headaches (5, 43, 45). In addition, differences in the rate of caffeine metabolism between individuals may manifest in varying response magnitude after supplementing MIPS-containing caffeine (5). As stated by Harty et al. (18), further research is warranted for direct comparisons between MIPS and principal ingredients to determine the uniqueness and effectiveness of proprietary blends. Therefore, the purpose of this study was to investigate the acute effects of 2 MIPS, one with beta-alanine and caffeine (BAC) and one without (NBAC), compared with placebo on anaerobic performance and \( \dot{V}O_2 \text{peak} \). In addition, we also sought to assess the effects of MIPS on mood state, cognitive function, and hormones associated with physical performance.

Methods

Experimental Approach to the Problem

This randomized, counterbalanced, cross-over, double-blind, placebo-controlled study investigated the acute effects of MIPS compared with placebo on physical performance, mood state, cognitive function, and hormone profiles. Subjects reported to the laboratory on 4 separate occasions, a familiarization visit and 3 experimental visits, each separated by 5–7 days. During the familiarization visit, subjects completed practice trials of all testing procedures that would be completed during the experimental visits; vertical jumps (VJs), ballistic jump squats (BJSs), ballistic bench press (BBP), 5-10-5 Pro-Agility test, Wingate Anaerobic cycling test (WAnT), Attention Switching Choice Reaction Test (ASCRT), and a cycle \( \dot{V}O_2 \) peak test, explained in further detail in the subsequent sections. Subjects were instructed to refrain from strenuous exertion, caffeine, and alcohol 24 hours before each trial. Before the first experimental visit, subjects completed a 24-hour dietary recall and were asked to replicate the same diet before each of their subsequent visits. Dietary recalls were collected at subsequent experimental visits to ensure compliance. Subjects reported to each experimental visit 8 hours fasted, aside from consumption of a standardized breakfast bar (men: 32 g carbohydrates, 6 g fat, 18 g protein; women: 19 g carbohydrates, 6 g fat, 6 g protein) eaten 1 hour before the research visit.

Subjects

Thirty exercise-trained individuals (mean ± SD: 24.4 ± 4.9 years, 173.2 ± 9.0 cm, 74.1 ± 15.1 kg, 15 men, 15 women) between the ages of 18–40 years participated in this study (Table 1). This study included an equal number of men and women to be representative of the exercise-trained population. Exercise-trained was defined as having a minimum of 1-year experience with high-intensity anaerobic or resistance training. Subjects were excluded if they were currently using preworkout products and had a current or recent musculoskeletal injury, inadequate training experience, tobacco use, or taking beta-adrenergic blocking medications. Subjects were instructed to maintain their current exercise regimen throughout the duration of the study. In addition, subjects were instructed to avoid the use of any preworkout products while enrolled in the study. This study was approved by the University of Pittsburgh’s Institutional Review Board, and subjects were informed of the benefits and risks of the investigation; written informed consent was obtained from each subject before any testing procedure.

Procedures

Familiarization Visit. During the familiarization session, body mass was collected using a standard physician’s scale [Health o meter, St. McCook, IL], and height was measured using a stadiometer (Seca, Hamburg, Germany). Subjects completed the Physical Activity Readiness Questionnaire (44) to ensure adequate health to complete the exercise protocol. If a subject answered yes to any question, physician clearance was required before continuing in the study. Body composition was assessed using dual X-ray absorptiometry (DEXA) (GE Healthcare, Wauwatosa, WI). Female subjects were required to perform a urine pregnancy test before the DEXA scan. Noninvasive ultrasond (SonoSite X-Porte, Bothell, WA) was used to measure upper-arm blood flow. During familiarization, ultrasound scans were taken on the brachial artery in the left bicep to determine the best location for subsequent measures during supplement visits. Distance from the medial epicondyle to the center of the probe was recorded and used across subsequent visits. After ultrasound, subjects completed 1RM testing for squat and bench press using the National Strength and Conditioning Association protocol for 1RM testing. After 1RM testing, subjects were exposed to a familiarization trial of all testing procedures that would be completed during the experimental visits.

Experimental Visits. The 3 subsequent supplement trials began at approximately the same time, within 1–2 hours, from 0600 to 1130 for each visit. Over the course of these trials, subjects were given 1 of 3 supplements in a randomized counterbalanced order: one with beta-alanine and caffeine (BAC; AMPED Nitro, 40 kcal/serving; Isagenix International, Gilbert, AZ), one without beta-alanine and caffeine (NBAC; AMPED Power, 30 kcal/serving; Isagenix International), or an isocaloric placebo (Kool-
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Aid Tropical Punch and MiO fruit punch, 30 kcals/serving; Kraft Heinz Company, Chicago, IL). Figure 1 demonstrates the experimental protocol used for this study.

Upon arrival to the laboratory, the research team confirmed the subject refrained from strenuous exercise, caffeine, and alcohol within the past 24 hours, and consumed similar meals to the previously completed 24-hour dietary recall. Subjects then completed a short Profile of Mood States (POMS) questionnaire assessing their current mood (16) and were fitted with a Polar heart rate monitor (Kempele, Findland). Resting heart rate, blood pressure, and arterial blood flow measured through brachial artery ultrasound were collected after completion of the POMS questionnaire. Subjects then completed ASCRT, a custom-built computer-based modified Stroop test with congruent and incongruent responses, using a finger-tapping response pad with accompanying SuperLab 5 software (Cedrus Corporation, San Pedro, CA). Subjects were instructed to answer as quickly, yet correctly, as possible. Subjects completed a practice round of 80 trials at the familiarization visit, followed by 80 scored trials for each subsequent test. During each test, reaction time and accuracy (i.e., percentage of trials answered correctly) were recorded for single trials.

**Supplementation Protocol.** Supplement dosing was based on the body mass of the subject (<68 kg = 1 scoop, 68–90 kg = 1.5 scoops, and >90 kg = 2 scoops) and was mixed with 20 ounces of water. Taste was similar between 2 of the supplements. A noncaloric, caffeine-free liquid water enhancer (1 teaspoon, MiO Cherry Blackberry; Kraft Heinz Food Company, Chicago, IL) was added to the third supplement-water mixture to maintain similar flavors across supplements. After baseline ASCRT were completed, subjects were given one half serving (i.e., 10 ounces of fluid) of the supplement to consume within 5 minutes. After 30 minutes, subjects completed the POMS questionnaire again, and heart rate, blood pressure, and brachial artery ultrasound measures were repeated. The other half of the serving was consumed upon completion of the second ultrasound measure. Supplementation was separated into 2 equal servings to account for the extended length of the experimental visits (~2 hours) and mimic consumption patterns in the field. Exercise-trained individuals often consume MIPS at the beginning and during workouts, rather than all at once before training.

**Physiological Testing.** Venous blood samples and upper-arm arterial blood flow were taken at 3 time points: (a) before supplement consumption, (b) 30 minutes after supplement consumption, and (c) after exercise. A 21- or 23-gauge needle (BD Vacutainer Eclipse 22 g and Vacutainer one-use holder; Becton, Dickinson and Company, Franklin Lakes, NJ) was used to collect 8 ml of blood at each of the 3 time points and placed into appropriate collection tubes (serum separator tube [SST] and Ethylenediaminetetraacetic acid [EDTA] BD Vacutainer, Becton, Dickinson and Company) by a trained phlebotomist. Serum was obtained from the serum separator tube tubes by allowing the blood to clot for 30 minutes then centrifuged at 1,500 g for 15 minutes at 4 °C. Plasma was obtained from the Ethylenediaminetetraacetic acid tubes and centrifuged immediately after collection at 1,500 g for 15 minutes at 4 °C. Plasma and serum samples were stored at −80 °C until ELISA assays were conducted for the analyte panel, including GH, IGF-I, testosterone, cortisol (ALPCO, Salem, NH), L-arginine (BioVision, Milpitas, CA), and serum osmolality, measured through an independent CORE laboratory facility. All samples were measured in duplicate and were within acceptable variance and above sensitivity provided from the manufacturer.

Blood pressure and blood lactate were also collected at the same time points after blood draws were conducted. Blood lactate concentration was also measured within 2–5 minutes after the completion of the BJS, BBP, and WAnT assessments using the Lactate Pro2 monitor (Arkray Inc., Kyoto Japan). Subjects

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**Table 1**

<table>
<thead>
<tr>
<th>Subject characteristics.†</th>
<th>N</th>
<th>Age (y)</th>
<th>Height (cm)</th>
<th>Body mass (kg)</th>
<th>BMI (kg·m⁻²)</th>
<th>Fat mass (kg)</th>
<th>Fat-free mass (kg)</th>
<th>Body fat (%)</th>
</tr>
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<tbody>
<tr>
<td>Overall</td>
<td>30</td>
<td>24.4 ± 4.9</td>
<td>173.2 ± 9.0</td>
<td>74.1 ± 15.1</td>
<td>24.5 ± 3.4</td>
<td>15.0 ± 7.4</td>
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<td>15</td>
<td>25.2 ± 5.4</td>
<td>179.2 ± 5.7</td>
<td>83.5 ± 14.1</td>
<td>25.9 ± 3.3</td>
<td>14.2 ± 8.3</td>
<td>66.4 ± 6.2</td>
<td>16.1 ± 6.3</td>
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<tr>
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<td>23.7 ± 4.4</td>
<td>167.2 ± 7.6</td>
<td>64.7 ± 9.1</td>
<td>23.2 ± 2.9</td>
<td>15.8 ± 6.4</td>
<td>46.7 ± 4.4</td>
<td>23.6 ± 7.2</td>
</tr>
</tbody>
</table>

*BMI = body mass index.
†Data are mean values ± SDs.

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Figure 1. Experimental visit study visit. Subjects completed baseline assessments then consumed one-half serving. Thirty minutes later, baseline assessments were repeated, subject consumed the second half-serving, then began the exercise protocol. Subjects received a 2-minute rest period between exercises. POMS = profile of mood states; HR = heart rate; BP = blood pressure; ASCRT = attention switching choice reaction test; MIPS = multi-ingredient preworkout supplement; BJS = ballistic jump squats; BBP = ballistic bench press; WAnT = Wingate anaerobic cycle test.
completed the testing battery in the order listed below, with a 2-minute rest period between each exercise. Heart rate and rating of perceived exertion (RPE) using the OMNI RPE visual scale (35) were collected after each physical assessment.

**Performance Testing Battery.** Study subjects completed a 5-minute warm-up on the treadmill at a self-selected walking pace, consistent across visits, before performing 3 maximum effort VJ assessed using Vertec (Sports Imports, Hilliard, OH) with 1-minute rest between jumps. A standard 2-minute rest period was implemented between exercises to allow for maximum ATP regeneration capacity, which is typically 2–3 minutes in trained individuals (6). Thirty repetitions of BJS, measuring lower-body explosive force and power, were conducted using a body mass equivalent to 30% of the subject’s 1RM squat determined at the familiarization visit. Subjects completed BJS in a MaxxRack (Star Trac, Vancouver, WA) with a Tendo unit linear transducer (Sorinex, Lexington, SC) connected to the bar and appropriate spotting by the research team to ensure safety. Power was assessed using Tendo Power Analyzer (Version 4) software. The same procedures were repeated for the BBP, which assessed explosive force and power of the upper body. Ballistic jump squats and BBP have previously been demonstrated to be reliable assessments of ballistic power (intraclass correlation coefficient [ICC] = 0.89–0.96 and ICC = 0.88–0.99, respectively) (1). Ballistic jump squats and BBP peak power (BJSPP and BBPPP, respectively) were determined as the highest peak power (Watts) for a single repetition across the 30 repetitions. BJS and BBP average peak power (BJSAPP and BBPAPP, respectively) were calculated as the average peak power across the 30 repetitions.

Subjects then completed the 5-10-5 Pro-Agility test, comprising quick directional changes and rapid accelerations from cones placed 5 meters apart in a “T”-shaped formation (17), which has shown to be a reliable and valid measure of change of direction speed (ICC = 0.90, SEM = 0.12) (39). Each subject completed 3 trials of the test for best effort. Subjects then performed the WAnT, consisting of a 2-minute warm-up at 50 Watts on a Velotron cycle ergometer (RacerMate, Inc., Seattle, WA) immediately followed by 30 seconds of maximum effort cycling against a resistance equivalent to 7.5% of their body mass. Peak power was calculated as the peak watts during the first 5 seconds of the 30-second test, and mean power was calculated as the average watts generated during the entire 30 seconds. Anaerobic power and anaerobic capacity were reported as peak and mean power relative to body mass (W·kg⁻¹), respectively. The Velotron has demonstrated to be a reliable measure of peak power (ICC = 0.70, SEM = 0.71) and mean power (ICC = 0.99, SEM = 0.18) (4).

Following post WAnT lactate measurement, subjects repeated the ASCRT then completed a 10-minute walking recovery on the treadmill at the same pace used for the warm-up walking speed. The VO₂peak Cycle Test was the final exercise assessment. Subjects completed the test on the Velotron cycle ergometer using the ParvoMedics TrueOne 2400 Metabolic Measurement System (ParvoMedics Inc., Sandy, UT). The TrueOne 2400 has been shown to be a reliable measure of oxygen consumption (r = 0.994, mean error = −0.04 L·min⁻¹, CV = 4.7%) (10). The VO₂peak protocol began with a 2-minute warm-up at 50 Watts followed by a ramp protocol in which the resistance increased by 1 Watt every 2 seconds. Subjects were required to maintain a cadence between 60 and 80 revolutions per minute (rpm) for the duration of the test. The test was terminated once the subject was no longer able to maintain a minimum pace of 60 rpm or volitional termination. VO₂peak was determined as the maximal oxygen consumption achieved during the test. Post VO₂peak RPE was recorded to determine if a maximal effort was given. No additional criteria were used to determine maximal effort provided that subjects had been exercising for approximately 30 minutes before the VO₂peak test. Upon completion of the VO₂peak Cycle Test, the final blood draw and brachial artery ultrasound were performed in addition to recording measures of heart rate and blood pressure.

**Statistical Analyses**

Descriptive statistics (mean ± SD, median, as appropriate) were calculated for all variables. Comparisons in dependent variables across supplements were conducted using one-way repeated-measures analysis of variance (ANOVA), followed by Bonferroni-adjusted pairwise comparisons, when appropriate. A two-way repeated-measures ANOVA (RMANOVA) was conducted to determine whether changes in mood states, ASCRT performance, blood concentrations, and brachial artery assessments throughout the exercise session differed over sessions across supplements (session × supplement). Significant interaction effects were followed by analysis of simple main effects. If interaction effects were not significant and main effects were significant, marginal Bonferroni-adjusted pairwise comparisons were conducted. The assumption of sphericity was examined using the Mauchly’s test, and Greenhouse–Geisser or HuynhFeldt corrections were appropriately used if the assumption of sphericity was not met. Residuals were examined for normality, outliers, and influential points. If the assumptions for ANOVA were not met, data transformations were conducted. In most cases, the statistical outcome and direction of differences did not differ between analysis of raw and transformed data. For the sake of simplicity, results of ANOVA using the raw (untransformed) data have been presented in the article because the ANOVA model is robust to violations of normality (23, p.145). Alpha was set at 0.05, a priori for all analyses. Partial eta-square (η²p) was calculated for significant outcome variables to measure the magnitude of difference between supplements as small, medium, or large (0.10, 0.25, and 0.50, respectively) (34). All analyses were conducted using IBM SPSS Statistics for Windows, Version 26 (IBM Corp., Armonk, NY).

**Results**

**Performance Outcomes**

Performance outcomes are depicted in Table 2. Wingate anaerobic power relative to body mass was 2.9% higher with NBCA (p = 0.014) and 3.8% higher with BAC (p = 0.022) compared with PLA. There was a significant supplement effect for Wingate peak power (p = 0.045, η²p = 0.113); NBCA and BAC demonstrated slightly greater peak power (2.3 and 3.9%, respectively) than PLA, although pairwise comparisons were not significant (p = 0.064 and p = 0.055, respectively). VO₂peak was significantly different across supplements (p = 0.024) in which BAC exhibited a slightly higher VO₂peak compared with PLA, although the pairwise comparison was not significant (p = 0.068). BAC also resulted in a longer duration of the VO₂peak test than PLA (p = 0.006) and NBCA (p = 0.015) by approximately 30 seconds (4.4%). There were no significant differences in VJ, 5-10-5 Pro-Agility, BJS, or BBP performance across supplements.
Physiological Outcomes

Heart rate was similar across conditions, with the exception of post $V_{O2}$peak ($p < 0.001$, $\eta^2_p = 0.351$) (Figure 2A). Post $V_{O2}$peak heart rate was higher with BAC compared with PLA ($p < 0.001$) and NBAC ($p = 0.013$); HR with NBAC was also significantly higher than PLA ($p = 0.009$). BLA concentrations remained similar across supplements during the exercise protocol before WAnT (Figure 2B). After WAnT, both NBAC and BAC supplements demonstrated an elevated BLA accumulation compared with PLA ($p = 0.012$ and $p = 0.010$, respectively). Differences in BLA across conditions persisted upon completion of the exercise protocol ($p < 0.001$, $\eta^2_p = 0.397$). BAC BLA remained significantly higher than both PLA ($p < 0.001$) and NBAC ($p = 0.001$).

There was a significant interaction effect for L-arginine concentrations throughout the exercise session by supplement ($p = 0.030$, $\eta^2_p = 0.114$). The simple main effect of exercise session on L-arginine concentration was significant for PLA ($p = 0.035$, $\eta^2_p = 0.123$) and BAC ($p = 0.044$, $\eta^2_p = 0.111$), but not NBAC ($p = 0.349$, $\eta^2_p = 0.037$). Post hoc Bonferroni-adjusted comparisons indicated that L-arginine concentrations increased 2.4% from baseline to post-supplement consumption of BAC ($p = 0.017$), and although pairwise comparisons were not significant for PLA, there was a 4.4% decrease in L-arginine concentration from baseline to postworkout (Figure 2C). There were no significant interaction effects or simple main effects for GH, IGF-I, testosterone, cortisol, or serum osmolality ($p > 0.05$).

Brachial artery ultrasound revealed a significant session by supplement interaction effect in brachial artery diameter ($p < 0.001$, $\eta^2_p = 0.160$) (Figure 3A), velocity ($p = 0.007$, $\eta^2_p = 0.122$) (Figure 3B), cross-sectional area (CSA) ($p = 0.001$, $\eta^2_p = 0.156$) (Figure 3C), and blood flow ($p < 0.001$, $\eta^2_p = 0.213$) (Figure 3D). There were no changes in brachial artery diameter or CSA across the exercise session with PLA ($p = 0.091$ and $p = 0.112$, respectively), but changes were identified in NBAC ($p = 0.001$) and BAC ($p < 0.001$).

Cognitive Outcomes

There was a significant interaction effect for ASCRT reaction time ($p = 0.043$, $\eta^2_p = 0.103$). Reaction time significantly decreased across session for all supplements (PLA: $p = 0.007$, $\eta^2_p = 0.226$; NBAC: $p = 0.040$, $\eta^2_p = 0.137$; BAC: $p < 0.001$, $\eta^2_p = 0.447$). There was also a significant interaction effect for ASCRT accuracy ($p = 0.027$, $\eta^2_p = 0.117$); however, simple main effect of exercise session for each supplement were not significant (PLA: $p = 0.053$, NBAC: $p = 0.065$, BAC: $p = 0.799$).

Self-Report Outcomes

Subjects reported similar RPE throughout most exercise sessions ($p > 0.05$). There was a difference in RPE after the 5-minute warm-up walk ($p = 0.047$, $\eta^2_p = 0.115$) and post BBP ($p = 0.037$, $\eta^2_p = 0.107$); however, pairwise comparisons were not significant (PLA: $p = 0.053$, NBAC: $p = 0.065$, BAC: $p = 0.799$). Average RPE reported post $V_{O2}$peak test were similar across supplements ($p = 0.699$; PLA: 9.3, NBAC: 9.4, BAC: 9.4), indicating subjects gave nearly maximal effort before terminating the $V_{O2}$peak test. There was no interaction between session and supplement on mood states of tension, anger, fatigue, esteem-related effect, and vigor ($p > 0.05$). Post hoc power was calculated for all variables and ranged from 6.70 to 57.76% for nonsignificant results.

Discussion

This study investigated the acute effects of 2 MIPS, with and without beta-alanine and caffeine, compared with placebo on anaerobic performance, endurance capacity, mood state, cognitive function, and anabolic hormones. The primary findings of this study indicate that acute ingestion of MIPS resulted in increased anaerobic power (~3%) and MIPS specifically containing beta-alanine and caffeine improved $V_{O2}$peak time to volitional exhaustion (~4.4%), accompanied by an increase in blood lactate accumulation (20.5%), during endurance cycling in exercise-trained men and women. Both NBAC and BAC demonstrated improved anaerobic power relative to body mass, and greater brachial artery CSA and diameter after workout compared with PLA. Provided that both supplements contain various...
Training adaptations require saturated creatine concentrations, efficacy of acute single-dose creatine supplementation because maximal effort exercise, there is limited evidence to support the effective ergogenic aid by maintaining ATP availability during although creatine supplementation has been reported to be an ingredient, it is unclear precisely which ingredients were most responsible for improvements in exercise performance.

Both supplements contained creatine monohydrate-, L-citrulline-, and inositol-stabilized arginine silicate (Nitrosigine). Although creatine supplementation has been reported to be an effective ergogenic aid by maintaining ATP availability during maximal effort exercise, there is limited evidence to support the efficacy of acute single-dose creatine supplementation because training adaptations require saturated creatine concentrations, accomplished through creatine “loading” or daily supplementation (27). Suzuki et al. (42) demonstrated L-citrulline supplementation increased mean power output by 2% in cyclists performing a 4-km time-trial test. In a similar study, mean power output during a 10-minute cycling test was significantly improved with a L-citrulline and L-arginine combination supplement compared with placebo (40). However, in both studies, subjects consumed the supplement for 7 consecutive days before the experimental test. The combination of L-arginine and L-citrulline has been shown to significantly increase plasma L-arginine concentrations within 1 hour (41). Therefore, the short-acting effects of the combination may have contributed to the 2.9% (NBAC) and 3.8% (BAC) increase in Wingate anaerobic power.

Martinez et al. (30) reported an increase in Wingate peak power (8.3%) with acute ingestion of a supplement containing a proprietary blend of beta-alanine and caffeine. We observed a significant effect of supplement on Wingate anaerobic power relative to body mass (p = 0.018) and absolute peak power (p = 0.045). Although NBAC and BAC increased anaerobic power by 0.3 and 0.4 W·kg⁻¹, respectively, a previous Velotron reliability study in recreationally active individuals reported the minimum difference to be 0.44 W·kg⁻¹ (4). However, the reliability study did not include a familiarization trial, which was implemented in this study. Similar to previous studies, MIPS did not improve VJ height (21,28,30). Provided that supplement dosing was based on body mass in the current study, it is possible that improvements in power were only detectible when power outcomes were relative to body mass.

Time to exhaustion during the VO2peak Cycle Test was significantly improved (4.4%) with BAC by approximately 30 seconds compared with NBAC and PLA. Similar to creatine, it is unlikely that beta-alanine significantly contributed to this acute increase in performance as a loading phase of approximately 4 weeks is necessary for beta-alanine to substantially increase muscle carnosine concentrations (45). Therefore, it is more likely that the increased time to exhaustion was attributed to caffeine and taurine, both ingredients unique to the BAC supplement blend. The ergogenic effects of caffeine are known to result from central nervous system activation by blocking A1 and A2a adenosine receptors, promoting the release of neurotransmitters such as glutamate and noradrenaline (31). A recent meta-analysis investigating the effects of caffeine on muscular endurance revealed a 14% improvement in muscular endurance with caffeine supplementation compared with placebo (49). More specifically, open endpoint endurance tests, such as sustained isometric, submaximal contractions, had a greater detectible caffeine effect compared with fixed endpoints, demonstrating an 18% improvement in muscular endurance with caffeine supplementation compared with placebo when comparing open endpoint endurance tests (49). Considering caffeine is quickly absorbed in the bloodstream and generally peaks within 1 hour of ingestion, the effects can be observed with acute supplementation (18).

Oral taurine ingestion has also been shown to improve endurance performance by a small amount, with no differences between acute and chronic supplementation (47). Balshaw et al. (7) demonstrated the benefits of acute taurine ingestion in which 1,000-mg taurine supplementation yielded a 1.3% improvement in 3-km timed-trial running performance. However, these findings were unable to be replicated in trained cyclists 4-km timed-trial performance (48). Taurine was also shown to improve fatigue index with repeated sprint cycling to volitional fatigue, defined as the percent change in mean power output between 6 sprints, accompanied by higher post-test blood lactate concentrations compared with...
placebo (46). These results are similar to the findings of the current study in which postworkout BLA was significantly elevated to NBAC and PLA. Waldron et al. (46) offers 2 explanations for this elevated BLA: (a) greater peripheral fatigue due to longer duration of exercise or (b) a decrease in the osmotic gradient within the muscle cell postexercise due to increased taurine concentration, reducing transport of solutes out of the cell, leading to cell swelling and potentially cellular damage.

Upper-body power, assessed through 30 repetitions of ballistic bench with 30% 1RM, was greater with BAC compared with NBAC, with no significant difference in upper-body power between PLA and NBAC. Improvements in upper-body power with MIPS were also identified by Lane and Byrd (28) in which bench press peak velocity was greater with MIPS containing beta-alanine and caffeine compared with placebo. However, Lane and Byrd (28) also reported similar improvements with dose-matched caffeine vs. placebo. Thus, given the nature of MIPS, we cannot conclude if caffeine, beta-alanine, or the combination elicited improvements in upper-body power.

The exercise protocol in this study elicited an endocrine response in which GH, testosterone, IGF-I, and cortisol significantly increased postworkout across all conditions, consistent with previous literature (25,26). For example, Rubin et al. (36) observed significant increases in plasma IGF-I and GH in both trained and untrained individuals immediately after an acute heavy resistance exercise protocol consisting of 6 sets of 10RM back squat with a load equivalent to 80–85% 1RM. In addition, serum osmolality increased after workout, regardless of supplementation, likely due to sweat loss and increase in blood lactate accumulation during exercise, leading to increased sodium concentrations (32,33). Therefore, the protocol used in this study was sufficiently metabolically demanding; however, MIPS were unsuccessful in altering the anabolic hormone response or hydration status.

L-arginine concentrations were elevated 30 minutes after consumption of BAC. A similar trend was observed with NBAC, although L-arginine concentrations did not significantly differ across the exercise session. Previous research has reported that a combination of L-citrulline and L-arginine can effectively increase plasma L-arginine concentrations to a greater extent than single amino acid supplementation or placebo (41). Unlike L-arginine, L-citrulline is not susceptible to hepatic metabolism and is converted to L-arginine in the urea cycle (13). As a result, L-citrulline yields more bioavailable L-arginine for nitric oxide synthesis, which can provide a more beneficial effect than L-arginine alone (12,41).

As previously stated, MIPS containing L-citrulline and L-arginine can improve nitric oxide synthesis and vascular function (12,41). In this study, brachial artery diameter and CSA were significantly increased after exercise in both MIPS conditions, whereas there was no significant change with PLA. These results suggest that L-citrulline and L-arginine, present in both BAC and NBAC, may have contributed to improved vascular function during exercise. Although both MIPS increased diameter and CSA after exercise, there was a significant decrease in both measures after consumption of BAC that was not observed with NBAC. Caffeine acts as an adenosine antagonist, releasing noradrenaline, which acts as a vasoconstrictor (8,31). Therefore, the effects of caffeine in the BAC blend likely attributed to the initial decrease in brachial artery and CSA 30 minutes after supplement consumption; however, the exercise-induced release of nitric oxide and progression of time may have diminished the initial vasoconstriction effects of caffeine resulting in an increase after exercise.

Reaction time, assessed by ASCRT, significantly improved across all supplements before to after exercise, whereas accuracy was stable. Likewise, Lefert et al. (29) demonstrated that acute exercise alone had no effect on memory recognition, 2-back, and
Eriksen Flanker task accuracy, but accelerated after exercise reaction time. Similar to Ellerbroek and Antonio (11), we did not observe a significant effect of supplement on mood state. However, we did observe a significant effect of time in which there was a small but significant increase in depression scores 30 minutes after supplement consumption in all 3 conditions. The authors hypothesize this may be attributed to the 30-minute wait period in which subjects sat and rested before the rigorous exercise protocol. Although no survey data regarding symptoms after supplement consumption were collected, data suggest subjects did not experience elevated heart rate 30 minutes after consuming supplements. However, some subjects did experience tingling after supplement consumption, consistent with the literature (45).

As previously stated, the supplements in this investigation consisted of proprietary blends containing various ingredients; therefore, it is not possible to definitively determine which ingredients led to exercise performance improvements. Although the Food and Drug Administration requires all dietary ingredients within a proprietary blend to be listed on the product label in descending order by body mass, some ingredients may be below their efficacy threshold (18). Therefore, a limitation of this study is the degree to which performance improvements can be attributed to individual ingredients within the proprietary blend. However, this study used only one container per supplement (i.e., BAC, NBAC, and PLA) for all subjects to ensure that the blends were consistent across subjects.

### Practical Applications

Acute ingestion of MIPS, both with and without beta-alanine and caffeine, demonstrated the potential to improve anaerobic power relative to body mass in exercise-trained men and women. Multi-ingredient preworkout supplements specifically containing beta-alanine and caffeine increases circulating L-arginine plasma concentrations and improves time to exhaustion (4.4%) during endurance cycling, accompanied by higher blood lactate accumulation. There seems to be no improvement in accuracy, mood, or anabolic hormone profile with acute ingestion of MIPS. Exercise-trained individuals looking to achieve modest improvements in power and endurance may benefit from consuming MIPS before exercise.

### Acknowledgments

This study was supported by an industry-sponsored grant through Isagenix International LLC. P.J. Arciero is a member of Scientific Advisory Board at Isagenix International LLC, the sponsor of this study. P.J. Arciero was not involved with data collection or data analysis for this study. The results of this study do not constitute endorsement of the product by the authors or the NSCA. This study was designed by P.J. Arciero, B.C. Nindl, M.E. Darnell, K. Beals, S.D. Flanagan, and B.J. Martin. M.E. Beckner and A.A. Piholer performed subject recruitment, data collection, and immunoassays. M. Lovalekar conducted data analysis. Data interpretation and manuscript preparation were undertaken by M.E. Beckner, B.J. Martin, A.A. Piholer, M.E. Darnell, F.S. Proessl, M. Lovalekar, B.C. Nindl, and P.J. Arciero assisted with manuscript preparation. All authors approved the final version of the article.

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