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Acute Ingestion of a Commercially Available Pre-workout Supplement Improves Anaerobic Power Output and Reduces Muscular Fatigue

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ABSTRACT

International Journal of Exercise Science 15(6): 455-472, 2022. The effect of a pre-workout supplement on anaerobic power output and muscular fatigue was examined. 18 participants took part in this double-blinded crossover study, reporting for testing on 3 occasions. Participants completed a 6x6 second repeated sprint test, with 20s recovery between sprints. Anaerobic power output was recorded as the highest power achieved during sprint test. Muscular fatigue was reported as a fatigue index across the six sprints ((maximum power – minimum power) ÷ total sprint time). During a baseline visit, participants consumed 250ml of water 30 minutes prior to testing, whilst in subsequent visits a taste-matched placebo (250ml water mixed with sugar-free juice) or a pre-workout supplement (250ml water mixed with one serving of 'THE PRE' myprotein.com). Anaerobic power output increased following pre-workout ingestion (pre-workout supplement, 885.8 ± 216.9W; Placebo, 853.6 ± 206.5W; Baseline, 839.3 ± 192.6W). Baseline vs pre-workout supplement ($p = 0.01$, $g = 0.30$); Placebo vs pre-workout supplement ($p = 0.01$, $g = 0.20$); Baseline vs Placebo ($p = 0.59$ $g = 0.09$). Muscular fatigue was reduced following pre-workout ingestion (Baseline, 4.92 ± 1.83W.s; Placebo, 4.39 ± 1.93W.s; pre-workout supplement, 3.31 ± 1.34W.s). Baseline vs pre-workout supplement ($p = < 0.01$ $g = 0.98$); Placebo vs pre-workout supplement ($p = 0.01$, $g = 0.63$); Baseline vs Placebo ($p = 0.20$, $g = 0.28$). Acute ingestion of a pre-workout supplement significantly improves anaerobic power output and attenuates muscular fatigue during repeated sprint cycling.

KEY WORDS: Nutrition, performance, sprint, cycling, training, placebo

INTRODUCTION

Pre-workout supplements continue to grow in popularity within the sports nutrition industry and are consumed by both recreational and competitive athletes (26). The majority of pre-workout supplements contain a blend of various key ingredients, such as Caffeine, β-alanine, Creatine, Citrulline Malate, and vasodilators such as L-Citrulline and L-Arginine (11), that when ingested, claim to enhance training adaptations and promote recovery (40).

Caffeine has been shown to act as a central nervous system stimulant, whilst also enhancing the release of calcium in skeletal muscle, which could potentially see an improvement in muscular

strength, power and endurance (38). The central nervous system response is facilitated by an antagonist response by caffeine on adenosine receptors, which has been reported to lead to an increase in the release of neurotransmitters, an increased firing rate of motor units, and an increase in the transmission of dopamine (21). Consequently, the performance benefits following ingestion of caffeine include improvements in endurance and power output and a delay in the time taken to fatigue (57). Cognitive benefits have also been associated with caffeine ingestion, including improvement in concentration and perceived energy, leading athletes to feel more alert and focused (15). Caffeine is most beneficial to performance when consumed in moderate to low amounts, with a serving suggestion of 3-6mg per kg body mass, 30-60 minutes before exercising (24). A meta-analysis by Grgic et al (30) supports acute caffeine intake as an effective ergogenic aid for achieving increases vertical jump height, a common measure of lower body anaerobic power. The magnitude of improvement in vertical jump height found for a single caffeine ingestion was roughly equivalent to the effects of ~ 4 weeks of plyometric training (44). In addition, a meta-analysis by Grgic (29) demonstrated a significant performance-enhancing effect of acute caffeine intake on Wingate test performance, a common cycling test of anaerobic power. The caffeine used in pre-workout supplement is in an anhydrous state, which some studies have shown to have a greater ergogenic effect in comparison to the caffeine found in tea, coffee, or soft drinks (24, 61).

Long-term ingestion of β-alanine has been reported to increase the rate of carnosine to skeletal muscle, therefore potentially improving performance in high intensity exercise, by enhancing power output and decreasing fatigue (Outlaw, et al., 2014). A meta-analysis by Saunders et al. (50) showed a significant positive effect of chronic β-alanine supplementation on exercise, supporting the efficacy of increased muscle carnosine to improve exercise. Short duration exercise (≤0.5 min) was not benefited from supplementation (d = 0.040), while effect sizes for moderate duration exercise (0.5-10 min) were significant (d = 0.224). β-alanine supplementation of 1.6 g ·day^{-1} for as little as two weeks has been shown to increase muscle carnosine (56), while improvements in exercise have been shown at doses ranging from 3.2-6.4 g ·day^{-1} for 4-12 weeks (34, 51). Ingesting 4-6 g ·day^{-1} of β-alanine over a ten-week period has been reported to improve muscle carnosine concentrations by up to 80% (34). Increased levels of carnosine may help an athlete reduce fatigue, as carnosine buffers hydrogen ions during exercise, thus influencing muscle pH level, and therefore potentially increasing work capacity (60). The acute effects of β-alanine ingestion appear limited, with Glenn et al., (23) reporting no significant performance or physiological benefits when cyclists ingested a single dose.

Watanabe et al., (62) states that upon ingestion, creatine is taken to the muscles and brain to be converted into phosphocreatine, which can later be produced into ATP. The energy used to rephosphorylate adenosine diphosphate (ADP) into ATP during an explosive movement is affected by phosphocreatine stored within the muscle (41), therefore increasing skeletal muscles phosphocreatine storage is believed to enhance sporting performance. Creatine supplementation has demonstrated positive results for repeated sprint performance trials, with Dawson et al. (14) reporting that supplementation (20 g ·day^{-1} for 5 days) significantly increased work performed during the first of 6 × 6s cycle ergometer sprints with 30s recovery between

sprints. These results are supported by Schneider et al. (52), who reported that supplementation (25 g·day^{-1} for 7 days) significantly improved 5 × 15s cycle ergometer sprints with 60s recovery between sprints. However in contrast, Finn et al. (19), reported no ergogenic effect of creatine supplementation (20 g·day-1 for 5 days) on 4 × 20s maximal cycle sprints, with each sprint separated by 20s of recovery. The effect of creatine supplementation on single maximal effort sprints is less clear, with Odland et al (48) demonstrating no ergogenic benefit on a 30s maximal cycling task after supplementation (20 g·day^{-1} for 3 days). It could be argued that a 3-day supplementation period was not long enough, contributing to the lack of performance increase. However, with a longer supplementation period (30 g·day^{-1} for 5 days), Snow et al. (55) also report no improvement in a single 20s maximal sprint on a cycle ergometer. Therefore, it may appear that creatine supplementation has no ergogenic benefit on single short duration high intensity cycling trials.

Research suggests that in order to facilitate an ergogenic effect following creatine supplementation, a chronic loading phase is needed, with the majority of creatine supplementation research reporting loading phases of 20-30 g.day^{-1} for 5-7 days (6). Following the initial loading phase, lower doses (around 2-3 g.day^{-1}) may act as a useful maintenance dose to prolong the ergogenic benefit of supplementation (36, 2, 46). However, positive performance effects have also been observed without an initial loading phase, following an extended low dose supplementation period. Hultman et al. (36) reported that a low dose of 3 g.day^{-1} for 28 days was as effective at raising creatine levels as protocols using chronic loading phases of higher doses for shorter periods. Similarly, Burke et al. (9) report that a dose of ~ 8 g.day^{-1} for 21 days produced an ergogenic benefit during short-duration, high-intensity activity. Finally, Hoffman et al. (35) demonstrated that a dose of 6 g.day^{-1} for just 6 days resulted in a reduced fatigue rate in subjects supplementing with creatine compared with a placebo. There appears to be no research investigating the performance effects following a single low dose ingestion of creatine.

L-citrulline is a nonessential amino acid found principally in watermelon, along with cucumber and other melons (25). L-citrulline supplements are often given in the form of citrulline malate (CM), with proposed benefits attributed to the combined effect of the supplements at the intramuscular metabolic level (25, 63). A range of work has investigated the effects of acute ingestion of citrulline malate prior to physical activity. The majority of studies ingest a dose of 6-8g of citrulline malate 60 min prior to exercise (25, 59). The main body of citrulline malate research has been conducted using resistance training protocols, with only two studies investigating its effects on high intensity cycling. Glenn et al. (22) investigated the effect of supplementation with 8g of citrulline malate 60 minutes prior to a single 30 second Wingate cycle test in female tennis players. Peak power and explosive power were both significantly higher in the citrulline malate trial. Cunniffe et al. (13) investigated the effects of 12g of citrulline malate 60 minutes prior to a repeated sprint cycle test (10 x 15s sprints, with 30s recovery) in well-trained males. No significant differences in peak power, mean power or fatigue index were reported between the citrulline malate and placebo conditions. In addition, there has been a limited range of work investigating the effects of a lower dose of citrulline malate

supplementation over an extended period of time. Hwang et al., (37) investigated the effects of 2g citrulline malate per day during an 8 week resistance training programme. Results revealed no significant differences for upper or lower body strength between citrulline malate and placebo conditions. There appears to be no research investigating the performance effects following a single low dose ingestion of citrulline malate.

There are conflicting evidence in the literature regarding the benefits of multi-ingredient pre-workout supplement ingestion on lower body power and anaerobic fatigue. A number of studies have reported no benfits of acute ingestion of a pre-workout supplement. Lane and Byrd (42) investigated the effects of a multi-ingredient pre-workout supplement, a placebo with added caffeine and a standard placebo on repeated sprint cycling (10 x 5s sprints, with 55s recovery between each sprint). Results report no significant differences in either peak or mean power output between conditions. Lane et al (43) repeated the same protocol in female subjects a year later, with identical results. Herbe et al. (31) investigated the effect of a multi-ingredient pre-workout supplement and a placebo on repeated sprint cycling (4 x 30s sprints, with 5 min recovery between each). Results report no significant differences in peak power, mean power, or fatigue management between conditions. Outlaw et al (49) investigated the effects of a multi-ingredient pre-workout supplement and a placebo on a single 30s sprint cycle test. Results report no significant differences in peak power between conditions. Contrary to the above research, there is some evidence of a potential benefit of ingesting a pre-workout supplement prior to exercise. Martinez et al (45) investigated the effects of a multi-ingredient pre-workout supplement and a placebo on a single 30s sprint cycle test. Results reported significantly higher peak and mean power in the supplementation trial, however no differences were seen in fatigue management between conditions. Furthermore, Jagim et al (38) investigated the effects of a multi-ingredient pre-workout supplement and a placebo on a single 25s sprint on a non-motorised treadmill. Whilst results reported no significant differences in peak power and fatigue management during the sprint, mean power was significantly higher during the supplement condition.

Due to the conflicting evidence within literature of the performance benefits associated with acute multi-ingredient pre-workout supplement ingestion, the purpose of this study was to examine the acute effect of a commercially available multi-ingredient (containing Caffeine, β-alanine, Creatine and Citrulline Malate) pre-workout supplement on anaerobic power output and muscular fatigue within recreationally active participants. It was hypothesised that participants would demonstrate increased power output and better ability to cope with fatigue following multi-ingredient pre-workout supplement ingestion.

METHODS

Participants

Eighteen recreationally trained participants (12 Male and 6 Female, Age, 23.8 ± 3.1y; Height, 174.2 ± 9.5cm; Body Mass, 71.7 ± 13.1kg) who regularly take part in spinning classes at least once per week were recruited to take part in the study. All participants completed a health history

questionnaire and provided written informed consent prior to testing. The study was granted a priori approval from the University of East London, School of Health, Sport and Bioscience ethics review board. This research was carried out fully in accordance to the ethical standards of the International Journal of Exercise Science (47). Participants were excluded from participating if they reported ingesting high doses of caffeine regularly, or had previously been consuming Creatine, Nitric Oxide, β-alanine, or any anabolic steroids on a consistent basis, within the past 3 months (54, 40, 45). The ingredients of the pre-workout supplement were fully explained to the participants, to ensure no allergies existed. For a minimum of two weeks prior to testing, participants were required to have had no consumption of any pre-workout supplement (45). The participants reported a 24-hour food diary prior to each test being completed.

Protocol

A double-blinded repeated measure crossover design was implemented, where participants completed a 6-second repeated sprint test on 3 separate occasions, including a baseline, placebo controlled, and a pre-workout supplement test. Due to participants taking part in repeated sprint cycle activity on a weekly basis, no familiarisation visit was provided prior to the three trials. Participants performed a 6x6 second maximal repeated sprint test on a WattBike Pro cycle ergometer (WattBike, Ltd, Nottingham, UK), with 20s rest intervals between each sprint. Anaerobic power output was recorded as the highest power output achieved during each six second sprint. Muscular fatigue was reported as a fatigue index across the six sprints ((maximum power - minimum power) ÷ total sprint time). A sprint test with maximal effort being present for 6 seconds, with a 20s rest interval, has been shown to be a valid test for researches and coaches to measure anaerobic power, and assess fatigue in athletes (1, 32). Each test was separated by exactly seven days and performed at the same time of day to account for any potential effect of circadian rhythm (18). A seven-day period is also deemed sufficient rest time between tests to ensure any potential effects of the supplement do not cross over between tests (45). Participants were instructed to refrain from caffeine for 24 hours prior to each test, and were asked to eat similarly 24-hours prior to each test. The participants performed each test individually in a private studio, with constant encouragement provided throughout. Each participant completed a 5-minute dynamic warm up protocol before each test, similar to Jagim et al., (38).

Supplementation: During the first visit the participants were provided with 250ml of water, to complete a baseline test, whilst in the second and third visit either a taste-matched Placebo (250ml water mixed with sugar-free juice) or a pre-workout supplement (250ml water mixed with one serving of 'THE PRE' from myprotein.com – see Table 1). Solutions were ingested 30 minutes prior to testing (45). Smith, et al. (54) used a similar amount of water when mixing their pre-workout supplement and placebo. The placebo was taste and colour matched prior to testing and a nose plug was employed during consumption (23).

Statistical Analysis

Data was checked for normality of distribution. A non-parametric Friedman test was deployed to assess for differences between both the absolute and scaled anaerobic power output variables across conditions. Wilcoxon Signed Ranks tests were used to subsequently compare paired test scores. A repeated measures ANOVA was used to assess for differences in the muscular fatigue variables across conditions. Paired sample t-tests were used to subsequently compare paired test scores. The criteria to accept the hypothesis was set at a $P < 0.05$. Effect sizes were used to determine the magnitude (Trivial = 0.0 – 0.2, Small = 0.2 – 0.6, Moderate = 0.6 – 1.2, Large = 1.2 – 2.0, Very large = 2.0 – 4.0, Extremely large = > 4.0) of effect present (10).

Table 1. Nutritional Information of Pre-Workout Supplement (MyProtein, 2020)

Nutritional Information		
Serving Size - 1 Scoop (14g)		
Servings Per Container - 30 Servings (based on 14g serving size) (420g)		
	Per 14g Serving	*RI
Vitamin C	75 mg	94%
Thiamin	1.1 mg	100%
Niacin	3.0 mg	19%
Vitamin B6	1.4 mg	100%
Folic Acid	250 µg	125%
Vitamin B12	1.5 µg	60%
Pantothenic Acid	1.0 mg	17%
L-Citrulline Malate 2:1	3000 mg	
Creatine Monohydrate	2000 mg	
Beta Alanine	1600 mg	
Leucine	1000 mg	
Betaine Anhydrous	1000 mg	
L-Glutamine	1000 mg	
L-Arginine AKG 2:1	500 mg	
L-Carnitine	500 mg	
N-Acetyl L-Tyrosine	500 mg	
Choline Bitartrate	200 mg	
Guarana Ext. (22% Caffeine)	75 mg	
Caffeine (inc Caffeine & Guarana Ext.)	175 mg	

*Reference intake of an average adult (8400kJ/2000kcal)

RESULTS

Participants produced their highest peak power for the pre-workout supplement condition (885.8 ± 216.9W), compared with the Placebo (853.6 ± 206.5W) and Baseline (839.3 ± 192.6W) conditions. Figure 1 establishes anaerobic power output in the pre-workout supplement condition was significantly higher than both the Baseline condition (Z = -2.81, P = 0.01, g = 0.30) and the Placebo condition (Z = -2.59, P = 0.01, g = 0.20). There was no significant difference between anaerobic power output in the Baseline and Placebo conditions (Z = -0.54, P = 0.59, g = 0.09).

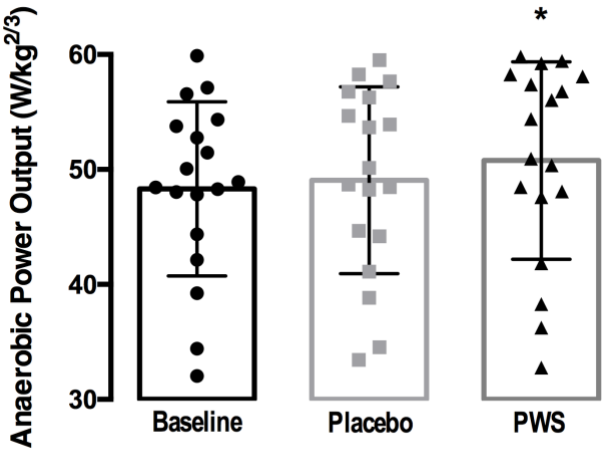

Figure 1. Difference in Anaerobic Power Output between the three supplementation conditions. Values are allometrically scaled ($W/kg^{2/3}$) and presented as mean ± SD. * Significantly higher than placebo and baseline (P = 0.01).

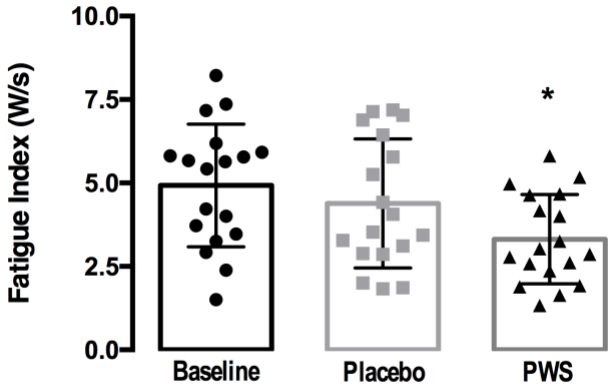

Figure 2. Difference in Fatigue Index between the three supplementation conditions. Values are presented as mean ± SD. * Significantly higher than placebo and baseline (P = 0.01).

A repeated measures ANOVA revealed a significant difference in muscle fatigue between the three supplementation conditions (F = 10.3 (2,34), *P* = < 0.01). Figure 2 demonstrates that fatigue was significantly attenuated in the pre-workout supplement condition (3.31 ± 1.34 W/s) compared with the Baseline (4.92 ± 1.83 W/s) and Placebo (4.39 ± 1.93 W/s) conditions ($t_{(17)}$ =

1.34, P = <0.01, g = 0.98; $t_{(17)}$ = 2.99, P = 0.01, g = 0.63 respectively). There was no significant difference in muscle fatigue index between the Baseline and Placebo conditions ($t_{(17)}$ = 5.00, P = 0.20, g = 0.28).

Hedge's g was calculated for each condition, examining both anaerobic power output and muscular fatigue (Table 2). A small magnitude of effect was seen for an improved anaerobic power output in the pre-workout supplement condition compared to the Placebo and Baseline conditions. A moderate magnitude of effect was seen for an improved fatigue index in the pre-workout supplement condition compared to the Placebo and Baseline conditions.

Table 2. Hedges g effect sizes and magnitude for Anaerobic Power Output and Muscular Fatigue. The magnitude of the significance between the conditions was determined using the limits outlined by Cohen (1992).

Conditions	Hedge's g (Anaerobic Power Output)	Magnitude	Hedge's g (Fatigue)	Magnitude
Pre-Workout Supplement vs. Placebo	0.20	Small	0.63	Moderate
Pre-Workout Supplement vs. Baseline	0.30	Small	0.98	Moderate
Baseline vs. Placebo	0.09	Trivial	0.28	Small

DISCUSSION

This study examined the effect of a commercially available pre-workout supplement on anaerobic power output and muscular fatigue. Results indicate that the supplement, containing Caffeine, β-alanine, Amino Acids, Creatine and Citrulline Malate, improved anaerobic power output (P = 0.01, g = 0.20), whilst blunting fatigue (P = 0.01, g =0.63) during repeated sprint cycling exercise, when compared with a placebo supplement.

These findings support the previous work by Martinez *et al*., (45), who demonstrated significant improvements in anaerobic power output (P < 0.01, d = 0.30) during a single 30s Wingate sprint cycle test, following acute ingestion of a multi-ingredient pre-workout supplement. However, contrary to the previous work of Martinez, *et al*., (45), the results of the present study also demonstrated a significant blunting of fatigue in the pre-workout supplement trial compared with the placebo.

Aside from the work of Martinez *et al*., (45), the results of the present study conflict with a number of past research studies which have all shown no significant differences in anaerobic power output or fatigue management during sprint cycling, following the acute ingestion of a multi-ingredient pre-workout supplement (31, 48, 42, 43). In addition the results of the present study conflict with the work of Jagim et al (38) who demonstrated no significant differences in anaerobic power output or fatigue management during sprint running, following the acute ingestion of a multi-ingredient pre-workout supplement. The present study therefore is the first

study to show a positive benefit of acute multi-ingredient pre-workout supplement ingestion on fatigue management during repeated sprint cycling, and one of only two study's to show a positive benefit on anaerobic power output.

Table 3. Comparison of research into multi-ingredient pre-workout supplements.

	Protocol	Supplement	Participants	Ingestion	Outcome
Current study	6x6s sprint, 20s recovery	Caffeine (175mg), β-alanine (1.6g), Creatine (2g), Citrulline Malate (3g))	12 Male, 6 Female 23.8 ± 3.1yr recreationally trained, regularly take part in high intensity cycle spin classes	30 minutes prior to testing	Improved anaerobic power output (P = 0.01), whilst blunting fatigue (P = 0.01)
Martinez et al. (45)	1x30s sprint	β-alanine (2g), Creatine (1g), Caffeine (200mg)	13 males (24 ± 6yr), recreationally trained.	20 minutes prior to testing	Improved anaerobic power output (P < 0.01). No significant differences in fatigue management
Herbe et al. (31)	4x30s sprint, 5 min recovery	β-alanine (2g), Creatine (1g), Caffeine (200mg)	7 males (25 ± 4yr) and 6 females (24 ± 2 yr, experience in high-intensity interval training, but no mention of specifically in cycling	20 minutes prior to testing	No significant difference in anaerobic power output or fatigue management
Outlaw et al. (49)	1x30s sprint	Creatine- β-alanine blend (8.4g), Caffeine (275mg)	20 males (22.4 ± 9.5yr), resistance-trained	30 minutes prior to testing	No significant difference in anaerobic power output or fatigue management
Lane and Byrd (42)	10x5s sprint, 55s recovery	Citrulline Malate (3g), Creatine (1.5g), β-alanine (2g), Caffeine (300mg)	23 recreationally trained men (22.9 ± 3.7yr), no mention of familiarity with high intensity cycling	20 minutes prior to testing	No significant difference in anaerobic power output or fatigue management

Jagim et al. (38)	1x25s sprint, on non-motorised treadmill	Creatine (2g), β-alanine (2g), Citrulline Malate (6g), Caffeine (300mg)	12 males (18.8 ± 1.2yr), resistance trained	20 minutes prior to testing	No significant difference in anaerobic power output or fatigue management

Table 3 provides a comparison of past research investigating the effects of multi-ingredient pre-workout supplements on sprint performance. The ingestion time-frame of the supplement prior to testing was similar across all studies. Protocols differed in durations of sprint efforts and recovery periods, which may have contributed to the conflicting findings between the current study and past research. Population groups across studies were similar in participant number and age, with studies using participants from a recreationally trained background. Unlike the current study, no previous study has tested a population group with specific experience in regular high-intensity interval cycling. The participants in the current study were all familiar with high-intensity short-sprint cycle training, conducting spinning classes weekly. This may provide some evidence that familiarity with the mode and intensity of the exercise used in the current study design may have contributed to the positive findings for anaerobic power output and fatigue management seen in this population group, following multi-ingredient pre-workout supplement ingestion.

Furthermore, when considering the potential causation of the current findings, it is important to consider each of the pre-workout supplement ingredients in turn.

The primary ingredient commonly associated with pre-workout supplements is caffeine, which claims numerous reported benefits within literature, such as an increased activation of the central nervous system from an inhibition of adenosine receptors (28). Caffeine's structure is comparable to adenosine, and therefore can fixate to cell membrane receptors, thus blocking their action and causing a wide range of reactions (27). Interestingly, Goldstein, et al., (24) states although caffeine has an influence on the contraction and excitation of muscle, the key effect may be less muscular and more neural, as caffeine crosses membranes in both nerve and muscle cells. The popular supplement is also associated with epinephrine stimulation, with Bell, et al., (4) reporting participants with high plasma epinephrine levels significantly improved peak anaerobic power output in a cycle sprint test ($P < 0.05$).

One of caffeine's primary sites of action is at the central nervous system, and it is believed to enhance cognitive functions, such as concentration and arousal (24). Testing sessions in the current study were conducted in the early morning, therefore caffeine's ability to increase arousal may have been augmented, contributing to the increases in anaerobic power output and fatigue management. Caffeine is also suggested to exhibit a supraspinal excitatory effect, which may enhance motor unit recruitment and rate coding (3). Improved recruitment and rate coding of the large lower-body muscle groups involved in sprint cycling may therefore partially explain the results for improved anaerobic power output in the current study (Figure 1).

Caffeine has also been associated with reductions in perceived effort, which may be a contributing factor to the improvement in muscular fatigue (Figure 2), as Doherty et al., (17) found participants rate of perceived of exertion to be significantly reduced during a cycling time trial, following caffeine ingestion ($P < 0.05$). The decrease in perception of effort due to caffeine may be caused by a reduced amount of neural activation needed for any changes in motor neurons or muscle contraction force, which therefore leads to a decrease in muscle sensory processing, whilst more motor units are still being employed alongside an enhanced force stimulus (53).

It has been suggested that a motor schema may play a role in the association between caffeine and muscle power (7). Bloms et al. (7) tested the effect of caffeine on muscle power among a cohort of athletes and reported significant increases in performance. Conversely, Gauvin (20) reported no effects of caffeine ingestion on muscle power in a group with no previous experience in the exercise test. The subgroup analysis for training status indicated a significant effect for athletes, but not for non-athletes (30). Grgic et al. (30) suggest that studies should control for the effect of training by including only participants with or without previous experience in the task. This is supported by the work of Collomp et al. (12), who reported a greater performance enhancement in trained athletes for supramaximal sprint exercise, following caffeine ingestion. In addition, Boyett et al. (8) suggest that trained athletes are more likely to derive ergogenic effects from caffeine in the morning. Participants in the current study were regular participants in high-intensity sprint cycle training and conducted testing in the early morning, both of which may have magnified the effects of the caffeine in the pre-workout supplement, contributing to the positive effects seen in the results for anaerobic power output and fatigue management.

The main body of previous research into the effects of citrulline malate has been conducted using resistance training protocols, with only two studies investigating its effects on high intensity cycling. Glenn, et al., (22) reported that an acute dose of citrulline malate (8g) significantly improved anaerobic cycling peak power ($p < .001$), compared with a placebo. Such improvements may be due to an increased blood flow caused by vasodilation of the blood vessels, which is commonly associated with the supplement (5), which in turn may increase exercise capacity during repeated sprints. Contrary to this, Cunniffe et al. (13) reported that an acute dose of citrulline malate (12g) produced no significant differences in peak power, mean power or fatigue index during repeated sprint cycling. The majority of citrulline malate studies ingest a dose of 6-8g 60 min prior to exercise (25, 59). The minimum effective dose appears to be 3g of L-citrulline, while the maximum effective dose may be as high as 10-15g. Citrulline malate products often provide a 1:1 or 2:1 ratio of citrulline to malate. Studies reporting ergogenic effects with citrulline malate tend to provide a 6 to 8g dose, providing at least 3g of L-citrulline at either ratio (25, 59). The level of citrulline malate used in the supplement in the present study was 3g, provided at a 2:1 ratio (2g of L-citrulline and 1g Mallate). This is significantly lower than the majority of research which previously demonstrated the benefits of citrulline malate ingestion across a variety of exercise modalities (25, 59).

There appears to be no previous research investigating the performance effects following a single low dose ingestion of citrulline malate. Whilst the results of the present study demonstrate significant improvements in anaerobic power output and fatigue management, following ingestion of a multi-ingredient pre-workout supplement containing a low dose of citrulline malate, it is difficult to ascertain how much of this improvement can be attributed to the citrulline malate in the multi-ingredient pre-workout supplement. Based on the fact that previous studies reporting positive effects of citrulline malate have used a dose of at least twice that used in the present study, one may conclude that the citrulline malate in the multi-ingredient pre-workout supplement had minimal impact on the improvements seen in anaerobic power output and fatigue management during the repeated sprint cycle exercise. Further research investigating the effects of isolated acute low dose ingestion of citrulline malate, rather than in the form of a multi-ingredient supplement, is warranted. In addition, there is scope for further research into the performance effects of an extended ingestion of a low dose of citrulline malate over a period of weeks, although preliminary research into this area suggests no benefit (37).

β-alanine ingestion has been reported to improve muscle carnosine concentrations (34, 56), aiding fatigue, influencing muscle pH level and increasing exercise work capacity (60). The impact of β-alanine ingestion on exercise performance is affected by the duration of the exercise protocol, with short duration exercise (≤ 0.5 min) demonstrating no benefit from β-alanine supplementation (d = 0.040), while improvements in moderate duration exercise (0.5-10 min) were significant (50). It is plausible therefore that the short duration sprint exercise (6s) used in the present study, may have been too short to have benefited from β-alanine ingestion. However conversely, the repeated sprint nature of the protocol used in the present study extended the total protocol duration (36s) within the timeframe where β-alanine ingestion has shown positive benefits. However, the body of research reporting benefits of β-alanine ingestion on exercise are based on chronic ingestion protocols over a period of 4-12 weeks (34, 50). In addition, these protocols ingest doses in the region of 3.2-6.4 g·day^{-1}, whereas the level of β-alanine in the current multi-ingredient pre-workout supplement was just 1.6g. A dose of this level (1.6g) may have a benefit for increased muscle carnosine levels, however only when ingested every day for 2-weeks (56). General advice for individuals looking to supplement with β-alanine, is to supplement daily for a minimum of 2-4 weeks at a dose of 3.2-6.4 g·day^{-1} ingested at several timepoints throughout the day (0.8-1.6g every 3-4h) to avoid acute side-effects (50). It is unlikely therefore that any of the improvements in anaerobic power output and fatigue management during the repeated sprint cycle exercise in the present study can be attributed to the single acute dose of β-alanine. This is supported by Glenn et al., (23), who reported no significant performance or physiological benefits when cyclists ingested a single dose of β-alanine.

Creatine supplementation is widely researched, often demonstrating positive improvements in repeated sprint performance trials (14, 52). However, in order to facilitate an ergogenic effect following creatine supplementation, a chronic loading phase is recommended, commonly in the region of 20-30 g.day^{-1} for 5-7 days (6). Post loading phase, lower doses (2-3 g.day^{-1}) may act as a maintenance dose prolonging ergogenic benefits (36, 2, 46). It is unlikely therefore, that the

single acute low dose of creatine (2g) provided in the multi-ingredient pre-workout supplement used in the present study, was responsible for the improvements seen in anaerobic power output and fatigue management during the repeated sprint cycle trials. There appears to be no previous comparable research investigating the performance effects following a single low dose ingestion of creatine, therefore further research investigating this area may be warranted. The low level of creatine used in the pre-workout supplement, may provide a positive benefit to repeated sprint performance if the pre-workout supplement is taken daily for an extended period of time, with Hultman et al. (36) suggesting that a low dose of around this level ingested daily for 28 days was as effective at raising creatine levels as protocols using chronic loading phases of higher doses for shorter periods. Future research may wish to investigate the chronic effect of daily pre-workout supplement ingestion on exercise performance.

The results of the present study will be of direct interest for cyclist coaches and athletes, due to the importance anaerobic power output and muscular fatigue can have on performance (33). A high anaerobic power output has also been associated with elite rugby, basketball and volleyball athletes, with coaches deeming muscle power an important component of physical fitness (39). Increased anaerobic power output could provide a performance benefit during competition, whilst training at an increased anaerobic power output may provide the foundation for greater adaptation, thus potentially leading to a greater sporting performance. Delaying the onset of fatigue would also be practically important for many athletes. A cyclist's ability to maintain high levels of force production for as long as possible can significantly improve cycle performance by having an effect on sprint time (16) and optimal pedal rate (58). Results of the current study demonstrate a multi-ingredient pre-workout supplement improves repeated sprint endurance, therefore sports involving multiple sprints such as football, basketball and other team sports may also draw interest from the outcomes of this study.

Table 4. Likely importance of the individual pre-workout supplement ingredients to the positive improvements in anaerobic power output and muscular fatigue.

Ingredient	Summary / Likely outcome effect
Caffeine	Numerous past research reports positive effect on lower body power tests. *Likely effect = positive impact on findings*
Citrulline Malate	Low dose compared to past research. *Likely effect = minimal impact on findings*
β-alanine	Low dose compared to past research. Only chronic ingestion appears to benefit. Chronic low dose may benefit. *Likely effect = minimal impact on findings*
Creatine	Low dose compared to past research. Only chronic ingestion appears to benefit. Chronic low dose may benefit. *Likely effect = minimal impact on findings*

The present study did not monitor RPE during the testing protocol, RPE measures during repeated sprint cycling across the three conditions, may have added further support to the

potential benefits of the pre-workout supplement. This may have provided further evidence for a potential positive effect of caffeine on repeated sprint cycling performance.

In conclusion, acute supplementation of a multi-ingredient pre-workout supplement containing Caffeine, β-alanine, Creatine and Citrulline Malate significantly augments anaerobic power output and attenuates muscular fatigue during repeated sprint cycling. The most likely cause of this outcome is the influence acute caffeine intake has as an ergogenic aid (24, 29). The remaining ingredients in the supplement are unlikely to have influenced the results, due to requiring a higher dose than used in the pre-workout supplement and/or a chronic loading phase to create performance benefits (6, 34, 51, 23, 25, 59). The results of this study will be of benefit to recreational and competitive cyclists, along with other sports where an increase in peak power and reductions in fatigue during repeated sprint tasks are important.

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