EFFECTS OF CREATINE, COFFEE, AND CAFFEINE ANHYDROUS ON STRENGTH AND SPRINT PERFORMANCE

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ABSTRACT

Eric T. Trexler: Effects of Creatine, Coffee, and Caffeine Anhydrous on Strength and Sprint Performance
(Under the direction of Abbie E. Smith-Ryan)

The current study sought to directly compare effects of caffeine-matched (300 mg) doses of caffeine anhydrous (CAF) and coffee (COF) on strength and sprint performance, and to determine if CAF or COF intake modulate the effects of creatine (CRE) loading. Resistance-trained males (n=54) completed baseline tests of strength and sprint performance, with post-tests occurring after acute and chronic supplementation periods. COF ingested 30 minutes pre-exercise improved leg press one-rep max to a greater extent than CAF (32.2±18.6 vs. 15.3±16.9 lbs; $P=0.03$), while both attenuated total work and peak power reductions compared to placebo ($P\leq0.05$). CRE loading (five days) did not improve strength or sprint performance compared to placebo ($P>0.05$). Effects of CRE were not influenced by chronic COF or CAF intake, but the combination of CRE+CAF resulted in mild gastrointestinal discomfort. Results may inform supplement dosing and timing strategies for concurrent use of CRE with CAF or COF.

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LIST OF ABBREVIATIONS

1RM	One-repetition maximum
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
BP	Bench press
CAF	Caffeine anhydrous
COF	Coffee
CRE	Creatine
CRN	Creatinine
HRT	Muscle half-relaxation time
LP	Leg press
PCr	Phosphocreatine
PLA	Placebo
PP	Peak power
RTF	Repetitions to fatigue
TW	Total work

CHAPTER I: INTRODUCTION

Use of nutritional supplements as ergogenic aids is widespread. In a survey of 207 division I collegiate athletes, 89% reported that they had previously used nutritional supplements (1). While many nutritional supplements lack data supporting their safety or efficacy, a number of supplements are considered safe, legal, and have ample evidence to support their efficacy. Two of the most commonly used and frequently studied ergogenic aids, which have been shown to improve exercise performance, are creatine and caffeine.

Creatine

Creatine is synthesized endogenously in the liver, kidneys, and pancreas by combining arginine, glycine, and methionine, with endogenous production yielding roughly one gram of creatine per day (2). Ninety-five percent of the creatine pool is stored within skeletal muscle, with the remaining 5% distributed throughout the liver, kidneys, brain, and testes (3). Sixty percent of the creatine pool is stored as phosphocreatine (PCr), while 40% is stored as free form creatine. Although the average 70 kg male has a creatine pool of 120-140 grams (4), creatine storage is affected by a number of factors including muscle mass, skeletal muscle fiber type, and dietary intake (3, 5-7). The most common dietary sources of creatine are meats, so vegetarians typically have a smaller pool of stored creatine (6).

The most widely used and studied form of supplemental creatine, creatine monohydrate, has been shown to increase the amount of creatine stored in skeletal muscle (3, 7, 8). A popular protocol of creatine supplementation is known as *creatine loading*, in which participants

consume 20 grams of creatine per day, split between four equal doses, for a period of 4-7 days (4). The loading phase is followed by a maintenance phase of 3-6 grams per day. Conversely, creatine supplementation can be efficacious without a structured loading phase. Ingesting a regular maintenance dose of 3-6 grams per day can effectively increase creatine storage, but saturation may take up to 3-4 weeks (9). Despite concerns over potential side effects, numerous studies have demonstrated the safety of creatine supplementation, and no deleterious side effects have been reported in controlled research (10).

Creatine supplementation has been consistently shown to increase muscle creatine storage, with a concomitant improvement in high intensity exercise performance. Previous studies have shown creatine supplementation to increase strength, single sprint performance, and repeated sprint performance (11). A minority of previous studies have failed to reveal a benefit from creatine supplementation (11-13), but such results may be attributed to ineffective dosing protocols, characteristics of the exercise tests employed, or a high prevalence of *nonresponders* in the selected samples (2). In high-intensity exercise, the rapid need for adenosine triphosphate (ATP) regeneration is accommodated, in part, by the creatine kinase reaction in which PCr rephosphorylates adenosine diphosphate (ADP). By increasing PCr storage, creatine supplementation increases ATP availability by enhancing the rate of ADP rephosphorylation (2), enhancing high-intensity, short-duration exercise (14). Rephosphorylation of ADP via the creatine kinase reaction also involves the consumption of a hydrogen ion (H^+) (15), indicating that enhanced H^+ buffering may be one mechanism contributing to creatine's effect on anaerobic exercise. Research in aerobic exercise has been less promising (14), as activities with longer duration rely more on oxidative phosphorylation for energy production and increases in total body weight may decrease locomotive efficiency.

Previous literature has also reported that creatine supplementation is advantageous for skeletal muscle hypertrophy. By enhancing PCr availability and ATP resynthesis, long-term supplementation allows for greater training intensity and work completed throughout a training program (16). Further, creatine supplementation has been shown to increase insulin-like growth factor 1 (IGF-1) (6, 17), satellite cell proliferation (18), and myogenic transcription factors such as myogenic regulatory factor 4 (MRF-4) (19, 20). These mechanisms are likely to contribute to the hypertrophic effect of creatine supplementation in combination with resistance training; this long-term hypertrophic response may potentially add to the ergogenic effect described in high-force, short-duration exercise. Weight often increases by 1-2 kg in response to short-term creatine loading (11). While the initial increase in total body weight is likely due to minor water retention, further increases in fat-free mass (FFM) are attributed to hypertrophic adaptations (11).

Caffeine

Caffeine (1,3,7-trimethylxanthine) is the most widely consumed psychoactive substance in the world (21), found in many common foods and beverages. Caffeine is also a commonly used ergogenic aid for endurance and strength athletes (1, 22). Caffeine's effect on endurance exercise has been studied extensively, with the majority of data indicating an ergogenic effect. Acute consumption of caffeine has been consistently shown to improve performance and increase time to exhaustion in endurance exercise (23-27). In previous research, doses commonly range from 3-9 mg of caffeine per kg of total body mass (mg/kg), when caffeine is ingested 30-60 minutes prior to exercise to allow caffeine levels in the bloodstream to reach peak values (28, 29). Caffeine may affect performance via both peripheral and central mechanisms. While caffeine's role as an adenosine antagonist is often considered its primary ergogenic mechanism, caffeine has also been shown to increase β-endorphin secretion, increase free fatty acid (FFA)

utilization, spare glycogen, increase circulating cortisol and epinephrine, and may peripherally affect neuromuscular function (21, 30).

Caffeine's effects on resistance exercise and sprint performance have been far less consistent. Multiple studies have found no benefit of caffeine consumption on anaerobic sprint performance in untrained participants (31-34), but contrary findings have been reported with moderately or highly trained participants (35-39). An ergogenic effect of caffeine on bench press endurance has been reported (39), but other studies have reported contrary findings (40-42). Similarly, studies investigating changes in bench press one-rep max (1RM) after caffeine ingestion have reported mixed results (40, 42). Previous investigations have revealed no significant improvements in 1RM or endurance for the leg press or leg extension exercises following acute caffeine intake (39, 40, 42). More research is needed to determine if caffeine induces an ergogenic effect in maximal strength and muscular endurance.

Coffee is a well-known and commonly consumed source of caffeine (22, 43). A cup of coffee yields roughly 65-110 mg per cup (44), prompting researchers to investigate its use as a caffeine source with ergogenic potential. The acute ingestion of coffee prior to exercise has been shown to enhance running performance (45). Although prior coffee consumption does not blunt the ergogenic effect of subsequent caffeine anhydrous intake (46), data from Graham et al. (47) indicate that caffeine anhydrous supplementation may improve running performance when compared to a caffeine-matched dose of coffee. To date, the comparative effects of coffee and caffeine have not been studied in strength or sprint performance.

Co-ingestion of Creatine and Caffeine

Creatine and caffeine are currently among the most popular nutritional ergogenic aids, with evidence indicating potential ergogenic effects for strength and sprint performance. Accordingly, supplements containing both ingredients have become popular in athletes participating in anaerobic exercise (48-50). Despite the popularity of such supplements, research on the concurrent use of caffeine and creatine is limited.

Vandenberghe et al. (51) reported that creatine loading increased dynamic torque production, but that the ergogenic effect of creatine was negated by chronic caffeine supplementation. Interestingly, chronic caffeine intake did not appear to influence muscle PCr saturation in response to creatine supplementation (51). Creatine supplementation was later shown to decrease muscle relaxation time, whereas chronic caffeine consumption increased relaxation time (52). These findings may indicate a potential interaction between chronic caffeine supplementation and creatine loading, as combined supplementation with both creatine and caffeine resulted in no significant change in relaxation time (52). Some authors have dismissed a potential interaction between creatine and chronic caffeine ingestion because previous creatine studies have administered the supplement dissolved into coffee or tea (53-55). This information, in combination with the findings of Vandenberghe et al. (51), may suggest that chronic caffeine anhydrous supplementation unfavorably affects creatine loading in a manner that is not replicated by coffee or tea. Differential effects may be explained by the numerous bioactive compounds that make coffee and tea markedly distinct from a dose of caffeine anhydrous (56, 57). Given the popular use of both creatine and caffeine in anaerobic athletes, further research is warranted to determine if chronic coffee or caffeine anhydrous consumption blunt the ergogenic effect of creatine loading on strength and sprint performance outcomes.

Purpose

1. The primary purpose of this study was to compare the effects of acute coffee and caffeine anhydrous ingestion on strength and sprint performance.
2. A secondary purpose was to determine if chronic coffee or caffeine anhydrous ingestion influence the ergogenic effects of creatine loading on strength and sprint performance.

Research Questions

1. Does acute caffeine ingestion improve strength and repeated sprint performance?
    a. In the context of strength and repeated sprint performance, how do the effects of acute coffee ingestion and acute caffeine anhydrous ingestion compare?
2. Does chronic consumption of caffeine anhydrous or coffee impact the ergogenic effect of creatine loading?

Hypotheses

1. Acute caffeine anhydrous ingestion would improve the given measures of strength and sprint performance to a greater extent than the coffee or placebo conditions.
2. Creatine loading would increase 1RM strength and repetitions to fatigue for leg press and bench press, as well as peak power and total work output during a repeated sprint protocol.
    a. Chronic caffeine anhydrous ingestion would inhibit the ergogenic effects of creatine loading.
    b. Chronic coffee ingestion would have no impact on the ergogenic effect of creatine loading.

Delimitations

1. Participants were moderately trained males, aged 18-35 years, with body mass between 60 to 100 kg.
2. The study consisted of four laboratory visits.
3. The duration of chronic supplementation for coffee, caffeine anhydrous, and creatine monohydrate was five days.
4. Participants did not consume performance-enhancing supplements containing creatine or beta-alanine for at least three months prior to the study.
5. Participants abstained from caffeine and/or coffee intake for at least 48 hours prior to exercise testing, and throughout the duration of chronic supplementation.
6. Participants were excluded if dietary analysis or pre-screening questionnaire answers indicate a sensitivity or history of adverse reactions to caffeine consumption, daily caffeine consumption in excess of 800 mg, or consumption of any food, supplement, or drug in amounts that may significantly influence the CYP1A2 isozyme.

Limitations

1. Participants were recruited from classes within the Department of Exercise and Sport Science and through fliers located near fitness facilities on the campus of the University of North Carolina at Chapel Hill (UNC-CH). Therefore, the sample was not selected in a truly random manner.
2. Results may not be applicable to females, or individuals below 18 or above 35 years of age.
3. Baseline and post-supplementation values of muscle creatine saturation were not directly measured.

Assumptions

Theoretical

1. Participants provided accurate information on all pre-screening materials, including questionnaires for health history, exercise and nutrition status, and 3-day diet logs.
2. Participants gave maximal effort during exercise testing.
3. Participants adhered to the supplementation protocol.
4. Participants complied with pre-testing instructions.
5. Participants maintained normal training and nutritional habits throughout the intervention.

Statistical

1. The sample was drawn from a population that is normally distributed.
2. Treatment groups were randomly assigned.
3. Variability was approximately equal in the selected sample (homogeneity of variance).

Operational Definitions

1. *Acute caffeine supplementation:* Oral administration of caffeine, commonly 3-5 mg/kg, 30-60 minutes prior to testing.
2. *Caffeine anhydrous (CAF):* The supplemental form of caffeine, commonly available as a powder or capsule (30).
3. *Chronic supplementation:* Daily oral administration of a supplement continued over multiple days. The chronic phase of the current study had groups ingesting caffeine anhydrous, coffee, creatine, and/or placebo treatments daily for 5 consecutive days.

4. *Coffee (COF):* A commonly consumed caffeinated beverage prepared from roasted coffee beans (58). Coffee is the most common source of caffeine consumed by adults in the United States (43).
5. *Creatine loading (CRE):* A creatine dosing protocol aimed at rapidly increasing muscle creatine storage. A typical protocol consists of 20 g/day, split evenly between 4 doses, administered for a period of 4-7 days (4).
6. *Creatine monohydrate:* The most widely used and researched form of supplemental creatine, used to enhance muscle creatine storage (2).
7. *Creatine nonresponder:* An individual who sees muscle creatine storage increase by < 10 mmol/kg dry weight in response to creatine supplementation, and is unlikely to obtain an ergogenic benefit from creatine supplementation (7, 55).
8. *Moderately trained:* Participants were considered moderately trained if they had regularly participated in resistance training for at least 30 minutes, 3 times per week, for at least 3 months prior to the study.
9. *One repetition maximum* (1RM): The greatest load with which an individual can complete one full repetition of a resistance exercise.
10. *Phosphagen energy system:* Energy system with the capacity to rapidly generate ATP. Phosphocreatine promotes the rephosphorylation of ADP via the creatine kinase reaction, resulting in the production of ATP and Cr (15).
11. *Phosphocreatine (PCr):* The phosphorylated form of creatine, which constitutes 60% of the stored creatine pool and contributes to ATP production via the creatine kinase reaction (2).

12. *Repetitions to fatigue (RTF):* The number of repetitions performed in a maximal set of a given resistance exercise. Used as an indicator of muscular endurance during a single maximal set of high-intensity (80% 1RM) resistance exercise. Repetitions conformed to standardized criteria to ensure proper form and safety, as determined by trained lab personnel.

Significance of study

This study aimed to evaluate the comparative effects of acute coffee and caffeine anhydrous intake on strength and repeated sprint performance. Previous studies on caffeine anhydrous have reported mixed results, and the comparative effects of coffee and caffeine anhydrous have not been investigated in the context of anaerobic exercise. This study also sought to determine if chronic caffeine anhydrous or coffee intake impact the ergogenic effect of creatine loading. While previous research has suggested a potential interaction between caffeine anhydrous and creatine supplementation, replication of these results is needed. Results of the current study may have important implications for the use and formulation of nutritional supplements for athletes engaged in anaerobic sports.

CHAPTER II: REVIEW OF LITERATURE

Nutritional supplementation is a common practice among competitive athletes (1). Creatine and caffeine are currently among the most commonly used nutritional ergogenic aids (1, 22), with ample evidence supporting their safety and efficacy. Despite concerns over potential adverse effects on hydration and renal function, evidence has shown creatine supplementation to be a safe practice (10). Similarly, consumption of moderate doses of caffeine (<400 mg) is not associated with adverse side effects (59), and average caffeine intake of adults in the United States may be as high as 4 mg/kg (43), equating to 280 mg/day for a 70-kg individual. Most athletic federations and organizations, including the World Anti-Doping Agency (60), do not consider creatine or caffeine to be banned substances.

Creatine supplementation increases muscle PCr storage, with a concomitant improvement in high-intensity exercise. Although some studies have failed to demonstrate an ergogenic effect (12, 13), the majority of studies investigating creatine supplementation have documented improvements in resistance exercise and sprint performance (11). Caffeine's effects on aerobic exercise have been studied extensively, but significantly less research has been conducted to investigate its impact on anaerobic exercise. Although promising findings have been reported (35-39), studies involving resistance exercise and sprint performance outcomes have reported conflicting results (31, 33, 39-42). While preliminary research (47) suggested that caffeine anhydrous may improve aerobic exercise performance to a greater extent than a caffeine-matched dose of coffee, these findings have recently been challenged (61). To date, authors of

the current review are unaware of any research comparing caffeine anhydrous and coffee in the context of anaerobic exercise.

Recently, many nutritional supplements have been formulated to include both caffeine and creatine (48-50). Although a number of previous studies on creatine have mixed the supplement in caffeinated coffee or tea (53-55), there is evidence suggesting a potential interaction between creatine and caffeine when supplemented concurrently (51). Such an interaction may relate to opposing effects on muscle relaxation time (52), but more research on the topic is warranted. The current review aims to evaluate the ergogenic potential of creatine and caffeine supplementation in anaerobic exercise. Research pertaining to the concurrent use of caffeine and creatine will also be discussed, along with direct comparison of caffeine and coffee as ergogenic aids.

Creatine

Creatine is synthesized endogenously in the liver, kidneys, and pancreas, with daily production yielding roughly 1 gram per day (2). Creatine is formed by combining arginine, glycine, and methionine in a series of reactions catalyzed by L-arginine:glycine amidinotransferase, guanidinoacetate methyltransferase, and methionine adenosyltransferase (2). Ninety-five percent of the creatine pool is stored within skeletal muscle (3); 60% of the creatine pool is stored as PCr, while 40% is stored as free form creatine. The average 70 kg male has a creatine pool of roughly 120-140 grams (4), but creatine storage is affected by a number of factors including muscle mass, skeletal muscle fiber type, and dietary intake (3, 5-7).

High-intensity exercise requires the rapid generation of large amounts of force. Sustaining high power outputs requires large quantities of adenosine triphosphate (ATP); during intense exercise, ATP demand can rise 1,000 times higher than resting levels (15). Because

intramuscular ATP storage is limited to roughly 8 mmol/kg wet weight of muscle (15), the urgent demand for ATP regeneration must be accommodated, in part, by PCr. By way of the creatine kinase reaction, PCr phosphorylates ADP to generate ATP for continued crossbridge cycling. Creatine supplementation therefore increases ATP availability by enhancing the rate of ADP rephosphorylation, enhancing performance in high-intensity, short-duration exercise (14). While creatine's primary ergogenic mechanism relates to its contribution to the phosphagen energy system, it may also increase the rate at which calcium (Ca^{2+}) is taken up by the sarcoplasmic reticulum, allowing more rapid crossbridge detachment and force production (2). It has been proposed that shortened muscle relaxation time may contribute to the ergogenic effects observed with creatine loading (52), although other research has shown no effect on relaxation time (62). Further, the creatine kinase reaction consumes a hydrogen ion in the process of rephosphorylating ADP (15), indicating a potential enhancement of pH buffering with creatine supplementation.

Creatine and strength outcomes

Since creatine supplementation's boost in popularity in the 1990's, hundreds of studies have investigated its ergogenic potential. The majority of studies (>70%) have reported statistically significant performance improvements in high-intensity exercise, such as resistance training (11). Short-term creatine loading has been shown to enhance resistance exercise performance, primarily by enhancing the phosphagen energy system. Volek et al. (63) documented improvements in bench press repetitions and peak power output after 7 days of creatine loading. Izquierdo et al. (64) reported increases in half-squat and bench press repetitions to fatigue, along with half-squat one-rep max, with five days of loading. It appears that improvements in maximal strength and resistance exercise performance are not only attributed to

the acute augmentation of PCr storage, but also by a chronic improvement in the quality of training sessions. In a 12-week study, Volek et al. (8) found that creatine supplementation led to statistically significant improvements in body mass, fat free mass, bench press, squat, muscle fiber cross-sectional area, and bench press volume during training sessions. Similarly, Aguiar et al. (65) found that 12 weeks of creatine supplementation improved training volume, bench press, knee extension, bicep curl, muscle mass, fat-free mass (FFM), and performance in submaximal-strength functional tests. By enhancing PCr availability and ATP resynthesis, long-term supplementation allows for greater training intensity and work completed throughout a training program (16). The chronic elevation of PCr storage allows for greater training volume in exercise programs, augmenting adaptations to resistance training.

Other mechanisms may also contribute to creatine's effects on resistance training adaptations. Creatine supplementation has been shown to increase insulin-like growth factor 1 (IGF-1) (6, 17), satellite cell proliferation (18), and myogenic transcription factors such as myogenic regulatory factor 4 (MRF-4) (19, 20). These mechanisms may contribute to the hypertrophic effect of creatine supplementation in combination with resistance training, and may enhance training adaptations to resistance training. More research is needed to determine the extent to which these mechanisms contribute to creatine's ergogenic effect. Weight often increases by 1-2 kg in response to short-term creatine loading (11), primarily due to water retention. Beyond this initial weight gain, further increases in FFM are likely attributed to hypertrophic adaptations (11).

Creatine appears to impart ergogenic benefits in a wide range of populations, including both males and females, trained and untrained participants, and a variety of age groups from children to the elderly (11). The preponderance of data indicate that creatine is an effective

ergogenic aid in the context of strength and resistance exercise performance, and applicable to a variety of populations. A meta-analysis by Branch (14) concluded that creatine supplementation improves isotonic strength (effect size, ES ≈ 0.44), one-rep maximum (1RM) strength (ES = 0.32), and repetitions lifted (ES = 0.64) in exercise lasting ≤30 seconds, indicating applications for a variety of resistance training programs and repetition ranges.

Creatine and sprint performance

Given creatine's critical role in the phosphagen energy system, creatine supplementation has been identified as a promising method of improving high-intensity sprint performance. To date, research has documented improvements in both single and repeated sprint performance after creatine supplementation. In highly trained male soccer players, creatine loading improved performance in a repeated sprint test (66). These findings are supported by those of Theodorou et al. (67), who found that creatine loading enhanced interval sprint times in elite swimmers. Preen et al. (68) investigated the effects of creatine supplementation in active, but not well-trained, male participants. Results indicated that creatine loading increased total work done and peak power in an extended repeated sprint test on a cycle ergometer. In agreement with the aforementioned studies, Skare et al. (69) showed that creatine supplementation improved performance in a series of 6 intermittent 60-meter sprints. Further, swimming velocity was improved in a single 100-meter sprint (69), indicating that the performance benefits of creatine supplementation are not exclusively limited to repeated sprint performance.

Conversely, other studies have reported no benefit of creatine supplementation on sprint performance. McKenna et al. (70) found no effect of creatine loading on an intermittent sprint test consisting of five 10-second sprints with variable recovery times between sprints. Finn et al. (71) examined the effects of creatine loading on a sprint test consisting of four 20-second sprints,

interspersed with 20-second rest periods. Despite trends toward improvement in 1-second peak power, 5-second peak power, and fatigue index, no performance measures improved to a statistically significant degree (71).

Despite conflicting results, the majority of data indicate an ergogenic effect of creatine in both single and repeated sprint performance. Roughly 70% of studies investigating creatine supplementation have reported an ergogenic effect, especially when measuring performance in high-intensity exercise (11). A meta-analysis by Branch (14) concluded that creatine supplementation produces an ergogenic effect in exercise bouts lasting less than 30 seconds, with an effect size of 0.24. Studies reporting negative results have often had methodological limitations including low statistical power, failure to control for non- or quasi-responders, failure to sufficiently familiarize participants to the exercise test prior to baseline testing, or exercise test protocols that are not sensitive to enhancement of the phosphagen energy system. Overall, the available data indicate that creatine supplementation improves sprint performance, particularly in repeated bouts lasting less than 30 seconds each.

Caffeine

Caffeine (1,3,7-trimethylxanthine) is the most widely consumed psychoactive substance in the world (21), found in many common foods and beverages. Caffeine is also a commonly used ergogenic aid (22), with potential applications in both aerobic and anaerobic sports. Antagonism of adenosine receptors is often considered caffeine's primary ergogenic mechanism (30). Caffeine may affect performance via both peripheral and central mechanisms by altering pain and effort perception (72), the release of calcium from the sarcoplasmic reticulum (73), and the activity of Na^+/K^+ ATPase pumps (74). Caffeine has also been shown to increase β-endorphin secretion, increase free fatty acid (FFA) utilization, spare glycogen, and increase circulating

epinephrine (21, 30); more research is needed to determine the extent to which these mechanisms contribute to the ergogenic effect of caffeine.

Caffeine and strength outcomes

While caffeine has been shown to consistently improve aerobic exercise performance (30), research investigating caffeine's effect on strength performance is mixed. Although Woolf et al. (39) reported an ergogenic effect of caffeine on chest press endurance, no improvement in leg press endurance was observed. Beck at al. (40) documented an improvement in bench press 1RM, but no improvement in bench press endurance, leg extension 1RM, or leg extension endurance. Subsequent research by Woolf et al. (41) revealed no significant effect of caffeine on bench press endurance, and Astorino et al. (42) did not find any significant improvements in bench press or leg press 1RM or endurance after acute caffeine ingestion. While the available data appear to be fairly equivocal, a number of positive outcomes were observed but failed to reach statistical significance. Although Woolf et al. (39) did not demonstrate a statistically significant increase in total weight lifted in a single maximal set of leg press ($P = 0.09$), 78% of participants saw an improvement of some magnitude following caffeine supplementation, and participants lifted 1,262 more pounds after caffeine ingestion compared to placebo. While a subsequent study by Woolf et al. (41) found no significant effects on bench press, 40-yard dash, or 20-yard shuttle, improvements were observed after caffeine ingestion in 59%, 59%, and 47% of participants, respectively. Astorino et al. (42) did not find any significant improvements in performance, but did report nonsignificant increases in bench press 1RM, leg press 1RM, and total weight lifted in both leg press and bench press.

Currently, the body of evidence does not conclusively demonstrate a consistent ergogenic effect of caffeine intake on strength outcomes. A mixture of positive and nonsignificant findings

suggest that further research on the topic is warranted. Inconsistent findings may be explained by insufficient sample sizes, variable responses between individuals, or specific methodological considerations regarding caffeine dosing and exercise protocols employed. Interestingly, significant findings are more commonly found in upper body strength compared to lower body strength; to date, a mechanism underlying this observation has yet to be identified. In light of mixed findings, more research is required to elucidate the effect of caffeine on strength outcomes.

Caffeine and sprint performance

Caffeine's effects on sprint performance have also been inconsistent in previous literature. A number of studies have found no benefit of caffeine consumption on anaerobic sprint performance (31-34), but many positive findings have been reported (36, 38, 39, 75). Discrepant findings in sprint performance may be attributed, in part, to the training status of study participants.

In studies with relatively untrained participants, caffeine has failed to improve sprint performance. Collomp et al. (31) investigated the effects of caffeine (5 mg/kg) on Wingate test performance. Despite altering catecholamine and blood lactate levels, no effect on performance was observed. Similarly, Lorino et al. (34) did not find any improvement in Wingate performance or an agility test following acute ingestion of caffeine (6 mg/kg). Negative findings have also been reported in the context of repeated sprint performance. Crowe et al. (32) found no ergogenic effect of caffeine (6 mg/kg) on a test consisting of two 60-second maximal cycling bouts; rather, the observed increases in time to peak power and blood lactate concentrations could be considered unfavorable effects on anaerobic performance. Greer et al. (33) administered caffeine (6 mg/kg) prior to a series of four Wingate tests, with 4-minute rests between sprints.

While there was no effect on sprint performance in the first two Wingates, an ergolytic effect was observed in sprints three and four (33). Despite unfavorable outcomes in untrained participants, caffeine has improved sprint performance in moderately and well-trained subjects. In addition to improved bench press performance, Woolf et al. (39) reported greater peak power in the Wingate test following 5 mg/kg of caffeine ingestion in trained athletes. Schneiker et al. (38) reported that 6 mg/kg of caffeine improved performance on a test consisting of prolonged, intermittent sprints lasting four seconds each in male team-sport athletes. In trained cyclists, Wiles et al. (75) found that 5 mg/kg of caffeine improved performance time, mean power, peak power, and mean speed in a 1-km time trial.

In light of these mixed findings, more research on caffeine's effects on sprint performance is warranted. However, the available evidence seems to indicate that caffeine's potential to improve sprint performance is dependent on training status. Accordingly, Collomp et al. (36) found that 250 mg of caffeine improved 100 meter sprint velocity in trained swimmers, with no improvement in untrained swimmers. While the authors speculate that the differential response may be due to an enhanced ability to buffer hydrogen ions in trained participants (36), it has been suggested that this difference may be attributed to greater variability in exercise performance in untrained participants (30). More research is needed to conclusively identify the underlying mechanisms of this observed difference. For trained athletes, the available evidence suggests that caffeine can improve both single and repeated sprint performance.

Comparison of caffeine and coffee

Caffeine is found in a number of common foods and beverages, including chocolate, soda, tea, and coffee. Coffee provides a relatively concentrated dose of caffeine, yielding 65-110 mg per cup (44). In athletes, coffee is a commonly used caffeine source prior to competition (22); accordingly, researchers have investigated the use of coffee as a practical source of caffeine with ergogenic potential. Costill et al. (76) showed that a dose of coffee yielding 330 mg of caffeine, ingested 60 minutes before exercise, enhanced cycling time trial performance. Further, data from Wiles et al. (45) indicate that acute ingestion of 3 g of coffee improves performance in a 1500-meter run. Although research has demonstrated an acute ergogenic effect from coffee intake, it has been suggested that coffee is less ergogenic than a caffeine-matched dose of caffeine anhydrous. Graham et al. (47) compared the effects of coffee and caffeine, with treatment arms including decaffeinated coffee, regular coffee, decaffeinated coffee + caffeine capsules, caffeine capsules, or a placebo. The caffeine capsule group outperformed all other treatment arms, including the group ingesting decaffeinated coffee with caffeine capsules. None of the coffee groups performed significantly better than the placebo group, prompting authors to conclude that supplemental caffeine improves performance to a greater extent than a caffeine-matched dose of coffee (47). Authors hypothesized that the differential responses to coffee and caffeine may be attributed to one or more of the numerous biologically active compounds found in coffee (47). One such group of bioactive compounds, known as chlorogenic acids, are present in coffee in varying amounts (77) and may antagonize caffeine's effects on adenosine receptors (78).

Contrary to this hypothesis, McLellan & Bell (46) found that coffee consumption 30 minutes prior to caffeine supplementation does not appear to blunt the ergogenic effect of

subsequent caffeine anhydrous intake. More recently, Hodgson et al. (61) found that caffeine and coffee both improved performance on a 45-minute, energy-based target time trial, with no difference between treatments. This study also directly measured chlorogenic acid content, indicating that 393 mg of chlorogenic acid present in the coffee treatment did not blunt its ergogenic effect on energy-based target time trial performance (61). To date, authors of the current review are unaware of any research investigating the comparative effects of coffee and caffeine in the context of strength or sprint performance. More research is needed to reconcile the discrepant findings regarding coffee and caffeine in endurance performance, and to compare coffee and caffeine in short-duration anaerobic exercise.

Concurrent use of caffeine and creatine

Creatine and caffeine are currently among the most commonly used ergogenic aids, with evidence indicating a potential ergogenic effect for strength and sprint performance. Accordingly, supplements containing both products have become popular in athletes participating in anaerobic sports (48-50). Such multi-ingredient supplements containing both caffeine and creatine have been shown to enhance performance and adaptations to anaerobic exercise. Spradley et al. (50) reported improvements in leg press performance, agility choice reaction performance, and multiple indices of subjective fatigue after the ingestion of a multi-ingredient pre-workout supplement. Smith et al. (49) investigated a similar supplement, consumed prior to exercise for a duration of three weeks. The supplementation, combined with a high-intensity interval training program, resulted in improvements in maximal oxygen consumption, critical velocity, and lean body mass (49). While these results may appear to indicate that creatine and caffeine can be ergogenic when used concurrently, results may be

confounded by the large number of purportedly ergogenic ingredients in the supplements used, including branched-chain amino acids, beta-alanine, and citrulline malate, among others (49, 50).

Controlled research directly investigating any potential synergy or interaction between caffeine and creatine is scarce. Vandenberghe et al. (51) investigated the effect of chronic caffeine supplementation on creatine loading. Treatment arms included a 6-day creatine load (0.5 g/kg per day), a creatine load with concurrent caffeine use on the final three days (5 mg/kg per day), and a placebo treatment. The creatine group saw a significant increase in dynamic torque production, while the groups ingesting a placebo or creatine with caffeine did not (51). Research has indicated that creatine and caffeine do not display pharmacokinetic interactions when ingested together (79). As such, concurrent ingestion of caffeine did not affect muscle phosphocreatine saturation (51), indicating that the blunted ergogenic effect of creatine is not likely attributable to disrupted absorption or uptake into skeletal muscle.

Subsequent research by Hespel et al. (52) sought to determine the effects of creatine and caffeine supplementation on muscle half-relaxation time (HRT). Results indicated that four days of creatine loading (20 g/day) decreased HRT, whereas HRT was increased by chronic caffeine supplementation (5 mg/kg per day for 3 days). Contrary to the creatine-only treatment, the creatine treatment with concurrent caffeine ingestion did not result in a significant reduction in HRT. Finally, acute caffeine ingestion (5 mg/kg, 1 hour prior to testing) did not have a significant impact on HRT (52). Together, these results indicate that chronic creatine and caffeine supplementation impart opposing effects on muscle HRT, offering a potential mechanism to explain the interaction reported by Vandenberghe et al. (51). Gastrointestinal (GI) discomfort may also explain a potential interaction with combined creatine and caffeine supplementation. In 2013, a study by Quesada & Gillum (80) found that GI discomfort was

reported in three of seven subjects receiving creatine and caffeine. In a published abstract, Harris et al. (81) sought to replicate the findings of Vandenberghe et al. (51). Authors found that caffeine did appear to blunt the effects of creatine loading, but attributed results to GI discomfort, which was reported in four of ten subjects receiving both supplements (81). As such, effects on muscle relaxation time and GI discomfort present plausible mechanisms by which chronic caffeine intake may counteract the ergogenic effects of creatine loading.

While research investigating concurrent use of caffeine and creatine is scarce, the data available suggest the possibility of an interaction between the two supplements. Despite this evidence, some authors have dismissed a potential interaction between creatine and chronic caffeine ingestion because many creatine studies have administered the product dissolved into coffee or tea (53-55). Tea and coffee contain a large number of bioactive compounds that distinguish them from a caffeine-matched dose of caffeine anhydrous (56, 57). It is therefore possible that the interaction described by Vandenberghe (51) may not necessarily be replicated by the chronic consumption of coffee or tea; more research is needed to address this research question directly. Given the popular use of both creatine and caffeine in anaerobic athletes, further research is warranted to determine if chronic coffee or caffeine anhydrous consumption blunt the ergogenic effect of creatine loading on strength and sprint performance outcomes.

Conclusion

Creatine and caffeine are popular ergogenic aids that are considered safe at effective doses (1, 10, 59). A large body of research has indicated that creatine is ergogenic for anaerobic exercise, improving both strength and sprint outcomes (14). Caffeine supplementation has been shown to improve sprint performance, but primarily in trained participants (36). Positive effects of caffeine on strength outcomes have been reported (39), but previous studies have failed to

reach statistical significance for a number of the measured strength outcomes (42). Although coffee is a commonly used source of caffeine in athletes (22), direct comparisons of the effects of coffee and caffeine anhydrous on aerobic exercise performance have yielded equivocal results (47, 61), and such research has not been performed in the context of strength and sprint performance. Despite the popularity of both caffeine and creatine, research on their concurrent use is limited. Although previous creatine studies have reported positive findings while mixing creatine into caffeinated beverages like coffee or tea, the scarce data available indicate a potential interaction between chronic supplementation of creatine and caffeine anhydrous (51), with GI discomfort and opposite effects on muscle half-relaxation time identified as potential mechanisms (52). Future research is warranted to evaluate the effects of acute caffeine consumption on strength outcomes, directly compare the effects of caffeine and coffee on anaerobic exercise, and further examine the concurrent use of creatine and caffeine.

CHAPTER III: METHODOLOGY

Experimental Design

The current study employed a double-blind, placebo-controlled experimental design consisting of an acute (Figure 1) and chronic (Figure 2) phase of supplementation. For visit 1, participants were screened for eligibility and completed medical, nutritional, and physical activity history questionnaires. Visit 2 consisted of a blood draw to determine baseline levels of serum creatinine, and strength testing to assess maximal upper- and lower-body strength and repetitions to fatigue at 80% of one repetition maximum (1RM). Ten minutes after strength testing, a repeated sprint test was completed. This exercise testing protocol was completed during visits 2, 3, and 4. Participants were randomly assigned to one of three treatment groups for acute (one-time) supplementation of: 1) caffeine anhydrous, 2) a caffeine-matched dose of caffeinated coffee, or 3) a placebo containing noncaloric flavoring. For visit 3, participants consumed their assigned treatment 30 minutes prior to assessments, which consisted of the same exercise tests completed in visit 2. After a washout period of at least 24 hours, participants were randomly assigned to one of four treatment groups for the chronic (5 day) phase of supplementation: 1) creatine monohydrate, 2) creatine monohydrate and caffeine anhydrous, 3) creatine monohydrate and coffee, or 4) placebo. Participants supplemented with their assigned treatment for 5 days, and returned for a fourth lab visit to complete the test protocol employed in visits 2 and 3, along with a blood draw to measure serum creatinine levels.

Subjects

Male participants (n=56) between the ages of 18 and 35 years were recruited. Power calculations were completed using G*Power 3.1 software (82) based on data from Branch (14), Anselme (35), and Woolf (39), with statistical power and α set *a priori* at 0.8 and 0.05, respectively. Calculations indicated that the intervention would be sufficiently powered with a total sample size of n=50; 56 participants were recruited to account for experimental dropout. Participants were assigned to treatment groups using a random number generator.

Participants were required to be moderately trained, defined by regular participation in resistance exercise for a minimum of 30 minutes, three times per week for the previous three months. Participants were excluded if they had supplemented with beta-alanine or creatine within the previous three months, or if dietary analysis or pre-screening questionnaires revealed habitual caffeine intake of >800 mg per day, a history of sensitivity or adverse reactions to caffeine consumption, or regular consumption of any food, supplement, or drug that may significantly influence the CYP1A2 isozyme. Participants were encouraged to maintain normal diet and exercise habits throughout the duration of the study, but to abstain from caffeine intake for at least 48 hours prior to exercise testing. Exercise testing sessions took place at a similar time of day (± 2 hrs), and participants were instructed to replicate similar food intake before all lab visits to ensure similar dietary habits prior to exercise testing.

Preliminary Testing

Instrumentation

The repeated sprint protocol was performed on a Monark friction-braked cycle ergometer, and peak power and total work output were evaluated using Monark ATS software (Monark, Stockholm, Sweden). This study also utilized questionnaires regarding the medical,

nutritional, and physical activity history of participants. The dietary history questionnaire provided information regarding the participant's past use of nutritional supplements, habitual caffeine consumption, and any potential adverse reactions or sensitivities to caffeine consumption. Participants each completed a three-day diet log, and logs were analyzed using The Food Processor software (ESHA Research, Salem, OR, USA) to evaluate habitual dietary intakes.

Serum Creatinine

At the beginning of the first and final exercise testing sessions, a 3.5 milliliter sample of blood was obtained from a vein in the antecubital region of the arm. Blood samples were immediately stored in a refrigerator, then transported to UNC hospitals for serum creatinine analysis as soon as possible (no more than 12 hours after obtaining the sample). Baseline and post-supplementation levels of serum creatinine were used to identify potential nonresponders, as high serum creatinine concentrations are indicative of increased creatine degradation.

One Rep Max (1RM) & Repetitions to Fatigue (RTF)

Participants completed a 1RM strength test for leg press (LP) and bench press (BP) using free weights and a spotter. To begin the warm up, each participant performed a set of 8-10 repetitions, with a weight that is approximately 50% of the anticipated 1RM. Participants rested for 1-2 minutes, after which a set of 4-6 repetitions was completed with a load of approximately 80% of the predicted 1RM. After a 2-minute rest period, the weight was increased to an estimated 1RM load, and the participants attempted a single repetition with the weight. After the completion of each successful 1RM attempt, the weight was increased until failure was reached, with 2-3 minutes of rest between 1RM attempts. After a 3-minute rest period, participants lifted a weight equivalent to 80% of their 1RM, for LP and BP, for as many continuous repetitions as

possible in a single set. Repetitions were counted by trained lab personnel; failure was considered the point at which a full repetition could no longer be completed with appropriate form. Upon failure, the spotter assisted the participant in returning the weight to the appropriate resting position. Maximal strength and repetition testing was completed at visits 2, 3, and 4. For all testing sessions, LP preceded BP.

Repeated Sprint Protocol

Participants completed a modified repeated sprint protocol, similar to the methods described by Wiroth et al. (83). The test began with a self-paced 5-minute warm up with a fixed load of 0.5 kg on a Monark friction-braked cycle ergometer (Monark, Stockholm, Sweden). Following the warm up, participants completed a series of 5 maximal sprints lasting 10 seconds each. After each sprint, participants were given 60 seconds of passive recovery while seated on the cycle ergometer. During sprints, a load of 95 g/kg of body mass was applied to the flywheel of the ergometer. During each sprint, peak power and total work output were evaluated. This protocol was completed on visits 2, 3, and 4.

Supplementation

In the acute phase of supplementation (visit 3), participants were assigned to one of three treatments: 300 mg of caffeine anhydrous mixed in a beverage with noncaloric flavoring (Crystal Light; Kraft Foods, Northfield, IL), a caffeine-matched serving of dehydrated coffee (8.9 g; Nescafé House Blend; Nestle, Vevey, Switzerland) mixed in hot water, or a placebo beverage with noncaloric flavoring. Thirty minutes after ingestion of the treatment, participants repeated the exercise tests performed in visit 2.

For the chronic phase of the study, participants were assigned to a treatment group for 5 continuous days of supplementation. One group consumed a loading dose (20 g/day, split

between 4 doses spaced evenly throughout the day) of creatine monohydrate (Micronized Creatine Powder; Optimum Nutrition, Aurora, IL, USA) mixed with noncaloric flavoring. A second group consumed the same creatine loading protocol, but with 300 mg of caffeine anhydrous added to the first dose of each day. A third group followed the creatine loading protocol, but mixed each day's first dose of creatine into a caffeine-matched serving of instant (dehydrated) coffee (8.9 g coffee; yielding 303 mg caffeine). The final group consumed a flavored, noncaloric placebo beverage, ingested 4 times throughout the day. Throughout the chronic phase, subjects were instructed to maintain normal diet and exercise habits, but to abstain from any extraneous caffeine consumption. To ensure compliance, participants were required to document their supplement intake in a written log. After 5 consecutive days of supplementation, participants reported for visit 4 to repeat the exercise testing protocol from visits 2 and 3. To avoid an acute ergogenic effect of coffee or caffeine intake, there was no supplementation on the day of visit 4, regardless of treatment group. At each lab visit, potential adverse effects were reviewed with each participant and recorded within their data collection folder.

Statistical Analysis

Acute Phase

Data are expressed as mean ± standard deviation. A series of one-way ANOVAs were used to evaluate baseline differences between groups for dietary intakes and strength and sprint outcomes. For strength outcomes (1RM and RTF for LP and BP), change scores were calculated from baseline testing to post-testing. Four separate one-way ANOVAs were used to compare change scores between groups; in the event of a significant interaction, *post hoc* comparisons were analyzed using the Bonferroni method. When baseline values were significantly different between groups (LP RTF), change scores were analyzed using a one-way ANCOVA, covaried

for baseline values. In addition, 95% confidence intervals (mean ± 1.96 × SEM) were constructed for all strength outcomes. When the 95% confidence interval did not include zero, the change was considered significant ($p \leq 0.05$).

For each sprint, change scores from baseline to post-testing were calculated for both PP and TW. A series of two-way (3 × 5; treatment × sprint) mixed model ANOVAs were used to compare change scores between treatments for each sprint. The Bonferroni method was used to evaluate *post hoc* comparisons. Peak power and TW values were averaged between all five sprints to calculate average PP and TW for the entire sprint protocol, and change scores were calculated for these values. Measures of PP and TW were also evaluated using 95% confidence intervals. Analyses were performed using SPSS software (Version 20.0; IBM, Armonk, NY, USA), and 95% confidence intervals were calculated and plotted in Microsoft Excel (Version 2011, Microsoft Corporation; The Microsoft Network, LLC, Richmond, WA, USA). Statistical significance was set *a priori* at $\alpha \leq 0.05$.

Chronic Phase

A series of one-way ANOVAs were used to evaluate baseline differences between groups for dietary intakes, strength outcomes, sprint outcomes, and serum creatinine values. Strength changes were evaluated using a series of two-way (2 × 4; time × treatment) repeated measures ANOVAs. In the event of a significant interaction, the Bonferroni method was used for *post hoc* comparisons. Peak power and TW values were compared using mixed factorial ANOVAs (2 × 5 × 4; time × sprint × treatment). In the event of a significant interaction effect, Bonferroni *post hoc* tests were used to decompose the model. Serum creatinine and body weight values were compared using mixed factorial ANOVAs (2 × 4; time × treatment). The Bonferroni method was used for *post hoc* comparisons when significant interaction effects were observed. In addition,

95% confidence intervals were calculated for change scores from baseline to post-testing for serum creatinine and all performance outcomes. For all analyses, the level of significance was set *a priori* at $\alpha \leq 0.05$.

CHAPTER IV: MANUSCRIPT I

Effects of coffee and caffeine anhydrous on strength and sprint performance

Introduction

Caffeine is the most widely consumed psychoactive substance in the world, with estimates of average daily intakes as high as 4 mg/kg of bodyweight in US adults (43). Caffeine was first shown to improve exercise performance by Costill et al. (76) in the 1970s, who demonstrated an increase in cycling time to exhaustion following coffee consumption. The primary ergogenic mechanism of caffeine is antagonism of adenosine receptors (30). Caffeine may also affect performance via both central and peripheral mechanisms by altering pain and effort perception (72), calcium kinetics in the sarcoplasmic reticulum (73, 84, 85), and sodium/potassium ATPase pump activity (74), among other potential mechanisms (30). Caffeine in doses of 3-6 mg/kg bodyweight has been consistently shown to enhance endurance performance (30), but findings have been equivocal in the context of strength and sprint performance. Evidence currently suggests that caffeine may improve sprint performance in trained subjects (36, 38, 39, 75), but not in untrained subjects (31-34). Collomp et al. (36) investigated the effects of caffeine supplementation on sprint performance in both trained and untrained swimmers. By stratifying the sample based on training status, authors showed that sprint velocity was improved only in the highly-trained group of swimmers. This distinction may be related to anaerobic training adaptations or reduced training variability in trained subjects, but more research is needed to identify the underlying mechanisms.

The reported effects of caffeine on strength performance have been largely equivocal. Although Woolf et al. (39) reported an ergogenic effect of caffeine on bench press endurance, no improvement in leg press endurance was observed. Beck at al. (40) documented an improvement in bench press one-rep max (1RM), but no improvement in bench press endurance, leg press 1RM, or leg press endurance. Subsequent research by Woolf et al. (41) revealed no significant effect on bench press endurance, and Astorino et al. (42) did not find any significant improvements in bench press or leg press 1RM or endurance after acute caffeine ingestion. It is possible that insufficient sample sizes, variation in individual responses, or differences in caffeine dosing and exercise protocols may partially explain inconsistent outcomes in research to date.

The first study reporting an ergogenic effect of caffeine used coffee as a caffeine source (76), but it was later suggested that coffee was less ergogenic than a caffeine-matched dose of caffeine anhydrous (47). Graham et al. (47) compared the effects of coffee and caffeine anhydrous, and found that only caffeine anhydrous resulted in significant improvements in running time to exhaustion. The authors hypothesized that the differential responses to coffee and caffeine may be attributed to one or more of the numerous biologically active compounds found in coffee. One such group of bioactive compounds, known as chlorogenic acids, are present in coffee in varying amounts (77) and may antagonize caffeine's effects on adenosine receptors (78). Contrary to this hypothesis, McLellan & Bell (46) reported that the ergogenic effect of caffeine supplementation was not affected by coffee consumption 30 minutes prior. More recently, Hodgson et al. (61) showed that energy-based target time trial performance was improved to a similar degree by caffeine-matched doses of coffee and caffeine anhydrous. While Graham et al. (47) found no significant performance improvement following coffee ingestion,

other studies have reported an ergogenic effect using similar doses of coffee before exercise (45, 61, 76). Therefore, the purpose of the current study was to compare the effects of caffeine-matched doses of coffee and caffeine anhydrous on strength and repeated sprint performance. It was hypothesized that caffeine anhydrous ingestion would improve strength and sprint performance to a greater extent than coffee or placebo.

Methods

Experimental Design

The current study consisted of two laboratory visits, separated by at least 48 hours (Figure 1). Baseline testing included one-rep maximum (1RM) and repetitions to fatigue (RTF) for leg press (LP) and bench press (BP), and a repeated sprint protocol to determine peak power and total work output. At least 48 hours after baseline testing, participants returned for a second visit. In a double blind fashion, participants were randomly assigned into one of three groups consisting of either 300 mg caffeine anhydrous (CAF), a caffeine-matched dose of coffee (COF), or placebo (PLA). Thirty minutes after ingestion, exercise testing was repeated. All methodology was approved by the University's Biomedical Institutional Review Board, and all participants signed an informed consent prior to participation.

Subjects

Fifty-four male participants (Mean ± SD; Age = 20.1 ± 2.1 yrs; stature = 177.3 ± 5.6 cm; body mass = 78.8 ± 8.8 kg) completed the current study. All participants were engaged in resistance training for at least 30 minutes a day, three days a week, for at least three months prior to the study. Participants were excluded if they had supplemented with creatine or beta-alanine in the three months prior to the study. Participants were also excluded if dietary analysis or pre-screening questionnaires revealed habitual caffeine intake of >800 mg per day, a history of

sensitivity or adverse reactions to caffeine consumption, or regular consumption of any food or drug in a quantity that may significantly influence caffeine metabolism, such as grapefruit juice, cigarette smoke, and a number of pharmaceutical drugs. Habitual caffeine intake for the sample was 32.9 ± 59.6 mg/day. Participants were encouraged to maintain normal diet and exercise habits throughout the duration of the study, but were asked to abstain from caffeine intake for at least 48 hours and vigorous exercise for at least 24 hours prior to all exercise testing. Participants each completed a three-day diet log, and logs were analyzed using The Food Processor software (ESHA Research, Salem, OR, USA) to evaluate habitual dietary intakes. Exercise testing sessions took place at a similar time of day (± 2 hrs), and participants were instructed to replicate similar food intake before all lab visits.

One Rep Max (1RM) & Repetitions to Fatigue (RTF)

Participants completed a 1RM strength test, for LP followed by BP, using free weights and a spotter. To begin the warm up, each participant performed a set of 8-10 repetitions, with a weight that is approximately 50% of the anticipated 1RM. Participants rested for 1-2 minutes, after which a set of 4-6 repetitions was completed with a load of approximately 80% of the predicted 1RM. After a 2-minute rest period, the weight was increased to an estimated 1RM load, and the participants attempted a single repetition with the weight. After the completion of each successful 1RM attempt, the weight was increased until failure was reached, with 2-3 minutes of rest between 1RM attempts. Once the 1RM was determined, participants were given a three minute rest period before the RTF test, in which participants lifted 80% of the 1RM load for as many continuous repetitions as possible in a single set, until a full repetition could no longer be completed with appropriate form. For subsequent post-testing, the load used for RTF

tests remained constant (80% of baseline 1RM). For all testing sessions, 1RM and RTF testing for LP preceded BP.

Repeated Sprint Protocol

Participants completed a repeated sprint test modified from the protocol described by Wiroth et al. (83) ten minutes after the conclusion of strength testing. The test began with a self-paced five minute warm up with a standardized load of 0.5 kg on a Monark friction-braked cycle ergometer (Ergomedic 894 E; Monark, Stockholm, Sweden). Following the warm up, participants completed a series of five maximal sprints lasting 10 seconds each, with a load of 95 g/kg of body mass. Participants were instructed to remain seated throughout the entire sprint and were provided verbal encouragement throughout the test. After each sprint, participants were given 60 seconds of passive recovery while seated on the cycle ergometer. Peak power (PP) and total work (TW) were evaluated for each sprint using the default software (Monark ATS Software; Monark, Stockholm, Sweden).

Supplementation

Participants were randomly assigned, according to a computer generated allocation sequence, to one of three treatments: 300 mg of caffeine anhydrous (CAF; Smart Powders, Graham, NC, USA) with noncaloric flavoring (Crystal Light; Kraft Foods, Northfield, IL, USA), 8.9 g of dehydrated coffee (COF; Nescafé House Blend; Nestle, Vevey, Switzerland), or a placebo beverage with noncaloric flavoring. To ensure a dose of 3-5 mg/kg, subjects had to weigh between 60-100 kg to participate. Blinding was completed by a member of the study staff not administering treatments. Supplements were mixed by study staff, with 350 mL of water, in opaque containers so neither participants nor investigators knew which treatment was being given. Supplements were consumed 30 minutes prior to exercise testing. Due to the distinct

coffee taste, this was considered a partially blinded study, although subjects were not aware if the coffee-flavored beverage was caffeinated or not. Previous research using a similar product verified a caffeine concentration of 3.4 g caffeine/100 g of instant coffee (61). As such, caffeine content was matched between CAF and COF (300 mg and 303 mg, respectively).

Statistical Analysis

Data are expressed as mean ± standard deviation. A series of one-way ANOVAs were used to evaluate baseline differences between groups for dietary intakes and strength and sprint outcomes. For strength outcomes (1RM and RTF for LP and BP), change scores were calculated from baseline testing to post-testing. Four separate one-way ANOVAs were used to compare change scores between groups; in the event of a significant interaction, *post hoc* comparisons were analyzed using the Bonferroni method. When baseline values were significantly different between groups (LP RTF), change scores were analyzed using a one-way ANCOVA, covaried for baseline values. In addition, 95% confidence intervals (mean ± 1.96 × SEM) were constructed for all strength outcomes. When the 95% confidence interval did not include zero, the change was considered significant ($p \leq 0.05$).

For each sprint, change scores from baseline to post-testing were calculated for both PP and TW. A series of two-way (3 × 5; treatment × sprint) mixed model ANOVAs were used to compare change scores between treatments for each sprint. The Bonferroni method was used to evaluate *post hoc* comparisons. Peak power and TW values were averaged between all five sprints to calculate average PP and TW for the entire sprint protocol, and change scores were calculated for these values. Measures of PP and TW were also evaluated using 95% confidence intervals. Analyses were performed using SPSS software (Version 20.0; IBM, Armonk, NY, USA), and 95% confidence intervals were calculated and plotted in Microsoft Excel (Version

2011, Microsoft Corporation; The Microsoft Network, LLC, Richmond, WA, USA). Statistical significance was set *a priori* at $\alpha \leq 0.05$.

Results

Descriptive characteristics and dietary intakes are listed by group in Table 1. No values were significantly different between groups at baseline.

Strength outcomes

There were no significant interaction effects for BP 1RM (p=0.78; Table 2) or BP RTF (p=0.47; Figure 3), indicating no difference in change scores between treatments. Only LP RTF was significantly different between groups at baseline (p=0.04).When covaried for baseline differences, change scores for LP RTF were not significantly different between groups (p=0.486; Figure 3). There was a significant interaction effect for LP 1RM (p=0.028); *post hoc* comparisons revealed a significantly greater improvement in LP 1RM for COF compared to CAF (p=0.039), with no differences between COF and PLA (p=0.99) or CAF and PLA (p=0.10; Table 2).

Analysis of 95% confidence intervals of change scores revealed significant improvements for all groups in all strength measures, with significantly greater improvements in LP 1RM for COF 95%CI [23.6, 40.8] lbs in comparison to CAF 95% CI [7.3, 23.3] lbs (Table 2; Figure 3).

Sprint outcomes

For changes in PP, no sprint × treatment interaction was observed (p=0.172), with no main effects for sprint (p=0.243) or treatment (p=0.248). For changes in TW, no sprint × treatment interaction was observed (p=0.145). There was no main effect for treatment (p=0.156), but there was a significant main effect for sprint (p=0.021). *Post hoc* analyses showed a

reduction in TW from Sprint 1 to Sprint 3 (p=0.030), with a nonsignificant TW reduction from Sprint 1 to Sprint 4 (p=0.056).

Analysis of 95% confidence intervals revealed a significant increase in Sprint 1 TW for CAF [81.4, 623.9] joules (J), but not for COF or PLA (Figure 4). Sprint 4 PP was significantly reduced in PLA 95%CI [-64.9, -2.5] watts (W), but not in COF 95%CI [-34.4, 36.6] or CAF 95%CI [-42.8, 22.2]. Total work was significantly reduced in Sprint 2 95%CI [-321.2, -66.1] and Sprint 4 95%CI [-403.1, -57.6] for PLA, but not for 95%CI in COF [-19.5, 439.3 and -291.2, 118.3] or CAF [-415.8, 103.3 and -300.9, 129.5]. Further, average TW was reduced in PLA [-219.0, -40.2], but not in COF or CAF (Figure 5).

Discussion

A number of studies have demonstrated that CAF supplementation improves endurance exercise performance (30). Far less research has investigated its effects on strength and sprint performance, and to our knowledge CAF and COF have not been directly compared in this context. Results of the current study revealed significant improvements in 1RM and RTF for BP and LP in all groups, although changes in LP 1RM were significantly greater for COF than CAF. Analysis of 95% confidence intervals suggests an ergogenic effect of both CAF and COF during a repeated sprint protocol. Sprint 1 TW was significantly improved in CAF only. Further, PLA experienced reductions in sprint 4 PP, sprints 2 and 4 TW, and average TW, while these reductions were attenuated by both CAF and COF. The current results may have implications for anaerobic athletes seeking to improve repeated sprint performance with pre-exercise CAF or COF ingestion.

In the current study, BP 1RM, BP RTF, and LP RTF improved in all groups, with no significant differences between treatments. Despite the recruitment of resistance-trained

participants, improvement in the PLA group suggests that increased familiarity with the testing protocol led to improved performance. Interestingly, no such effect was observed for the cycling test, which was likely a more unfamiliar exercise test based on inclusion criteria. Coffee improved LP 1RM more than CAF, but not more than PLA. Previous CAF studies investigating similar strength measurements have reported equivocal results. Results of Beck et al. (40) suggested that pre-exercise CAF supplementation increased BP 1RM, but did not affect BP endurance, leg extension 1RM, or leg extension endurance. Conversely, Woolf et al. (39) found an improvement in chest press endurance, but not LP endurance. Other studies by Woolf et al. (41) and Astorino et al. (42) found no effect on BP endurance or on BP or LP 1RM or endurance, respectively. Graham et al. (47) previously indicated that CAF improved endurance performance more than a caffeine-matched dose of COF; more recent research has failed to support these findings (61). To our knowledge, the current study is the first to investigate this question in the context of strength and sprint performance, and results do not support the hypothesis that CAF is superior to COF for performance. Coffee improved LP 1RM more than CAF, but the underlying mechanism for this difference is not yet known. Previous research has indicated that individual responses to CAF can vary (30), and it is therefore possible that individuals in the CAF group reacted less favorably to caffeine than the COF group. Self-reported habitual daily caffeine intake was similar between CAF and COF groups (41.0 ± 64.5 mg vs. 33.3 ± 59.4 mg, respectively), and participants abstained from caffeinated products for a minimum of 48 hours prior to all testing sessions to eliminate short-term habituation. It is also possible that one or more of the numerous bioactive compounds in coffee (86) may influence strength performance independently or synergistically with CAF, but more research is needed to investigate this finding.

In the current study, sprint 1 TW was significantly improved by CAF, but not by COF or PLA. Further, the PLA group experienced reductions in PP and TW during post-testing, while PP and TW were maintained with CAF or COF. These results indicate that both COF and CAF had an ergogenic effect on the repeated sprint test, with no clear difference between the two groups. These findings are consistent with previous research documenting improvements for both single (39) and repeated sprint performance (38) in trained subjects with CAF use, and suggest that improvements are similar with COF. In the context of high-intensity sprints, the ergogenic effects of caffeine are most likely related to its effects on adenosine receptors (30) and pain/effort perception (72). Participants in a previous study reported reduced leg pain during cycling to exhaustion at 60% of VO_2peak following caffeine ingestion (87). While leg pain was not directly assessed in the current study, attenuation of pain or effort perception would likely be advantageous in the strenuous sprint protocol employed. Additionally, caffeine's effects on cycling performance may relate to direct effects of caffeine on muscle. Electronically stimulated cycling time to exhaustion was improved by caffeine in a sample of paraplegic and tetraplegic participants (88). Results indicated that this effect was not influenced by catecholamines, substrate utilization, or central factors, and was possibly attributable to peripheral effects on excitation-contraction coupling (88). Power reductions are not likely related to caffeine withdrawal, as habitual caffeine intake was low in the PLA group (24.2 ± 56.8 mg/day), and participants observed a standardized, 48-hour abstention from caffeinated products prior to baseline testing. While baseline testing and post-testing were separated by at least 48 hours, power reductions in the PLA group may relate to the strenuous nature of the testing session, or residual soreness or fatigue from pre-testing. These power reductions were attenuated by both

CAF and COF, suggesting that CAF and COF may be beneficial for athletes completing multiple strenuous training sessions within a given week.

Previous research has suggested that one or more of the bioactive compounds in COF, such as chlorogenic acid, may attenuate the ergogenic effect of caffeine (30, 47). In agreement with more recent research (46, 61), results of the current study suggest that COF and CAF yield similar benefits for high-intensity exercise. While caffeine has previously been associated with negative health outcomes, recent research has suggested that habitual COF consumption may be beneficial after controlling for confounding variables such as smoking and heavy alcohol consumption (86, 89). Coffee contains numerous biologically active compounds, and authors have proposed that some of these compounds may counteract the negative health effects associated with caffeine intake (86). As such, COF may be considered a suitable source of caffeine prior to high-intensity exercise, with results of the current study suggesting a similar ergogenic effect when compared to CAF.

A limitation of the current study is the provision of a flat dose of caffeine (300 mg) rather than scaling the dosage to bodyweight. Ergogenic effects of caffeine are commonly observed with doses around 3-5 mg/kg of body mass (30), so inclusion criteria required participants to be between 60-100 kg to ensure an appropriate dose. Many caffeine studies provide supplements one hour prior to exercise, whereas supplements were ingested 30 minutes prior to exercise in this study. This time frame was selected because 90% of caffeine is cleared from the stomach within 20 minutes of ingestion, with peak values occurring as early as 30-45 minutes after ingestion (86), and because the exercise test protocol took roughly one hour to complete. Despite the recruitment of resistance trained participants, it appears that a learning effect was observed for strength outcomes, but not for sprint outcomes. Finally, reductions in power output in the

PLA group may suggest that CAF and COF attenuated fatigue throughout the series of exercise tests in post-testing. These power reductions could also indicate that participants were not fully recovered between pre- and post-testing sessions; if so, attenuation of power decrements may reveal a valuable effect of CAF and COF for athletes completing multiple strenuous exercise bouts in the same week. Future research should seek to determine optimal dosing strategies for COF and CAF prior to high-intensity exercise.

Conclusion

The current study demonstrated an ergogenic effect of caffeine-matched doses of CAF and COF on repeated sprint exercise, with no clear difference between treatments. Neither CAF nor COF improved strength outcomes compared to placebo. In trained males, caffeine dosed at 3-5 mg/kg may improve high-intensity sprint performance when ingested 30 minutes prior to exercise. Caffeine anhydrous does not appear to improve performance more than coffee. Given the health benefits of habitual coffee consumption (86, 89), coffee appears to be a suitable source of caffeine to improve sprint performance, particularly when multiple strenuous bouts are completed in the same week.

CHAPTER V: MANUSCRIPT II

Effects of coffee and caffeine anhydrous intake during creatine loading

Introduction

Creatine and caffeine supplements are commonly used by athletes to improve performance. Surveys conducted in 2004 and 2005 revealed that 37% of Division I collegiate athletes had used creatine supplements (1), and 89% of triathletes intended to use caffeine either before or during their upcoming race (22). A number of pre-workout supplements have been formulated to include both creatine and caffeine (90-93), as both ingredients are widely popular and thought to improve performance. While early creatine studies reported performance improvements when administering creatine in caffeinated tea or coffee (53, 54), the scarce research directly investigating concurrent creatine and caffeine anhydrous supplementation has suggested that caffeine may blunt the effectiveness of creatine supplementation (51, 52, 81).

Demand for adenosine triphosphate (ATP) is elevated during intense exercise, to allow for sustained crossbridge cycling and force output. The rapid provision of ATP is facilitated by stored phosphocreatine (PCr), which phosphorylates ADP in the creatine kinase reaction. Creatine is synthesized endogenously in the liver, kidneys, and pancreas at a rate of roughly one gram per day (2), with 95% of the creatine pool stored within skeletal muscle (3). The average 70 kg male has a creatine pool of roughly 120-140 grams (4), but creatine storage is affected by a number of factors including muscle mass, skeletal muscle fiber type, and dietary intake (3, 5-7). Creatine supplementation has been shown to increase muscle creatine storage (94), which increases ATP availability and improves exercise performance (16). To date, hundreds of studies

have investigated the effects of creatine supplementation on exercise performance, with the majority of studies demonstrating an ergogenic effect on strength and sprint performance lasting less than 30 seconds (11, 14).

Caffeine (1,3,7-trimethylxanthine) was first shown to improve exercise performance by Costill et al. (76), who found that coffee consumption prior to exercise led to an improvement in running time to exhaustion. While the primary ergogenic mechanism of caffeine is antagonism of adenosine receptors, caffeine may affect performance by a number of both central and peripheral mechanisms (30). In previous literature, caffeine doses ranging from 3-6 mg/kg of body mass have been consistently shown to improve endurance performance (30). While caffeine's effects on strength and sprint performance have been fairly equivocal, there is evidence to suggest that caffeine improves strength (39, 40) and sprint (38, 39, 75) performance, particularly in trained subjects (36).

Creatine and caffeine do not display pharmacokinetic interactions when ingested together (79), and are thought to improve performance via independent mechanisms, which has prompted interest in the potential for combined supplementation. Previous studies have reported performance improvements from creatine mixed into caffeinated tea or coffee (53, 54), and a number of multi-ingredient supplements including both creatine and caffeine have been shown to improve outcomes for strength, power, and body composition (90-93). However, the scarce research directly investigating combined supplementation has indicated that chronic caffeine anhydrous consumption may blunt the ergogenic effect of creatine loading (51, 81). This apparent incompatibility could potentially be explained by creatine and caffeine imposing opposite effects on muscle relaxation time (52), or by gastrointestinal distress caused by concurrent ingestion of both ingredients (80, 81). It is currently unclear if these discrepant

findings may relate to differences in the dose or source of caffeine consumed. Given the popular use of both creatine and caffeine, further research is warranted to determine if chronic coffee or caffeine anhydrous consumption blunt the ergogenic effect of creatine loading.

The purpose of the current study was to determine if chronic (five days) coffee or caffeine anhydrous ingestion blunts the ergogenic effect of creatine loading on strength and sprint performance. It was hypothesized that creatine loading would improve outcomes for strength and power output, and that this effect would be blunted by chronic consumption of caffeine anhydrous, but not coffee.

Methods

Experimental Design

The current study consisted of two laboratory visits, with a five day supplementation period preceding the second visit (Figure 2). Baseline testing included serum creatinine levels, one-rep maximum (1RM) and repetitions to fatigue (RTF) for leg press (LP) and bench press (BP), and a repeated sprint protocol to determine peak power (PP) and total work (TW) output. At least 72 hours after baseline testing, participants began supplementation with either creatine monohydrate (CRE; 20 g/day), creatine and caffeine (CRE+CAF; CRE + 300 mg/day of caffeine), creatine and coffee (CRE+COF; CRE + 8.9 g/day of instant coffee), or placebo (PLA). Participants supplemented for five days and returned to the laboratory on day six for serum creatinine analysis and exercise testing. All methodology was approved by the University's Biomedical Institutional Review Board, and all participants signed an informed consent prior to participation.

Subjects

Fifty-four male participants (Mean ± SD; Age = 20.1 ± 2.1 yrs; stature = 177.3 ± 5.6 cm; body mass = 78.8 ± 8.8 kg) completed the current study. Participants were between 18 and 35 years of age, and had been engaged in habitual resistance training for at least 30 minutes per day, three days per week, for at least three months leading into the study. Exclusion criteria included supplementation with creatine or beta-alanine in the three months prior to the study, habitual caffeine intake of over 800 mg per day, or a history of adverse reactions to caffeine. Participants were also excluded if they consumed substances known to influence the CYP1A2 isozyme, such as grapefruit juice, cruciferous vegetables, cigarette smoke, antidepressant drugs, and a number of other pharmaceutical drugs, in doses large enough to impact caffeine metabolism. Habitual caffeine intake for the sample was 32.9 ± 59.6 mg/day. Participants were instructed to abstain from caffeine intake within 48 hours of baseline testing, and to avoid strenuous exercise within 24 hours of all testing sessions. Aside from these restrictions, participants were encouraged to maintain their normal training and dietary habits, and to replicate similar food intake before visits to the laboratory. Participants each completed a three-day diet log; logs were analyzed using The Food Processor software (ESHA Research, Salem, OR, USA) to evaluate habitual dietary intakes. Laboratory visits occurred at a similar time of day (± 2 hrs).

Serum Creatinine

Prior to exercise at each lab visit, a 3.5 milliliter sample of blood was obtained from a vein in the antecubital region of the arm using a BD Vacutainer Serum Separator Tube (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Blood samples were immediately stored in a refrigerator, then transported to an independent laboratory (Core Laboratory, McLendon

Clinical Labs, UNC Hospitals, Chapel Hill, NC) for serum creatinine analysis no more than 12 hours after sample collection.

One Rep Max (1RM) & Repetitions to Fatigue (RTF)

One-rep max for leg press (LP) and bench press (BP) were determined using free weights and a spotter. Before testing, participants were allowed time for a brief warm up and technique familiarization. The first warm up set consisted of 8-10 repetitions performed with a load estimated to be 50% of 1RM. After 1-2 minutes of rest, participants performed a second warm up set consisting of 80% of the predicted 1RM, lifted for 4-6 repetitions. Participants were allowed two minutes of rest, after which participants completed their first 1RM attempt. For all subsequent sets, the load was adjusted and a single repetition was performed until the 1RM was identified, with 2-3 minutes of rest provided between attempts.

Following the final 1RM attempt for a given lift, participants rested for three minutes, after which the RTF test was completed. This test consisted of lifting 80% of the 1RM load for as many repetitions as possible, until failure was reached. Testing was supervised by trained laboratory personnel, and failure was defined as the point at which a full repetition could no longer be completed with appropriate technique. For the second laboratory visit, participants used the same load (80% of baseline 1RM) for the RTF test, regardless of any changes in 1RM. In all testing sessions, leg press 1RM and RTF testing preceded the bench press.

Repeated Sprint Protocol

Ten minutes after the conclusion of strength testing, participants completed a repeated sprint test consisting of five, 10-second sprints on a Monark friction-braked cycle ergometer (Ergomedic 894 E; Monark, Stockholm, Sweden). Participants completed a self-paced, five minute warm up with a standardized load of 0.5 kg. After the warm up, participants completed

the sprint protocol adapted from Wiroth et al. (83), completing five maximal sprints lasting ten seconds each, with a load of 95 g/kg of body mass applied to the weight basket. Each sprint was followed by a passive rest period of 60 seconds. Participants remained in a seated position for the duration of each sprint, and verbal encouragement was provided by lab personnel. For each sprint, peak power (PP) and total work (TW) output were evaluated using the default software (Monark ATS Software; Monark, Stockholm, Sweden).

Supplementation

Participants were randomly assigned to a treatment group for five continuous days of supplementation, using a computer generated allocation sequence. One group (CRE) consumed a loading dose (20 g/day, split between 4 servings) of creatine monohydrate (Micronized Creatine Powder; Optimum Nutrition, Aurora, IL, USA) mixed with noncaloric flavoring (Crystal Light; Kraft Foods, Northfield, IL, USA). A second group (CRE+CAF) followed the same creatine loading protocol, but with 300 mg of caffeine anhydrous (CAF; Smart Powders, Graham, NC, USA) added to the first dose of each day. A third group (CRE+COF) followed the creatine loading protocol, but mixed each day's first dose of creatine into a caffeine-matched serving (8.9 g) of instant coffee (COF; Nescafé House Blend; Nestle, Vevey, Switzerland). The final group (PLA) consumed a flavored, noncaloric placebo beverage, ingested four times daily. All participants weighed between 60-100 kg, to ensure that 300 mg caffeine doses were between 3-5 mg/kg of bodyweight. The current study was considered partially blinded, as coffee has a distinct taste. However, laboratory personnel were blinded to the groups, and participants did not know if the coffee-flavored beverage contained caffeine. Previous research has used a similar instant coffee product and verified a caffeine concentration of 3.4 g caffeine/100 g of instant coffee (61).

As such, caffeine content was approximately matched between CRE+CAF and CRE+COF treatment arms (300 mg and 303 mg, respectively).

Throughout supplementation, subjects were instructed to maintain normal diet and exercise habits, but to abstain from any extraneous caffeine consumption. To ensure compliance, participants were required to document their supplement intake in a written log. After five consecutive days of supplementation, participants reported for the second exercise testing session on day six. To avoid an acute ergogenic effect of coffee or caffeine intake, there was no supplementation on day six, regardless of treatment group.

Statistical Analysis

Data are expressed as mean ± standard deviation. A series of one-way ANOVAs were used to evaluate baseline differences between groups for dietary intakes, strength outcomes, sprint outcomes, and serum creatinine values. Strength changes were evaluated using a series of two-way (2 × 4; time × treatment) repeated measures ANOVAs. In the event of a significant interaction, the Bonferroni method was used for *post hoc* comparisons. Peak power and TW achieved during each sprint were compared using mixed factorial ANOVAs (2 × 5 × 4; time × sprint × treatment). In the event of a significant interaction effect, Bonferroni *post hoc* tests were used to decompose the model. For each subject, average PP and TW were calculated by averaging values from all five sprints. Serum creatinine and body weight values were compared using mixed factorial ANOVAs (2 × 4; time × treatment). The Bonferroni method was used for *post hoc* comparisons when significant interaction effects were observed.

For serum creatinine values and all performance outcomes, 95% confidence intervals were calculated for change scores from baseline to post-testing (mean ± 1.96 × SEM). A change was considered significantly significant ($p < 0.05$) if zero did not fall within the 95% confidence

interval. Microsoft Excel (Version 2011, Microsoft Corporation; The Microsoft Network, LLC, Richmond, WA, USA) was used to calculate and plot 95% confidence intervals. All other statistical analyses were performed using SPSS software, (Version 20.0; IBM, Armonk, NY, USA), with the level of significance set *a priori* at $\alpha \leq 0.05$.

Results

Descriptive characteristics and dietary intakes are listed by group in Table 3. No values were significantly different between groups at baseline.

Strength outcomes

Baseline strength values were not significantly different between groups (Table 4; Figure 6). No interaction effect was observed for BP 1RM ($p=0.95$). A significant main effect was observed for time ($p<0.001$), but not for treatment ($p=0.60$). For BP RTF, there was no significant interaction ($p=0.12$) or main effect for treatment ($p=0.98$), but a main effect for time was observed ($p<0.001$). For LP 1RM, the interaction effect ($p=0.44$) and main effect for treatment ($p=0.99$) were not significant, while the main effect for time was significant ($p<0.001$). There was no significant interaction for LP RTF ($p=0.86$) and no main effect for treatment ($p=0.41$), but there was a significant main effect for time ($p<0.001$).

Analysis of 95% confidence intervals for change scores indicated that all groups significantly improved BP 1RM, LP 1RM, and LP RTF from baseline to post-testing (Table 4; Figure 6). For BP RTF, 95%CIs indicated significant increases in repetitions for PLA [0.6, 2.4], CRE+CAF [0.2, 1.2], and CRE [0.4, 1.4], but not for CRE+COF [-0.6, 1.1] (Figure 6).

Sprint outcomes

Baseline values were not significantly different between groups (Table 5). For PP, the time × sprint × treatment interaction was not significant ($p=0.48$). Similarly, there were no

significant time × sprint (p=0.13), sprint × treatment (p=0.38), or time × treatment (p=0.94) interactions. A significant main effect was observed for sprint (p<0.001), but not for time (p=0.99) or treatment (p=0.99). *Post hoc* comparisons of marginal means revealed that PP dropped significantly between all five sprints (all p<0.01; Table 5).

For TW, the time × sprint × treatment interaction was not significant (p=0.71). A significant time × sprint interaction was observed (p=0.02). There were no significant differences between pre- and post-test values for TW for any sprint; *post hoc* comparisons showed significant reductions in TW between all five sprints for pre-testing (p<0.001; Table 5), while the TW drop between sprints 4 and 5 were not significant in post-testing (p=0.99). Sprint × treatment and time × treatment interactions were not significant (p=0.53 and p=0.80, respectively). A significant main effect was observed for sprint (p<0.001), but not for time (p=0.87) or treatment (p=0.90).

Analysis of 95% confidence intervals for change scores revealed no statistically significant changes in PP or TW for any sprint, or in average PP or TW, in any treatment group (Table 5). Confidence intervals for average TW are presented in Figure 7.

Serum creatinine values

A significant time × treatment interaction was observed for serum creatinine (CRN) values (p=0.04). After decomposing the model, a one-way ANOVA revealed a significant interaction between post-test CRN values (p=0.04), with CRE+COF greater than PLA (p=0.04). Paired samples T-tests revealed significant increases from pre- to post-testing in CRE+COF (p=0.02), CRE+CAF (p=0.01), and CRE (p=0.04), but not in PLA (p=0.70). Analysis of 95% confidence intervals for change scores revealed a significant increase in serum CRN values for

CRE [0.01, 0.15] mg/dL, CRE+COF [0.05, 0.35], and CRE+CAF [0.06, 0.25], but not for PLA (Figure 8).

Reported side effects

Analysis of supplement logs indicated 99% compliance for supplementation. Four participants given CRE+CAF reported mild gastrointestinal (GI) disturbance, which was not reported in any other group. For changes in body weight, there was no significant time × treatment interaction (p=0.22), with no main effects for time (p=0.59) or treatment (p=0.75). Weight was slightly reduced in PLA (-0.2 ± 1.0 kg) and CRE+COF (-0.2 ± 1.2 kg), with small increases observed in CRE+CAF (0.5 ± 0.7 kg) and CRE (0.1 ± 0.7 kg).

Discussion

Previous research has suggested that chronic caffeine anhydrous intake blunts the ergogenic effect of creatine loading (51, 52, 81). In contrast, multiple studies have demonstrated the efficacy of CRE supplementation when mixed into caffeinated tea or coffee (53, 54). With many pre-workout supplement formulations including both CRE and CAF (90-93), further research on the potential interaction is needed. While previous studies on CRE+CAF supplementation have employed unique exercise tests consisting of electrical stimulation (52) or isokinetic knee extension (51, 81), the current study used dynamic exercise tests relating more closely to the training of many athletes. Strength outcomes were improved in all groups, except for a nonsignifcant improvement in BP RTF for CRE+COF (Table 4; Figure 6). For sprint outcomes, no group interactions were observed for PP or TW. Although not statistically significant, average TW was reduced in PLA, but not in CRE+CAF, CRE+COF, or CRE (Figure 7). Serum creatinine values were elevated in all treatment groups, except PLA. Minor GI disturbance was reported by four subjects in CRE+CAF, with no such reports in other groups.

In the current study, upper and lower body strength measurements improved in all treatment groups, with the exception of BP RTF for CRE+COF, which did not change. A meta-analysis published in 2003, which included both acute and chronic interventions, indicated that CRE supplementation led to significant improvements in 1RM and RTF (14). Acute (5-7 days) CRE loading interventions have demonstrated increases in muscle creatine storage (55), while changes in strength have been mixed. Although CRE loading has been shown to increase indices of maximal strength (8, 64) and RTF (63, 64), Zuniga et al. (95) found no effect of CRE loading on 1RM for leg extension or BP. Izquierdo et al. (64) documented improvements in half-squat 1RM, half-squat RTF, and BP RTF, while BP 1RM did not improve. Volek et al. (8) reported improvements in BP 1RM with one week of CRE loading, but no change in squat 1RM or BP RTF. Strength outcomes improved to a similar degree in all CRE groups in the current study, but these improvements were not significantly greater than PLA. Single-rep strength performance is not likely limited by the phosphagen energy system, which may explain the results observed for 1RM. For RTF, null findings could possibly be explained by the presence of non-responders in the sample, as up to 30% of individuals may not see performance benefits from CRE (7, 55). In previous research, longer CRE supplementation protocols (10-12 weeks) with concurrent resistance training have yielded greater improvements in strength outcomes than short-term loading (8, 96). As such, more sensitive measures of strength may be needed to consistently detect strength improvements from short-term CRE loading. Results indicate that CRE did not improve strength outcomes more than PLA, and that the addition of CAF or COF did not modulate strength outcomes.

For sprint outcomes, no significant changes in PP or TW were observed for any group. It was hypothesized that CRE would improve sprint performance, as CRE is thought to be

particularly effective for repetitive high-intensity bouts lasting ≤30 seconds (14). A number of previous studies have shown CRE supplementation to improve performance on both single (69) and repeated (66-69) sprint tests. However, McKenna et al. (70) demonstrated that CRE did not improve performance on a repeated sprint test consisting of five ten-second sprints with variable rest periods. Finn et al. (71) also reported no benefit of CRE on a protocol consisting of four 20-second sprints with 20 seconds of rest between sprints. Using a sprint protocol similar to that of the current study, Wiroth et al. (83) found CRE to improve performance in both young and old sedentary participants, but not in well-trained participants. It is not clear if the lack of improvement in the current study is a result of the sprint protocol employed, or characteristics of the sample. Despite the nonsignificant results, it does appear that average TW declined in PLA, but was maintained in all groups consuming CRE (Figure 7). This may suggest a modest benefit of CRE loading for athletes reliant on repetitive, intermittent bursts of high-intensity activity. Similarly to strength outcomes, the inclusion of CAF or COF with CRE did not appear to affect sprint performance.

While no pharmacokinetic interaction has been observed between CRE and CAF (79), Vandenberghe et al. (51) demonstrated that CAF blunted the ergogenic effect of CRE loading. Subsequent research suggested that opposing effects on muscle relaxation time may explain this interaction (52). Results of Vandenberghe et al. (51) may have been influenced by an insufficient washout period (three weeks), or CAF withdrawal, as the final CAF dose was ingested at least 20 hours before post-testing. In a published abstract, Harris et al. (81) sought to replicate previous findings, but employed a six week washout period and included post-test sessions both two and 24 hours after CAF cessation. While CAF appeared to blunt the ergogenic effect of CRE, authors suggested that this effect was explained by GI discomfort with CRE+CAF, which was reported

in four out of ten subjects completing the study. Gastrointestinal discomfort was also noted in another recent study involving CRE+CAF supplementation (80), in which three out of seven subjects reported symptoms. In agreement with past studies, GI discomfort was reported in four of thirteen participants in the CRE+CAF group in the current study. While mild GI distress has been reported anecdotally with CRE supplementation (3), it was not reported by any participants in PLA, CRE, or CRE+COF. Future studies should seek to determine if athletes can successfully combine CRE and CAF by employing supplement timing strategies to mitigate GI distress and CAF withdrawal before exercise.

In agreement with previous research (3), serum CRN values in the present study were significantly elevated in all groups supplementing with CRE (Figure 8). Creatinine is the breakdown product of creatine, and can be influenced by a number of factors including age, sex, lean mass, diet, physical activity, and creatine supplementation (3, 97, 98). Changes in serum CRN were greater in CRE+COF and CRE+CAF compared to CRE, but differences were not statistically significant, and it is not known if differences are physiologically significant. Supplement logs indicated a compliance rate of 99%, which was supported by changes in serum CRN. In individuals supplementing with CRE, high serum CRN levels should be interpreted with caution, as increased creatine breakdown may not reflect an impairment of kidney function (3, 98). Creatine is osmotically active, and CRE loading generally results in a weight gain of 1-2 kg due to water retention (3). Despite excellent compliance, the current study did not find a significant time × treatment interaction for body weight, with no group increasing by more than 0.5 kg. Elevated serum CRN and lack of weight gain could potentially be indicative of poor muscle CRE uptake, although these values are often variable and influenced by a number of factors. While Syrotuik et al. (7) found that creatine non- and quasi-responders gained less

weight and had greater increases in urinary CRN compared to responders after CRE loading, differences were nonsignificant and varied between individuals. As such, these values may not be valid indicators for identifying CRE non-responders.

Limitations of the current study must be noted. While the 5-day CRE loading protocol has been shown to increase muscle creatine stores (55), more pronounced performance effects would likely be observed with a longer supplementation period accompanied by progressive resistance training. Despite the recruitment of resistance-trained participants, strength improvements in the PLA group may indicate a learning effect or placebo effect for 1RM and RTF, but not for sprint outcomes. Although 1RM and RTF are highly applicable to the training of athletes, more sensitive indices of strength may be favorable for future studies on short-term creatine loading. Finally, the current study did not directly measure muscle creatine content, and therefore cannot rule out the presence of non-responders in the sample.

Conclusions

In the current study, five days of CRE supplementation did not improve strength or sprint outcomes more than PLA. The addition of CAF and COF did not appear to influence performance outcomes of CRE supplementation. While differences were not statistically significant, reductions in total work output were attenuated to a similar extent by CRE, CRE+CAF, and CRE+COF, compared to PLA. Results support previous research indicating that GI discomfort is observed with combined CRE+CAF supplementation, while this was not observed with CRE+COF. Future research should investigate the effects of COF and CAF with a longer period of CRE supplementation combined with progressive resistance training, and seek to determine if supplement timing and dosing strategies can be employed to obtain the ergogenic benefits of CRE and CAF without experiencing GI discomfort.

CHAPTER VI: CONCLUSION

Results of this study demonstrated a modest ergogenic effect of CAF and COF on high-intensity sprint exercise, particularly in the context of multiple strenuous bouts of exercise. Caffeine doses at 3-5 mg/kg may improve sprint performance when ingested by resistance-trained males 30 minutes prior to exercise. Results did not support the hypothesis that CAF is more ergogenic than a caffeine-matched dose of COF, with no clear difference between treatments. Five days of CRE loading did not improve exercise performance to a significant degree, although a nonsignificant maintenance of average total work output was observed in all groups consuming CRE. The addition of CAF or COF did not influence performance outcomes compared to consuming CRE alone. Results indicated that the combination of CRE+CAF may cause mild GI discomfort, while none was reported with CRE or CRE+COF. Future studies should seek to determine if COF or CAF influence the effects of longer durations of CRE supplementation, and if supplement timing and dosing strategies can be employed to obtain the ergogenic benefits of CRE and CAF without experiencing GI discomfort. Results of this study may have important implications for pre-exercise supplementation strategies and formulations.

TABLES

Table 1. Baseline characteristics and habitual dietary intakes by group for caffeine (CAF), coffee (COF), and placebo (PLA) (acute phase). Values are mean ± SD.

	CAF (n=18)	COF (n=18)	PLA (n=18)
Age (yrs)	20.3 ± 2.3	20.3 ± 2.3	19.7 ± 1.6
Height (cm)	178.0 ± 4.8	176.4 ± 6.0	177.4 ± 6.2
Weight (kg)	77.9 ± 7.8	79.0 ± 10.7	79.4 ± 8.3
Calories (kcal)	2472.6 ± 410.2	2808.4 ± 651.3	2554.5 ± 604.6
CHO (g)	275.6 ± 64.4	309.6 ± 114.9	309.3 ± 99.1
FAT (g)	97.7 ± 28.0	111.8 ± 34.3	97.4 ± 33.6
PRO (g)	128.4 ± 31.6	142.4 ± 33.9	117.7 ± 34.0
CAF (mg)	41.0 ± 64.5	33.3 ± 59.4	24.2 ± 56.8

CHO = carbohydrate intake, PRO = protein intake, CAF = habitual caffeine intake

Table 2. Effects of acute supplementation on leg press and bench press 1RM (lbs)

	LP 1RM (lbs)		BP 1RM (lbs)	
Treatment	Pre	Post	Pre	Post
CAF	623.8 ± 138.3	639.1 ± 141.0*	204.7 ± 40.8	208.3 ± 40.8*
COF	680.6 ± 189.9	712.8 ± 189.6*§	210.0 ± 39.0	212.8 ± 39.9*
PLA	614.4 ± 152.0	644.2 ± 148.5*	191.1 ± 38.1	195.3 ± 37.7*

* Significant change from baseline

§ Change score significantly greater than CAF

Table 3. Baseline characteristics and habitual dietary intakes by group (chronic phase)

	PLA (n=14)	CRE+COF (n=13)	CRE+CAF (n=13)	CRE (n=14)
Age (yrs)	19.9 ± 2.0	20.0 ± 1.3	20.2 ± 2.7	20.3 ± 2.3
Height (cm)	175.2 ± 7.0	177.6 ± 5.0	177.7 ± 5.3	178.7 ± 5.0
Weight (kg)	77.7 ± 9.2	77.9 ± 7.6	80.8 ± 10.6	78.7 ± 8.4
Calories (kcal)	2659.5 ± 737.6	2630.2 ± 753.4	2540.4 ± 406.5	2628.7 ± 333.8
CHO (g)	282.6 ± 78.0	326.4 ± 120.3	257.7 ± 78.6	323.8 ± 88.3
FAT (g)	113.1 ± 43.5	97.7 ± 25.9	107.6 ± 29.1	91.9 ± 26.5
PRO (g)	130.0 ± 46.1	123.9 ± 31.3	134.1 ± 33.5	132.3 ± 25.8
CAF (mg)	18.4 ± 57.5	12.1 ± 29.1	54.6 ± 76.0	46.4 ± 60.9

CHO = carbohydrate intake, PRO = protein intake, CAF = habitual caffeine intake

Table 4. Effects of chronic supplementation on leg press and bench press 1RM (lbs) for placebo (PLA), creatine + coffee (CRE + COF), creatine + caffeine (CRE + CAF), or creatine alone (CRE). Values are mean ± SD.

	LP 1RM (lbs)		BP 1RM (lbs)	
Treatment	Pre	Post	Pre	Post
PLA	633.6 ± 161.0	679.6 ± 157.6*	197.1 ± 28.1	202.5 ± 29.3*
CRE+COF	635.8 ± 115.6	672.3 ± 114.4*	193.8 ± 34.3	198.5 ± 32.7*
CRE+CAF	659.2 ± 204.6	690.4 ± 213.4*	213.8 ± 45.9	218.5 ± 45.8*
CRE	633.6 ± 174.0	680.7 ± 167.3*	203.2 ± 47.7	208.9 ± 48.6*

* Significant change from baseline

Table 5. Changes in peak power (PP; watts) and total work (TW; joules) following chronic supplementation. Values are mean ± SD.

Peak Power (W)						
Treatment	Sprint 1	Sprint 2	Sprint 3	Sprint 4	Sprint 5	Average
PLA	20.4 ± 93.9	-1.5 ± 76.4	-6.8 ± 77.0	-17.9 ± 57.8	-20.3 ± 128.0	-5.2 ± 45.0
CRE+COF	20.3 ± 72.0	-18.2 ± 78.2	-38.4 ± 83.1	4.0 ± 113.7	38.3 ± 105.0	1.2 ± 65.6
CRE+CAF	23.6 ± 105.3	20.1 ± 94.3	8.5 ± 73.1	-19.0 ± 74.8	2.1 ± 57.9	7.0 ± 59.0
CRE	3.8 ± 72.2	0.46 ± 69.3	-4.9 ± 60.8	-30.8 ± 71.4	16.9 ± 71.5	-2.9 ± 50.5

Total Work (J)						
Treatment	Sprint 1	Sprint 2	Sprint 3	Sprint 4	Sprint 5	Average
PLA	-43.7 ± 700.8	-169.4 ± 607.9	-208.4 ± 520.9	-169.8 ± 509.7	37.6 ± 843.7	-110.7 ± 434.3
CRE+COF	201.5 ± 612.1	-189.5 ± 682.0	-214.6 ± 639.0	2.4 ± 751.2	288.1 ± 633.6	17.6 ± 489.2
CRE+CAF	233.0 ± 615.5	-26.9 ± 522.4	18.3 ± 491.3	-112.2 ± 442.5	-1.2 ± 470.5	22.2 ± 405.5
CRE	80.1 ± 491.4	26.6 ± 476.7	-11.0 ± 366.8	-63.9 ± 585.8	126.3 ± 636.5	31.6 ± 389.9

FIGURES

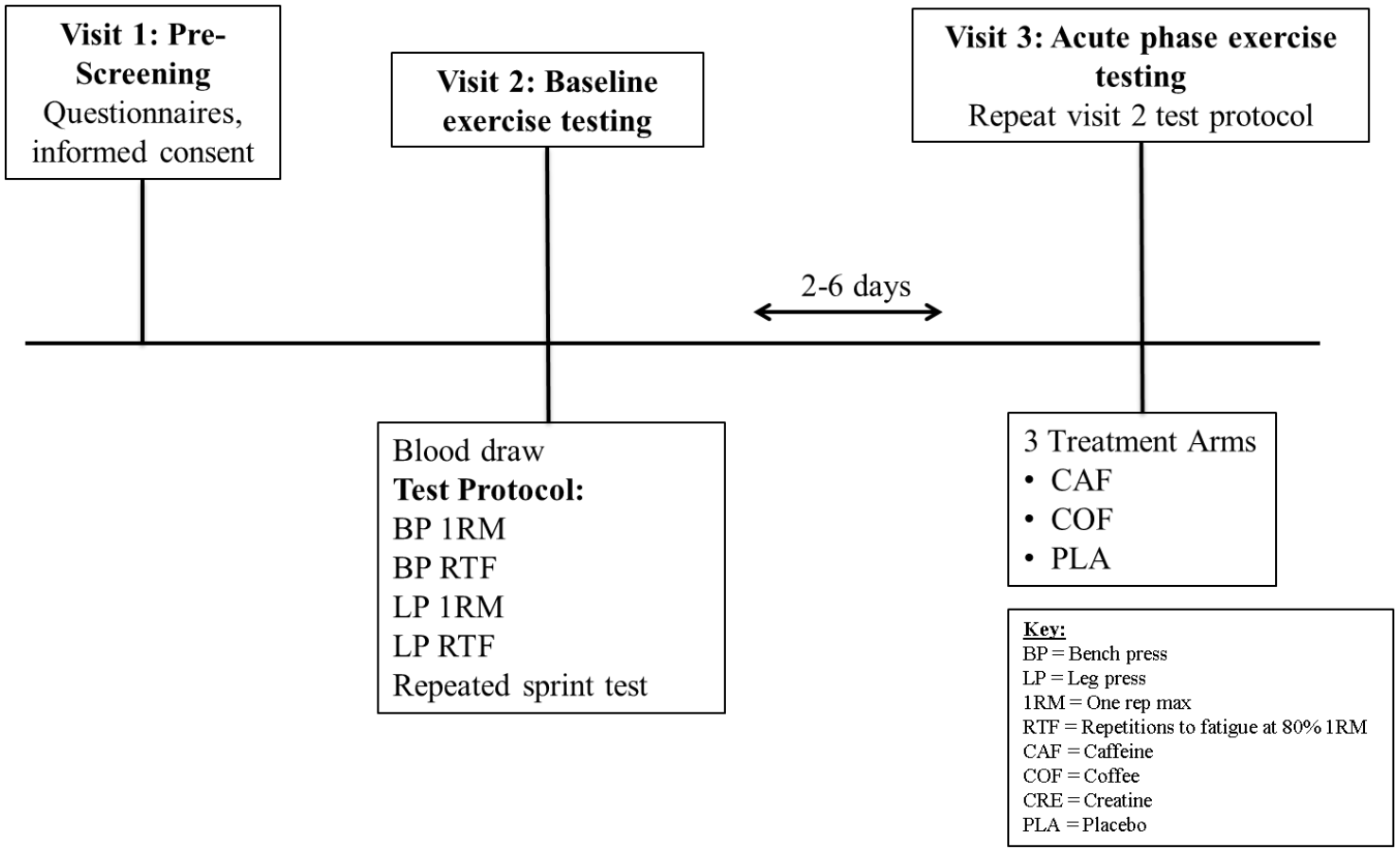

Figure 1: Experimental protocol schematic for the acute phase of supplementation

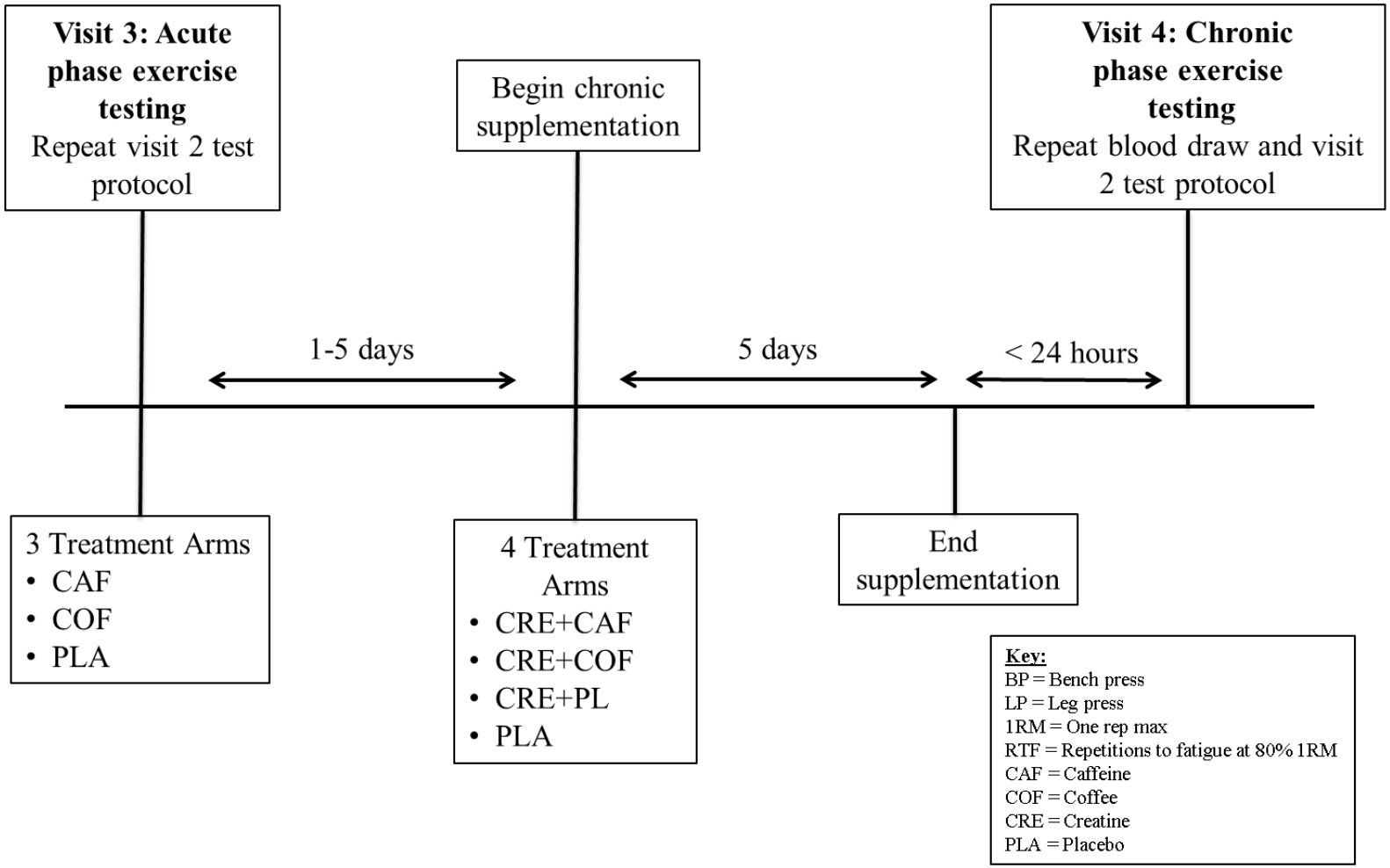

Figure 2: Experimental protocol schematic for the chronic phase of supplementation

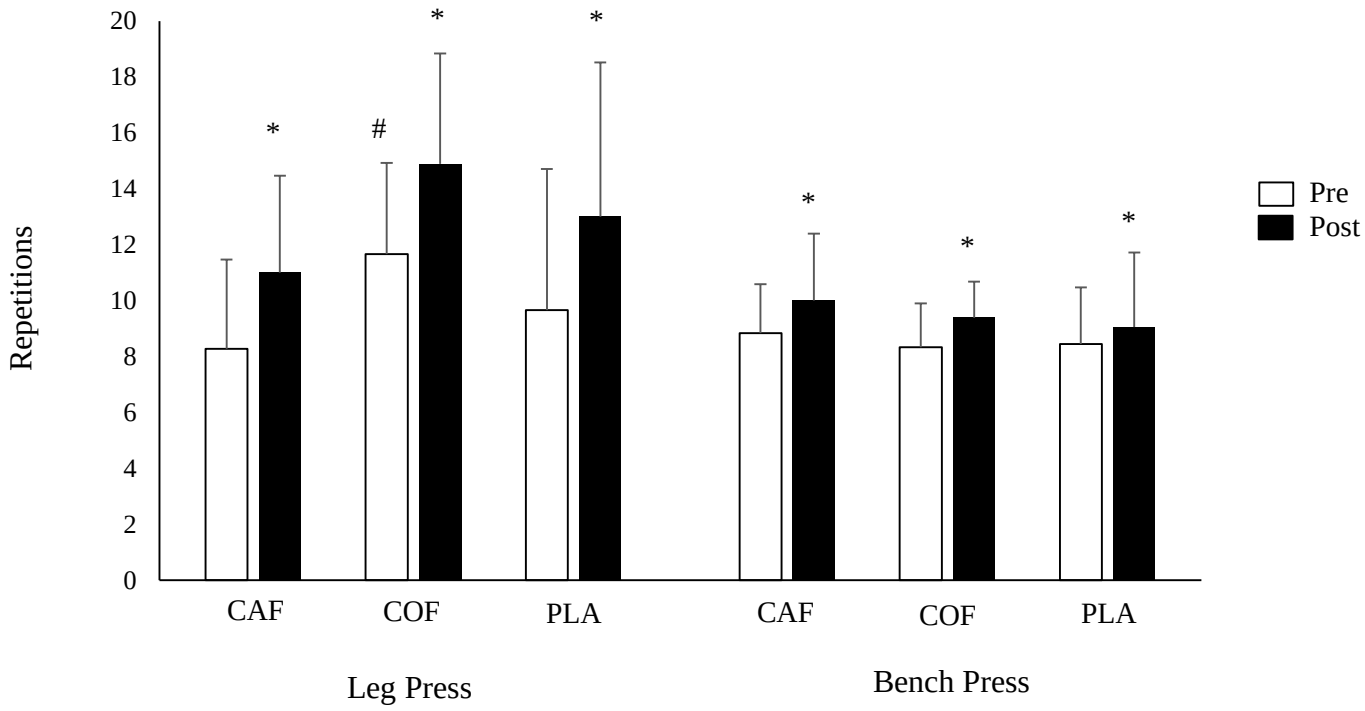

Figure 3. Effects of acute supplementation on leg press and bench press repetitions to fatigue. *Significant change from baseline; #significantly greater than CAF at baseline

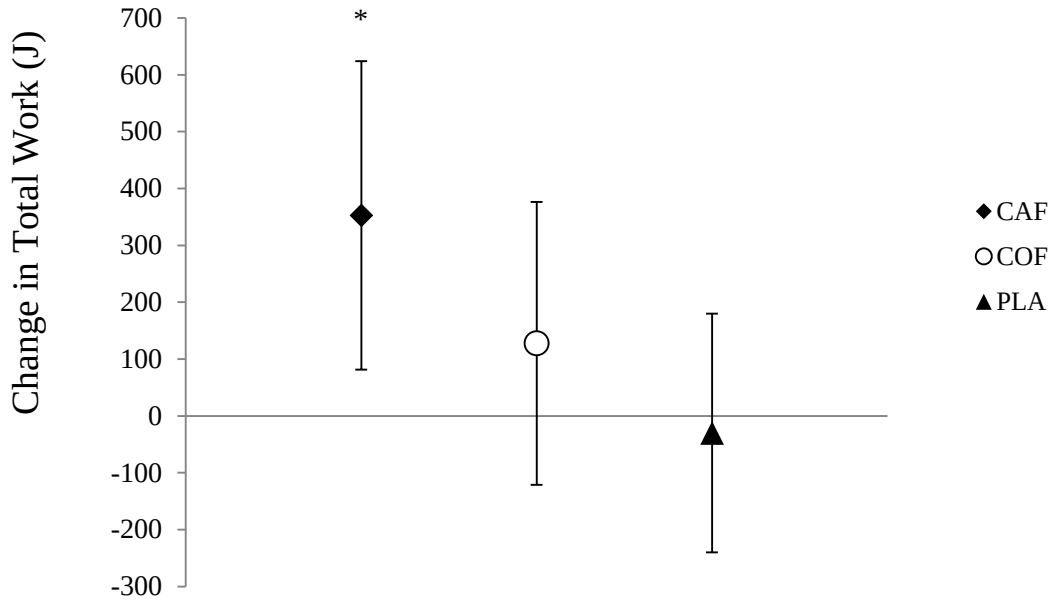

Figure 4: Changes in sprint 1 total work (TW; joules) after acute supplementation. *Significant change from baseline

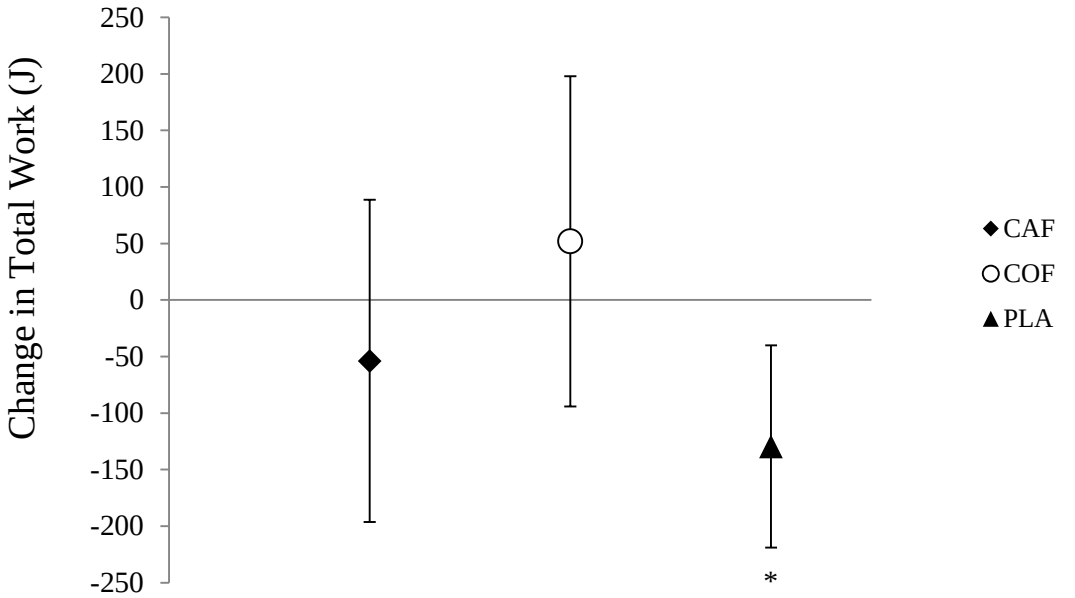

Figure 5. Changes in average total work (TW; joules) after acute supplementation. *Significant change from baseline

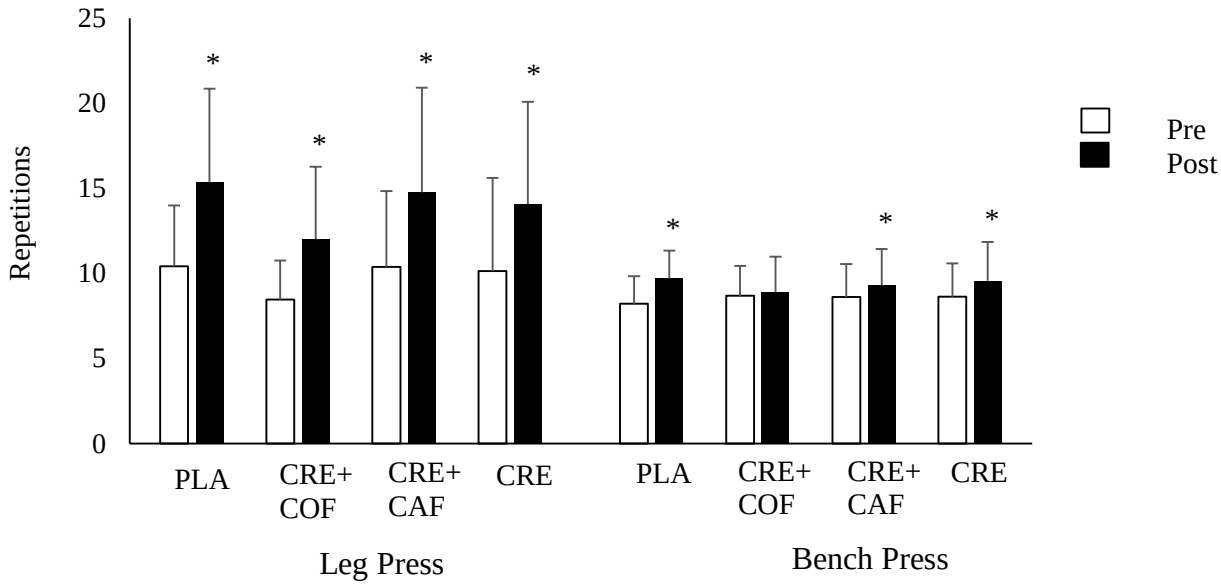

Figure 6: Effects of chronic supplementation on leg press and bench press repetitions to fatigue.

*Significant difference from baseline

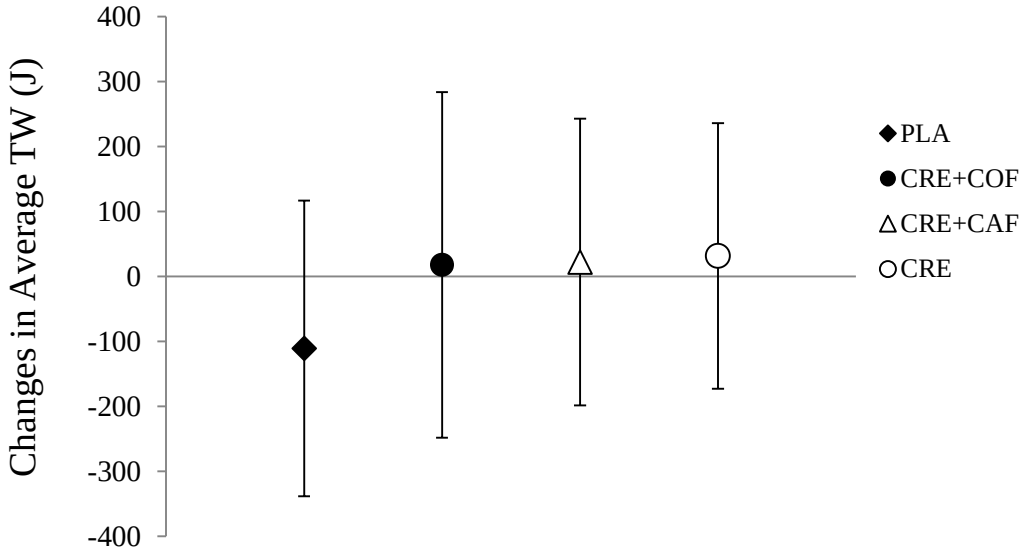

Figure 7: Changes in average total work (TW; joules) after chronic supplementation.

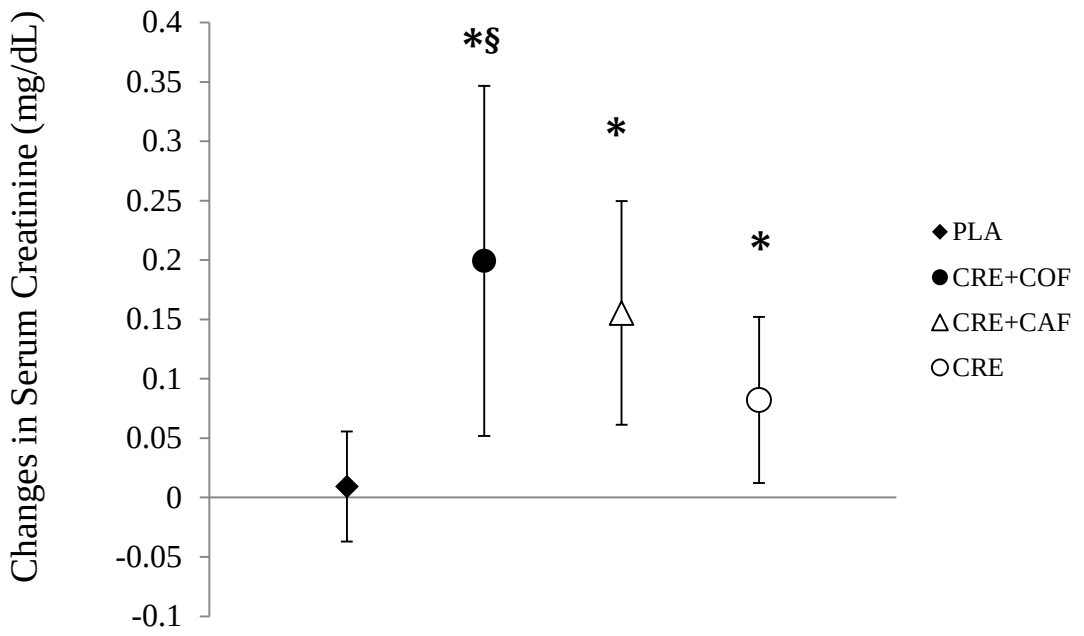

Figure 8: Changes in serum creatinine (mg/dL) after chronic supplementation. *Significant change from baseline value; § significantly greater change than placebo

REFERENCES

1. Froiland K, Koszewski W, Hingst J, Kopecky L. Nutritional supplement use among college athletes and their sources of information. International journal of sport nutrition and exercise metabolism. 2004 Feb;14(1):104-20. Epub 2004/05/08.

2. Cooper R, Naclerio F, Allgrove J, Jimenez A. Creatine supplementation with specific view to exercise/sports performance: an update. Journal of the International Society of Sports Nutrition. 2012;9(1):33. Epub 2012/07/24.

3. Persky AM, Brazeau GA. Clinical pharmacology of the dietary supplement creatine monohydrate. Pharmacological reviews. 2001 Jun;53(2):161-76. Epub 2001/05/18.

4. Bemben MG, Lamont HS. Creatine supplementation and exercise performance: recent findings. Sports medicine. 2005;35(2):107-25. Epub 2005/02/15.

5. Brosnan JT, da Silva RP, Brosnan ME. The metabolic burden of creatine synthesis. Amino acids. 2011 May;40(5):1325-31. Epub 2011/03/10.

6. Burke DG, Candow DG, Chilibeck PD, MacNeil LG, Roy BD, Tarnopolsky MA, et al. Effect of creatine supplementation and resistance-exercise training on muscle insulin-like growth factor in young adults. International journal of sport nutrition and exercise metabolism. 2008 Aug;18(4):389-98. Epub 2008/08/19.

7. Syrotuik DG, Bell GJ. Acute creatine monohydrate supplementation: a descriptive physiological profile of responders vs. nonresponders. Journal of strength and conditioning research / National Strength & Conditioning Association. 2004 Aug;18(3):610-7. Epub 2004/08/24.

8. Volek JS, Duncan ND, Mazzetti SA, Staron RS, Putukian M, Gomez AL, et al. Performance and muscle fiber adaptations to creatine supplementation and heavy resistance training. Medicine and science in sports and exercise. 1999 Aug;31(8):1147-56. Epub 1999/08/17.

9. Hultman E, Soderlund K, Timmons JA, Cederblad G, Greenhaff PL. Muscle creatine loading in men. Journal of applied physiology. 1996 Jul;81(1):232-7. Epub 1996/07/01.

10. Persky AM, Rawson ES. Safety of creatine supplementation. Sub-cellular biochemistry. 2007;46:275-89. Epub 2008/07/26.

11. Kreider RB. Effects of creatine supplementation on performance and training adaptations. Molecular and cellular biochemistry. 2003 Feb;244(1-2):89-94. Epub 2003/04/19.

12. Bemben MG, Witten MS, Carter JM, Eliot KA, Knehans AW, Bemben DA. The effects of supplementation with creatine and protein on muscle strength following a traditional resistance training program in middle-aged and older men. The journal of nutrition, health & aging. 2010 Feb;14(2):155-9. Epub 2010/02/04.

13. Jakobi JM, Rice CL, Curtin SV, Marsh GD. Contractile properties, fatigue and recovery are not influenced by short-term creatine supplementation in human muscle. Experimental physiology. 2000 Jul;85(4):451-60. Epub 2000/08/05.

14. Branch JD. Effect of creatine supplementation on body composition and performance: a meta-analysis. International journal of sport nutrition and exercise metabolism. 2003 Jun;13(2):198-226. Epub 2003/08/30.

15. Baker JS, McCormick MC, Robergs RA. Interaction among skeletal muscle metabolic energy systems during intense exercise. Journal of nutrition and metabolism. 2010;2010:905612. Epub 2010/12/29.

16. Buford TW, Kreider RB, Stout JR, Greenwood M, Campbell B, Spano M, et al. International Society of Sports Nutrition position stand: creatine supplementation and exercise. Journal of the International Society of Sports Nutrition. 2007;4:6. Epub 2007/10/03.

17. Deldicque L, Louis M, Theisen D, Nielens H, Dehoux M, Thissen JP, et al. Increased IGF mRNA in human skeletal muscle after creatine supplementation. Medicine and science in sports and exercise. 2005 May;37(5):731-6. Epub 2005/05/05.

18. Olsen S, Aagaard P, Kadi F, Tufekovic G, Verney J, Olesen JL, et al. Creatine supplementation augments the increase in satellite cell and myonuclei number in human skeletal muscle induced by strength training. The Journal of physiology. 2006 Jun 1;573(Pt 2):525-34. Epub 2006/04/04.

19. Willoughby DS, Rosene JM. Effects of oral creatine and resistance training on myogenic regulatory factor expression. Medicine and science in sports and exercise. 2003 Jun;35(6):923-9. Epub 2003/06/05.

20. Hespel P, Op't Eijnde B, Van Leemputte M, Urso B, Greenhaff PL, Labarque V, et al. Oral creatine supplementation facilitates the rehabilitation of disuse atrophy and alters the expression of muscle myogenic factors in humans. The Journal of physiology. 2001 Oct 15;536(Pt 2):625-33. Epub 2001/10/16.

21. Sokmen B, Armstrong LE, Kraemer WJ, Casa DJ, Dias JC, Judelson DA, et al. Caffeine use in sports: considerations for the athlete. Journal of strength and conditioning research / National Strength & Conditioning Association. 2008 May;22(3):978-86. Epub 2008/04/29.

22. Desbrow B, Leveritt M. Awareness and use of caffeine by athletes competing at the 2005 Ironman Triathlon World Championships. International journal of sport nutrition and exercise metabolism. 2006 Oct;16(5):545-58. Epub 2007/01/24.

23. Graham TE, Spriet LL. Performance and metabolic responses to a high caffeine dose during prolonged exercise. Journal of applied physiology. 1991 Dec;71(6):2292-8. Epub 1991/12/01.

24. Graham TE, Spriet LL. Metabolic, catecholamine, and exercise performance responses to various doses of caffeine. Journal of applied physiology. 1995 Mar;78(3):867-74. Epub 1995/03/01.

25. Mc Naughton LR, Lovell RJ, Siegler JC, Midgley AW, Sandstrom M, Bentley DJ. The effects of caffeine ingestion on time trial cycling performance. The Journal of sports medicine and physical fitness. 2008 Sep;48(3):320-5. Epub 2008/11/01.

26. Pasman WJ, van Baak MA, Jeukendrup AE, de Haan A. The effect of different dosages of caffeine on endurance performance time. International journal of sports medicine. 1995 May;16(4):225-30. Epub 1995/05/01.

27. Spriet LL, MacLean DA, Dyck DJ, Hultman E, Cederblad G, Graham TE. Caffeine ingestion and muscle metabolism during prolonged exercise in humans. The American journal of physiology. 1992 Jun;262(6 Pt 1):E891-8. Epub 1992/06/01.

28. Robertson D, Frolich JC, Carr RK, Watson JT, Hollifield JW, Shand DG, et al. Effects of caffeine on plasma renin activity, catecholamines and blood pressure. The New England journal of medicine. 1978 Jan 26;298(4):181-6. Epub 1978/01/26.

29. Mumford GK, Benowitz NL, Evans SM, Kaminski BJ, Preston KL, Sannerud CA, et al. Absorption rate of methylxanthines following capsules, cola and chocolate. European journal of clinical pharmacology. 1996;51(3-4):319-25. Epub 1996/01/01.

30. Goldstein ER, Ziegenfuss T, Kalman D, Kreider R, Campbell B, Wilborn C, et al. International society of sports nutrition position stand: caffeine and performance. Journal of the International Society of Sports Nutrition. 2010;7(1):5. Epub 2010/03/09.

31. Collomp K, Ahmaidi S, Audran M, Chanal JL, Prefaut C. Effects of caffeine ingestion on performance and anaerobic metabolism during the Wingate Test. International journal of sports medicine. 1991 Oct;12(5):439-43. Epub 1991/10/01.

32. Crowe MJ, Leicht AS, Spinks WL. Physiological and cognitive responses to caffeine during repeated, high-intensity exercise. International journal of sport nutrition and exercise metabolism. 2006 Oct;16(5):528-44. Epub 2007/01/24.

33. Greer F, McLean C, Graham TE. Caffeine, performance, and metabolism during repeated Wingate exercise tests. Journal of applied physiology. 1998 Oct;85(4):1502-8. Epub 1998/10/07.

34. Lorino AJ, Lloyd LK, Crixell SH, Walker JL. The effects of caffeine on athletic agility. Journal of strength and conditioning research / National Strength & Conditioning Association. 2006 Nov;20(4):851-4. Epub 2006/12/30.

35. Anselme F, Collomp K, Mercier B, Ahmaidi S, Prefaut C. Caffeine increases maximal anaerobic power and blood lactate concentration. European journal of applied physiology and occupational physiology. 1992;65(2):188-91. Epub 1992/01/01.

36. Collomp K, Ahmaidi S, Chatard JC, Audran M, Prefaut C. Benefits of caffeine ingestion on sprint performance in trained and untrained swimmers. European journal of applied physiology and occupational physiology. 1992;64(4):377-80. Epub 1992/01/01.

37. MacIntosh BR, Wright BM. Caffeine ingestion and performance of a 1,500-metre swim. Can J Appl Physiol. 1995 Jun;20(2):168-77. Epub 1995/06/01.

38. Schneiker KT, Bishop D, Dawson B, Hackett LP. Effects of caffeine on prolonged intermittent-sprint ability in team-sport athletes. Medicine and science in sports and exercise. 2006 Mar;38(3):578-85. Epub 2006/03/17.

39. Woolf K, Bidwell WK, Carlson AG. The effect of caffeine as an ergogenic aid in anaerobic exercise. International journal of sport nutrition and exercise metabolism. 2008 Aug;18(4):412-29. Epub 2008/08/19.

40. Beck TW, Housh TJ, Schmidt RJ, Johnson GO, Housh DJ, Coburn JW, et al. The acute effects of a caffeine-containing supplement on strength, muscular endurance, and anaerobic capabilities. Journal of strength and conditioning research / National Strength & Conditioning Association. 2006 Aug;20(3):506-10. Epub 2006/08/30.

41. Woolf K, Bidwell WK, Carlson AG. Effect of caffeine as an ergogenic aid during anaerobic exercise performance in caffeine naive collegiate football players. Journal of strength and conditioning research / National Strength & Conditioning Association. 2009 Aug;23(5):1363-9. Epub 2009/07/22.

42. Astorino TA, Rohmann RL, Firth K. Effect of caffeine ingestion on one-repetition maximum muscular strength. European journal of applied physiology. 2008 Jan;102(2):127-32. Epub 2007/09/14.

43. Barone JJ, Roberts HR. Caffeine consumption. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association. 1996 Jan;34(1):119-29. Epub 1996/01/01.

44. Lieberman HR. Nutrition, brain function and cognitive performance. Appetite. 2003 Jun;40(3):245-54. Epub 2003/06/12.

45. Wiles JD, Bird SR, Hopkins J, Riley M. Effect of caffeinated coffee on running speed, respiratory factors, blood lactate and perceived exertion during 1500-m treadmill running. British journal of sports medicine. 1992 Jun;26(2):116-20. Epub 1992/06/01.

46. McLellan TM, Bell DG. The impact of prior coffee consumption on the subsequent ergogenic effect of anhydrous caffeine. International journal of sport nutrition and exercise metabolism. 2004 Dec;14(6):698-708. Epub 2005/01/20.

47. Graham TE, Hibbert E, Sathasivam P. Metabolic and exercise endurance effects of coffee and caffeine ingestion. Journal of applied physiology. 1998 Sep;85(3):883-9. Epub 1998/09/08.

48. Ormsbee MJ, Thomas DD, Mandler WK, Ward EG, Kinsey AW, Panton LB, et al. The effects of pre- and post-exercise consumption of multi-ingredient performance supplements on cardiovascular health and body fat in trained men after six weeks of resistance training: a stratified, randomized, double-blind study. Nutrition & metabolism. 2013;10(1):39. Epub 2013/05/18.

49. Smith AE, Fukuda DH, Kendall KL, Stout JR. The effects of a pre-workout supplement containing caffeine, creatine, and amino acids during three weeks of high-intensity exercise on aerobic and anaerobic performance. Journal of the International Society of Sports Nutrition. 2010;7:10. Epub 2010/02/17.

50. Spradley BD, Crowley KR, Tai CY, Kendall KL, Fukuda DH, Esposito EN, et al. Ingesting a pre-workout supplement containing caffeine, B-vitamins, amino acids, creatine, and beta-alanine before exercise delays fatigue while improving reaction time and muscular endurance. Nutrition & metabolism. 2012;9:28. Epub 2012/04/03.

51. Vandenberghe K, Gillis N, Van Leemputte M, Van Hecke P, Vanstapel F, Hespel P. Caffeine counteracts the ergogenic action of muscle creatine loading. Journal of applied physiology. 1996 Feb;80(2):452-7. Epub 1996/02/01.

52. Hespel P, Op't Eijnde B, Van Leemputte M. Opposite actions of caffeine and creatine on muscle relaxation time in humans. Journal of applied physiology. 2002 Feb;92(2):513-8. Epub 2002/01/18.

53. Birch R, Noble D, Greenhaff PL. The influence of dietary creatine supplementation on performance during repeated bouts of maximal isokinetic cycling in man. European journal of applied physiology and occupational physiology. 1994;69(3):268-76. Epub 1994/01/01.

54. Dentkowski A, Opaszwoski B, Blachnio D, Polanowski B. Effect of creatine supplementation on the performance in supramaximal, intermittent exercise. Biology of Sport. 1997;14:291-8.

55. Greenhaff PL, Bodin K, Soderlund K, Hultman E. Effect of oral creatine supplementation on skeletal muscle phosphocreatine resynthesis. The American journal of physiology. 1994 May;266(5 Pt 1):E725-30. Epub 1994/05/01.

56. Komes D, Horžić D, Belščak A, Ganić KK, Vulić I. Green tea preparation and its influence on the content of bioactive compounds. Food research international. 2010 1//;43(1):167-76.

57. Rodrigues NP, Bragagnolo N. Identification and quantification of bioactive compounds in coffee brews by HPLC–DAD–MSn. Journal of food composition and analysis. 2013 12//;32(2):105-15.

58. Heckman MA, Weil J, Gonzalez de Mejia E. Caffeine (1, 3, 7-trimethylxanthine) in foods: a comprehensive review on consumption, functionality, safety, and regulatory matters. Journal of food science. 2010 Apr;75(3):R77-87. Epub 2010/05/25.

59. Nawrot P, Jordan S, Eastwood J, Rotstein J, Hugenholtz A, Feeley M. Effects of caffeine on human health. Food additives and contaminants. 2003 Jan;20(1):1-30. Epub 2003/01/10.

60. The World Anti-Doping Code: The 2014 Prohibited List. World Anti-Doping Agency, 11 September 2013.

61. Hodgson AB, Randell RK, Jeukendrup AE. The metabolic and performance effects of caffeine compared to coffee during endurance exercise. PloS one. 2013;8(4):e59561. Epub 2013/04/11.

62. Smith-Ryan AE, Ryan ED, Fukuda DH, Costa PB, Cramer JT, Stout JR. The effect of creatine loading on neuromuscular fatigue in women. Medicine and science in sports and exercise. 2013 Oct 22. Epub 2013/10/25.

63. Volek JS, Kraemer WJ, Bush JA, Boetes M, Incledon T, Clark KL, et al. Creatine supplementation enhances muscular performance during high-intensity resistance exercise. Journal of the American Dietetic Association. 1997 Jul;97(7):765-70. Epub 1997/07/01.

64. Izquierdo M, Ibanez J, Gonzalez-Badillo JJ, Gorostiaga EM. Effects of creatine supplementation on muscle power, endurance, and sprint performance. Medicine and science in sports and exercise. 2002 Feb;34(2):332-43. Epub 2002/02/06.

65. Aguiar AF, Januario RS, Junior RP, Gerage AM, Pina FL, do Nascimento MA, et al. Long-term creatine supplementation improves muscular performance during resistance training in older women. European journal of applied physiology. 2013 Apr;113(4):987-96. Epub 2012/10/12.

66. Mujika I, Padilla S, Ibanez J, Izquierdo M, Gorostiaga E. Creatine supplementation and sprint performance in soccer players. Medicine and science in sports and exercise. 2000 Feb;32(2):518-25. Epub 2000/02/29.

67. Theodorou AS, Cooke CB, King RF, Hood C, Denison T, Wainwright BG, et al. The effect of longer-term creatine supplementation on elite swimming performance after an acute creatine loading. Journal of sports sciences. 1999 Nov;17(11):853-9. Epub 1999/12/10.

68. Preen D, Dawson B, Goodman C, Lawrence S, Beilby J, Ching S. Effect of creatine loading on long-term sprint exercise performance and metabolism. Medicine and science in sports and exercise. 2001 May;33(5):814-21. Epub 2001/04/27.

69. Skare OC, Skadberg, Wisnes AR. Creatine supplementation improves sprint performance in male sprinters. Scandinavian journal of medicine & science in sports. 2001 Apr;11(2):96-102. Epub 2001/03/17.

70. McKenna MJ, Morton J, Selig SE, Snow RJ. Creatine supplementation increases muscle total creatine but not maximal intermittent exercise performance. Journal of applied physiology. 1999 Dec;87(6):2244-52. Epub 1999/12/22.

71. Finn JP, Ebert TR, Withers RT, Carey MF, Mackay M, Phillips JW, et al. Effect of creatine supplementation on metabolism and performance in humans during intermittent sprint cycling. European journal of applied physiology. 2001 Mar;84(3):238-43. Epub 2001/04/26.

72. Doherty M, Smith PM. Effects of caffeine ingestion on rating of perceived exertion during and after exercise: a meta-analysis. Scandinavian journal of medicine & science in sports. 2005 Apr;15(2):69-78. Epub 2005/03/19.

73. Tallis J, James RS, Cox VM, Duncan MJ. The effect of physiological concentrations of caffeine on the power output of maximally and submaximally stimulated mouse EDL (fast) and soleus (slow) muscle. Journal of applied physiology. 2012 Jan;112(1):64-71. Epub 2011/10/08.

74. Mohr M, Nielsen JJ, Bangsbo J. Caffeine intake improves intense intermittent exercise performance and reduces muscle interstitial potassium accumulation. Journal of applied physiology. 2011 Nov;111(5):1372-9. Epub 2011/08/13.

75. Wiles JD, Coleman D, Tegerdine M, Swaine IL. The effects of caffeine ingestion on performance time, speed and power during a laboratory-based 1 km cycling time-trial. Journal of sports sciences. 2006 Nov;24(11):1165-71. Epub 2006/10/13.

76. Costill DL, Dalsky GP, Fink WJ. Effects of caffeine ingestion on metabolism and exercise performance. Medicine and science in sports. 1978 Fall;10(3):155-8. Epub 1978/01/01.

77. Crozier TW, Stalmach A, Lean ME, Crozier A. Espresso coffees, caffeine and chlorogenic acid intake: potential health implications. Food & function. 2012 Jan;3(1):30-3. Epub 2011/12/02.

78. de Paulis T, Schmidt DE, Bruchey AK, Kirby MT, McDonald MP, Commers P, et al. Dicinnamoylquinides in roasted coffee inhibit the human adenosine transporter. European journal of pharmacology. 2002 May 10;442(3):215-23. Epub 2002/06/18.

79. Vanakoski J, Kosunen V, Meririnne E, Seppala T. Creatine and caffeine in anaerobic and aerobic exercise: effects on physical performance and pharmacokinetic considerations. International journal of clinical pharmacology and therapeutics. 1998 May;36(5):258-62. Epub 1998/06/18.

80. Quesada T, Gillum T. Effect of acute creatine supplementation and subsequent caffeine ingestion on ventilatory anaerobic threshold. Journal of Exercise Physiology Online. 2013;16(4):112-20.

81. Harris RC, Sale C, Delves SK. Modification of the ergogenic effects of creatine loading by caffeine [Abstract]. Medicine and science in sports and exercise. 2005;37(5):S348-S9.

82. Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. Behavior research methods. 2009 Nov;41(4):1149-60. Epub 2009/11/10.

83. Wiroth JB, Bermon S, Andrei S, Dalloz E, Hebuterne X, Dolisi C. Effects of oral creatine supplementation on maximal pedalling performance in older adults. European journal of applied physiology. 2001 Jun;84(6):533-9. Epub 2001/08/03.

84. Allen DG, Westerblad H. The effects of caffeine on intracellular calcium, force and the rate of relaxation of mouse skeletal muscle. The Journal of physiology. 1995 Sep 1;487 (Pt 2):331-42. Epub 1995/09/01.

85. Weber A, Herz R. The relationship between caffeine contracture of intact muscle and the effect of caffeine on reticulum. The Journal of general physiology. 1968 Nov;52(5):750-9. Epub 1968/11/01.

86. Gonzalez de Mejia E, Ramirez-Mares MV. Impact of caffeine and coffee on our health. Trends in endocrinology and metabolism: TEM. 2014 Oct;25(10):489-92. Epub 2014/08/16.

87. Cramer JT. Creatine Supplementation in Endurance Sports. In: Stout JR, Antonio J, Kalman D, editors. Essentials of Creatine in Sports and Health: Humana Press; 2008. p. 45-99.

88. Mohr T, Van Soeren M, Graham TE, Kjaer M. Caffeine ingestion and metabolic responses of tetraplegic humans during electrical cycling. Journal of applied physiology. 1998 Sep;85(3):979-85. Epub 1998/09/08.

89. Freedman ND, Park Y, Abnet CC, Hollenbeck AR, Sinha R. Association of Coffee Drinking with Total and Cause-Specific Mortality. New England Journal of Medicine. 2012;366(20):1891-904.

90. Lowery RP, Joy JM, Dudeck JE, Oliveira de Souza E, McCleary SA, Wells S, et al. Effects of 8 weeks of Xpand(R) 2X pre workout supplementation on skeletal muscle hypertrophy, lean body mass, and strength in resistance trained males. Journal of the International Society of Sports Nutrition. 2013;10(1):44. Epub 2013/10/11.

91. Ormsbee MJ, Mandler WK, Thomas DD, Ward EG, Kinsey AW, Simonavice E, et al. The effects of six weeks of supplementation with multi-ingredient performance supplements and resistance training on anabolic hormones, body composition, strength, and power in resistance-trained men. Journal of the International Society of Sports Nutrition. 2012;9(1):49. Epub 2012/11/17.

92. Spillane M, Schwarz N, Leddy S, Correa T, Minter M, Longoria V, et al. Effects of 28 days of resistance exercise while consuming commercially available pre- and post-workout supplements, NO-Shotgun(R) and NO-Synthesize(R) on body composition, muscle strength and mass, markers of protein synthesis, and clinical safety markers in males. Nutrition & metabolism. 2011;8:78. Epub 2011/11/05.

93. Shelmadine B, Cooke M, Buford T, Hudson G, Redd L, Leutholtz B, et al. Effects of 28 days of resistance exercise and consuming a commercially available pre-workout supplement, NO-Shotgun(R), on body composition, muscle strength and mass, markers of satellite cell activation, and clinical safety markers in males. Journal of the International Society of Sports Nutrition. 2009;6:16. Epub 2009/08/07.

94. Harris RC, Soderlund K, Hultman E. Elevation of creatine in resting and exercised muscle of normal subjects by creatine supplementation. Clinical science. 1992 Sep;83(3):367-74. Epub 1992/09/01.

95. Zuniga JM, Housh TJ, Camic CL, Hendrix CR, Mielke M, Johnson GO, et al. The effects of creatine monohydrate loading on anaerobic performance and one-repetition maximum strength. Journal of strength and conditioning research / National Strength & Conditioning Association. 2012 Jun;26(6):1651-6. Epub 2011/09/17.

96. Vandenberghe K, Goris M, Van Hecke P, Van Leemputte M, Vangerven L, Hespel P. Long-term creatine intake is beneficial to muscle performance during resistance training. Journal of applied physiology. 1997 Dec;83(6):2055-63. Epub 1998/02/14.

97. Baxmann AC, Ahmed MS, Marques NC, Menon VB, Pereira AB, Kirsztajn GM, et al. Influence of muscle mass and physical activity on serum and urinary creatinine and serum cystatin C. Clinical journal of the American Society of Nephrology : CJASN. 2008 Mar;3(2):348-54. Epub 2008/02/01.

98. Yoshizumi WM, Tsourounis C. Effects of creatine supplementation on renal function. Journal of herbal pharmacotherapy. 2004;4(1):1-7. Epub 2004/07/27.