THE EFFECT OF CAFFEINE INGESTION AFTER CREATINE SUPPLEMENTATION ON PEAK TORQUE PRODUCTION

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ABSTRACT

The purpose of this study was to examine the acute effects of caffeine ingestion on isokinetic torque production after a period of oral creatine supplementation and caffeine abstinence. Twelve sports university students performed 5 sets of 30 unilateral knee extensions under baseline (BASE), creatine (CRE) and creatine plus caffeine (CRE+CAF) conditions. Each set of knee extensions was performed at maximal intensity and was separated by 60 seconds of rest. Following the establishment of the BASE condition, participants were administered CRE (0.3 mg.kg^{-1}.day^{-1} of creatine for 6 days followed by a placebo 1 h prior to testing) or CRE+CAF (0.3 mg.kg^{-1}.day^{-1} of creatine for 6 days followed by 3 mg.kg^{-1} of caffeine 1 h prior to testing) in a randomized order, with a 4-week washout period separating the conditions. CRE (164.7 ± 31.3 N·m, 4858.7 ± 977.2 W) and CRE+CAF (170.8 ± 33.2 N·m, 5037.9 ± 946.9 W) significantly increased ($p<0.05$) peak torque and total work in set 1 compared to BASE (154.8 ± 35.2 N·m, 4513.2 ± 1105.6 W). However, there was no significant difference in peak torque or total work between CRE and CRE+CAF in set 1 ($p>0.05$). In addition, neither peak torque nor total work significantly differed in subsequent sets between conditions ($p>0.05$). Heart rate (HR) was significantly elevated ($p<0.05$) in CRE+CAF (156.6 ± 5.4 bpm) compared to BASE (146.1 ± 4.6 bpm) in all sets. In conclusion, these data suggest short-term creatine supplementation can augment initial torque production in the first bout of isokinetic exercise. However, a single dose of caffeine following creatine loading may not provide an additive ergogenic effect on isokinetic performance.

INTRODUCTION

Creatine is an essential source of chemical energy for muscle contraction because it facilitates phosphorylation that is both rapid, with the formation of phosphocreatine (PCr), and reversible, with donation of the phosphate group to adenosine diphosphate (ADP) to form adenosine triphosphate (ATP). This phosphorylation-dephosphorylation reaction, catalysed by the enzyme creatine kinase, is a rapid source of high-energy phosphate for performing high-intensity, short-duration exercise (Izquierdo et al., 2002). However, the supply of muscle PCr is limited, and its depletion is associated with a decline in force production during such activity (Harris et al., 1992). Supplementing with creatine has consistently been shown to enhance PCr stores in skeletal muscle (e.g., Greenhaff et al., 1994; Kreider et al., 2003) and provides

additional substrate that can be used to augment ATP resynthesis during episodes of muscle relaxation (Terjung et al., 2000; Buford et al., 2007).

Another popular ergogenic aid that has routinely been used by athletes and investigated over several decades is caffeine. While the effect caffeine elicits on endurance performance is well founded (e.g., Lindinger et al., 1993; Graham et al., 1998), there is now introductory evidence supporting its use to improve high-intensity exercise performance (e.g., Bliss et al., 2008; Pallarés et al., 2013) and isokinetic peak torque (Jacobson et al., 1992). Considering the regularity with which creatine and caffeine are administered concurrently, and their well-established efficacy in isolation, it is logical to speculate that co-ingesting creatine and caffeine might have an additive effect on performance. Accordingly, Vandenberge et al. (1996) investigated the potential synergistic effects of creatine and caffeine co-ingestion and suggested that caffeine, a potent sympathomimetic, might facilitate the uptake of exogenous creatine via adrenergic stimulation of the sarcolemma (Clausen, 1986; 2003). Results revealed that creatine administration enhanced torque production, but surprisingly this ergogenic effect was completely eliminated when caffeine was ingested simultaneously with creatine. Creatine alone and in combination with caffeine both increased muscle PCr stores to the same extent, but only the former was beneficial to performance. This concept is consistent with a report by Greenhaff et al. (1994) showing the muscle PCr resynthesis, rather than an elevated muscle PCr pool *per se*, to be critical to the ergogenic action of creatine loading. Thus, these findings suggest that chronic caffeine administration does not impair muscle PCr availability, but may inhibit rapid ATP rephosphorylation during episodes of muscle relaxation. Subsequent studies (Vanakoski et al., 1998; Hespel et al., 2002) have substantiated this finding. It appears that caffeine prolongs muscle relaxation time and by this action overrides the shortening of relaxation time induced by creatine supplementation (Hespel et al., 2002). However, Hespel et al. (2002) also noted that acute caffeine intake does not impair muscle relaxation. Thus, an alternative strategy to optimize the independent effects of creatine and caffeine, and to minimize the interference of combining these nutritional aids, would be to supplement with creatine while abstaining from caffeine for the duration of the loading period. Ingestion of a single dose of caffeine, theoretically, could then be taken before exercise for an additive ergogenic effect.

Doherty et al. (2002) explored this notion and found that an acute dose of caffeine after a period of creatine loading with caffeine abstinence improved running time to exhaustion at an exercise intensity equivalent to 125% VO_2max. The magnitude of this performance improvement (10.7%) exceeds the reported error of measurement for this exercise protocol (5%, coefficient of variation; Doherty et al., 2000), and therefore can be considered 'worthwhile'. Consistent with this, Lee et al. (2011) also found this strategy to improve intermittent high-intensity sprint performance on a cycle ergometer. More recently, Lee et al. (2012) found acute caffeine ingestion following creatine supplementation significantly lengthened time to exhaustion during a maximal incremental exercise test. This provides further evidence to support the assertion that caffeine is ergogenic after creatine loading and caffeine abstinence. Notwithstanding, the aforementioned studies have administered either 5 mg.kg^{-1} (Doherty et al., 2002) or 6 mg.kg^{-1} (Lee et al., 2011; 2012) of caffeine to participants, despite substantial evidence demonstrating no additional performance benefits of caffeine doses above 3 mg.kg^{-1} (Kovacs et al., 1998; Graham, 2001; Desbrow et al., 2012). Furthermore,

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doses of between 6-9 mg.kg^{-1} have been associated with various side effects that may be ergolytic during sustained exercise (Magkos & Kavouras, 2005; Pallarés et al*.,* 2013). Thus, the available literature infers that 3 mg.kg^{-1} of caffeine may be the optimal dose to induce performance improvements with a minimal propensity for side effects (Graham, 2001).

While an acute caffeine dose following creatine supplementation has consistently been shown to augment endurance performance, no evidence exists to clarify whether this supplemental regime could facilitate improvements in isokinetic strength. Gains in muscular strength may subsequently induce improvements in performance during short-term, high-intensity exercise (Hickson et al*.,* 1988) by enhancing muscle economy (Støren et al*.,* 2008) and the rate of force development (Aagaard et al*.,* 2002). Therefore, the purpose of this study was to evaluate the effect of acute caffeine ingestion (3 mg.kg^{-1}) on peak torque production after pre-loading with creatine for 6 days (0.3 mg.kg^{-1}.day^{-1}) and abstinence from caffeine.

METHODS

Participants and Sampling
Twelve male sports university students (mean ± SD; age: 21 ± 1 years; body mass: 83 ± 11 kg; height: 179 ± 6 cm) volunteered for the study. All participants were physically active, but none were highly trained. An important inclusion criteria for participants was that they were exercising at a moderate to high intensity two or more times a week. Additional inclusion criterion were that participants had experience in both resistance training and maximal exercise within the last month and were also accustomed to ingesting caffeine via food and/or beverages at >50 mg.day^{-1}, which was monitored via a caffeine consumption questionnaire. Participants were informed of the experimental procedures to be undertaken and received instructions regarding the supplementation during the study. All participants gave their written consent and completed a pre-exercise screening questionnaire before participating in the study. This study was approved by York St John's University Ethics Committee.

Experimental Design
A double-blind, randomized crossover and counterbalancing experimental design was used in the study (Fig. 1). Participants completed a familiarization session to become habituated to the test protocol. One week later, participants completed a BASE trial without any ergogenic aids, which included the exercise test and taking capillary blood samples during the exercise test. Subsequently, participants completed two experimental trials that were separated by a washout period of four weeks and randomized using the William's square technique (see Kuehl, 2000). Before both experimental trials, participants were loaded with creatine for a 6 d period. On the next day, participants then ingested either an acute dose of caffeine (CRE+CAF) or a placebo beverage (CRE) 1 h prior to the exercise test. After the final trial, participants completed a questionnaire that asked whether they had abstained from caffeine for the duration of the study.
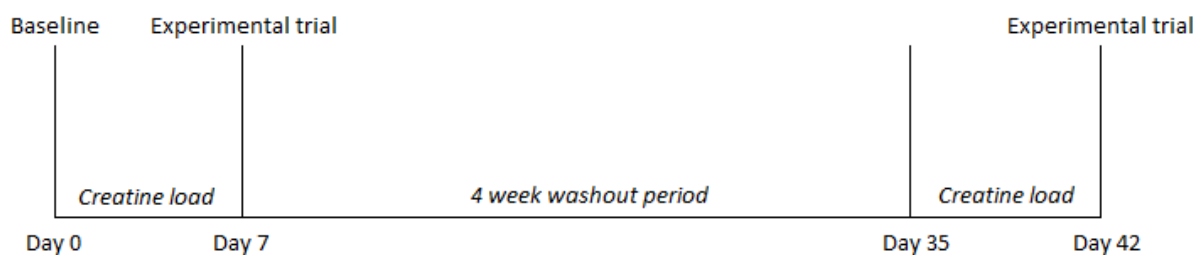
Figure 1. Schematic of the experimental period

Supplementation

Participants ingested creatine monohydrate (0.3 g.kg^{-1}.day^{-1}) over a 6 d period, with the dose split over 4 times per day. Participants were instructed to mix the creatine in 250 mL of warm water for improved dissolution (Harris et al., 1992) and ingested the solution with their morning, mid-day, afternoon and evening meals. Participants then returned to the laboratory and ingested caffeine at a dosage of 3 mg.kg^{-1} in powder dissolved in solution, or a placebo beverage of equal volume, 60 min prior to the exercise trial. Both creatine and caffeine were sourced via Sports Supplements Ltd, Colchester, UK, and distributed by an experienced technician.

Exercise Protocol

The exercise test consisted of unilateral knee extensions performed with the non-dominant leg in a sitting position on a Cybex II isokinetic dynamometer (Computer Sports Medicine Inc., Stoughton, MA, USA). During each experimental trial (Fig. 2), participants completed 5 sets of 30 isokinetic knee extensions at a constant angular velocity of 180°/s. Each set of knee extensions was performed at maximal intensity and was separated by 60 seconds of rest. One bout of 30 maximal voluntary contractions at this velocity has previously been shown to result in significant PCr degradation in both type I and type II skeletal muscle fibres of the quadriceps muscle group (Tesch et al., 1989). Each maximal voluntary contraction was initiated from a position of 90° knee flexion and continued through to the point of full knee extension. Before each test, participants completed a standardized warm up of 10 isokinetic knee extensions at an angular velocity of 180°/s and then rested for 2 min before the testing began. Subsequently, participants were instructed to fully extend and flex the knee with no pause between contractions. Participants were encouraged using positive, verbal praise throughout and frequently reminded to maintain a maximal intensity rather than pace their efforts. During testing, each participant was secured in a sitting position with a strap to restrain the thigh of the test leg and the upper trunk. After each set, participants were instructed to take 1 min of rest before the onset of the next set. At the commencement of each rest period, transient values for heart rate, using a Polar heart rate monitor (Polar Electro, Kempele, Finland), and rating of perceived exertion (RPE), using a standard 6-20 Borg scale (Borg, 1998), were recorded. The knee strap was released during each rest period to ensure blood flow to the quadriceps was not restricted. Knee torque production (N·m), fatigue index (N·m) and total work (W) for extension were recorded during each contraction. Capillary blood samples were collected by an experienced technician immediately before and after the exercise test. Subsequently, blood samples were analysed for plasma lactate concentration using an Analox Microstat P-GM7 (Analox Instruments Ltd, London, UK). Following the cessation of the exercise test, participants self-selected an appropriate cool down procedure.
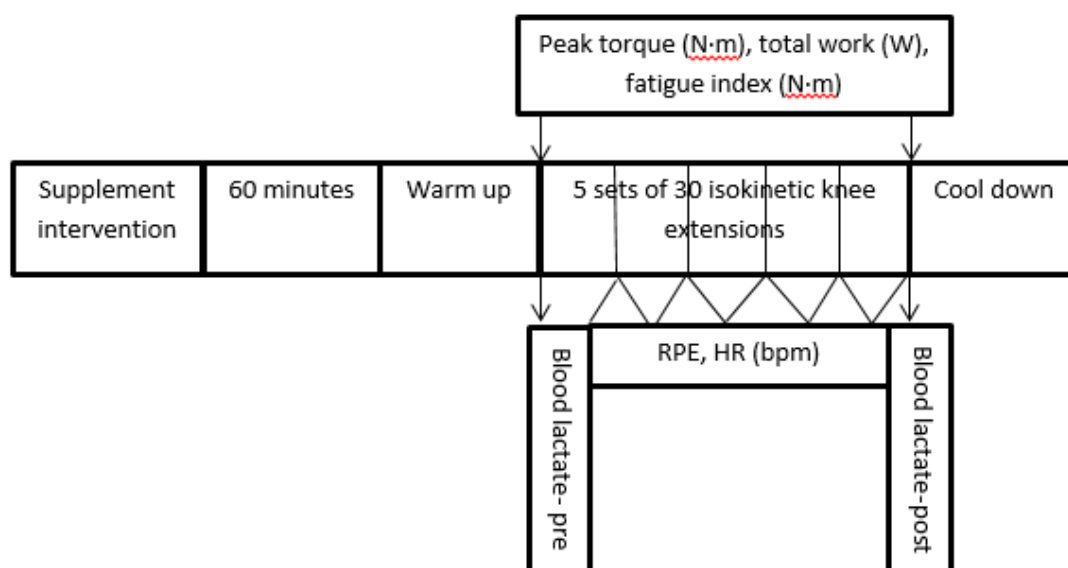
Figure 2. Design of the experimental trials on day 7, following 6 d of creatine supplementation

Dietary and Exercise Standards
Participants were instructed to avoid changes in their usual level of physical activity and dietary habits during the entire experimental period, which was monitored via a self-report diary. 48 hours prior to each experimental trial, participants were also instructed to refrain from heavy exercise to minimize interference. In addition, participants were educated about caffeine-containing food and beverages via the caffeine consumption questionnaire and subsequently instructed to abstain from caffeine for the entire duration of the experimental period. Furthermore, any participants previously supplemented with creatine stopped at least four weeks prior to the beginning of the study to ensure PCr stores had returned to normal levels after cessation of creatine supplementation (Vandenberghe et al*.,* 1997).

Data analysis
The SPSS statistical program was used for evaluation and calculation of the data. Data homogeneity and normality were confirmed using Mauchly's test of Sphericity. Throughout the exercise test, peak torque, total work and fatigue index values were analysed using two-way ANOVA with repeated measures for conditions (BASE, CRE, and CRE+CAF) and time (set number). Plasma lactate, heart rate and RPE were analysed in the same manner. 95% confidence intervals (CI) were computed for all relevant statistics. An alpha level of $\leqslant 0.05$ was chosen to indicate statistical significance.

RESULTS

Out of the thirteen participants that were recruited, twelve male participants completed the study. One participant did not complete all three trials due to scheduling conflicts. All participants maintained that they had abstained from caffeine consumption during the entire experimental period.

Knee extension torque

Mean peak torque scores across all 5 sets were as follows: BASE, 121.5 ± 30.5 N·m; CRE, 121.1 ± 30.7 N·m; CRE+CAF, 122.8 ± 32.6 N·m (Fig. 3). Repeated-measures ANOVA revealed a non-significant effect between conditions (p=0.824). However, CRE (164.7 ± 31.3 N·m, p=0.030, 95% CI 4.1–19.7 N·m) and CRE+CAF (170.8 ± 33.2 N·m, p=0.004, 95% CI 6.7–27.7 N·m) significantly increased peak torque in set 1 compared to BASE (154.8 ± 35.2 N·m). There was no significant difference in peak torque between CRE and CRE+CAF in set 1 (p=0.175, 95% CI -15.0–2.8 N·m). There was also a marked individual variation in the peak torque response to caffeine (Fig. 4). Although 4 of the 12 participants increased isokinetic torque with CAF ingestion (range, 8.2 to 12.4 N·m), 8 participants failed to show any substantial change from CRE (range, -20.6 to 4 N·m).
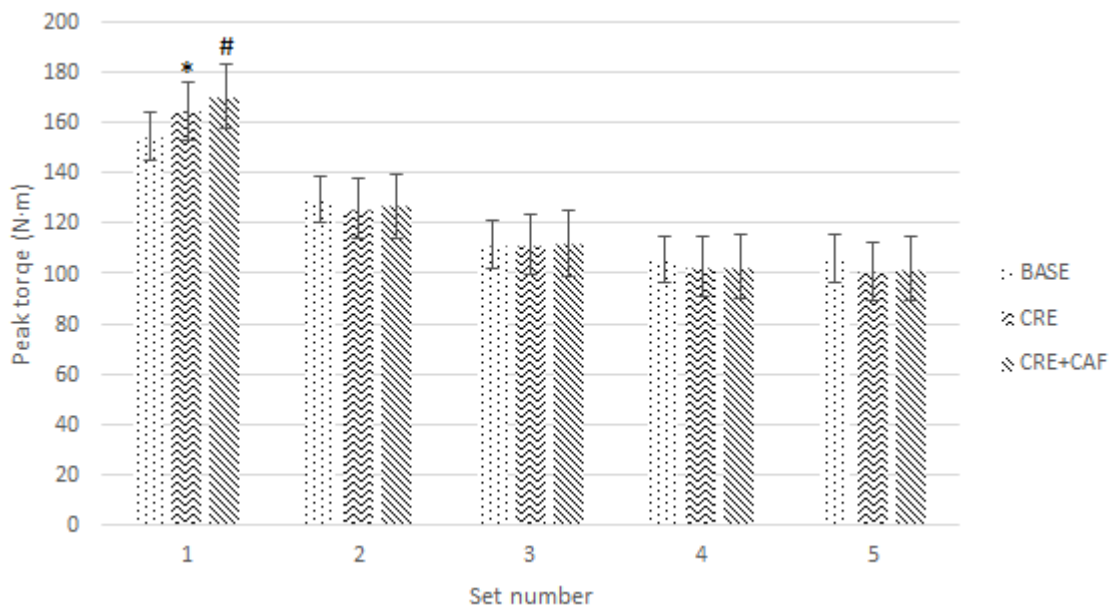

Figure 3. Peak torque performance in BASE, CRE and CRE+CAF conditions. *CRE is significantly different from BASE (p<0.05). #CRE+CAF is significantly different from BASE (p<0.05). Values are presented as mean ± SE
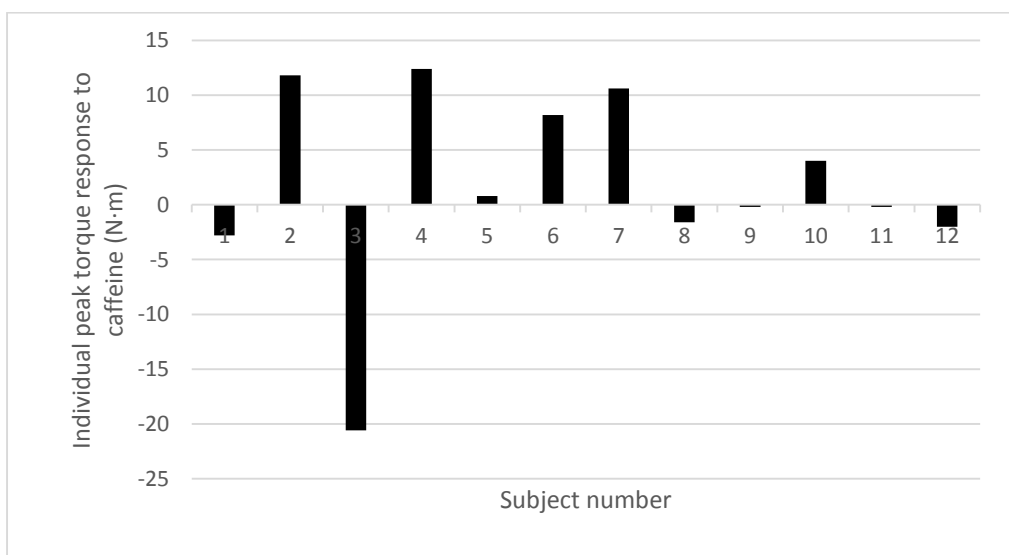

Figure 4. The individual peak torque response to caffeine in comparison to the mean of the CRE trial

Total work

Mean total work scores across all 5 sets were as follows: BASE, 3236.7 ± 248.2 W; CRE, 3292.7 ± 199.2 W; CRE+CAF, 3330.2 ± 168.4 W. There was no significant difference in total work between conditions (p=0.591). However, CRE (4858.7 ± 977.2 W, p=0.46, 95% CI -124.9−815.9 W) and CRE+CAF (5037.9 ± 946.9 W, p=0.034, 95% CI -12.2−1061.7 W) significantly increased total work in set 1 compared to BASE (4513.2 ± 1105.6 W). There was no significant difference in total work between CRE and CRE+CAF in set 1 (p=0.265, 95% CI -527.0–104.4 W).

Blood Lactate, RPE and Fatigue Index

All blood lactate, RPE and fatigue index data are presented in Table 1. No significant differences in blood lactate, RPE or fatigue index were observed among conditions (p>0.05).

Table 1. Mean ± SD data for blood lactate, RPE and fatigue index

Condition	Pre-exercise blood lactate (mmol.L^{-1})	Post-exercise blood lactate (mmol.L^{-1})	RPE (6-20)	Fatigue index (N·m)
BASE	0.9 ± 0.5	5.5 ± 1.0	15.1 ± 0.2	38.1 ± 3.2
CRE	0.8 ± 0.4	5.3 ± 1.1	14.7 ± 0.2	40.1 ± 3.4
CRE+CAF	1.0 ± 0.5	6.4 ± 2.2	14.6 ± 0.2	39.9 ± 1.9

Heart Rate

There was a significant difference in mean HR across all 5 sets between conditions (p=0.001): BASE, 146 ± 5 bpm; CRE, 153 ± 6 bpm; CRE+CAF, 157 ± 5 bpm (Fig. 5). CRE+CAF significantly increased HR compared to BASE in all sets (p=0.001). In contrast, CRE (156 ± 17 bpm) significantly increased (p=0.019) HR only in set 3 compared to BASE (145 ± 17 bpm).
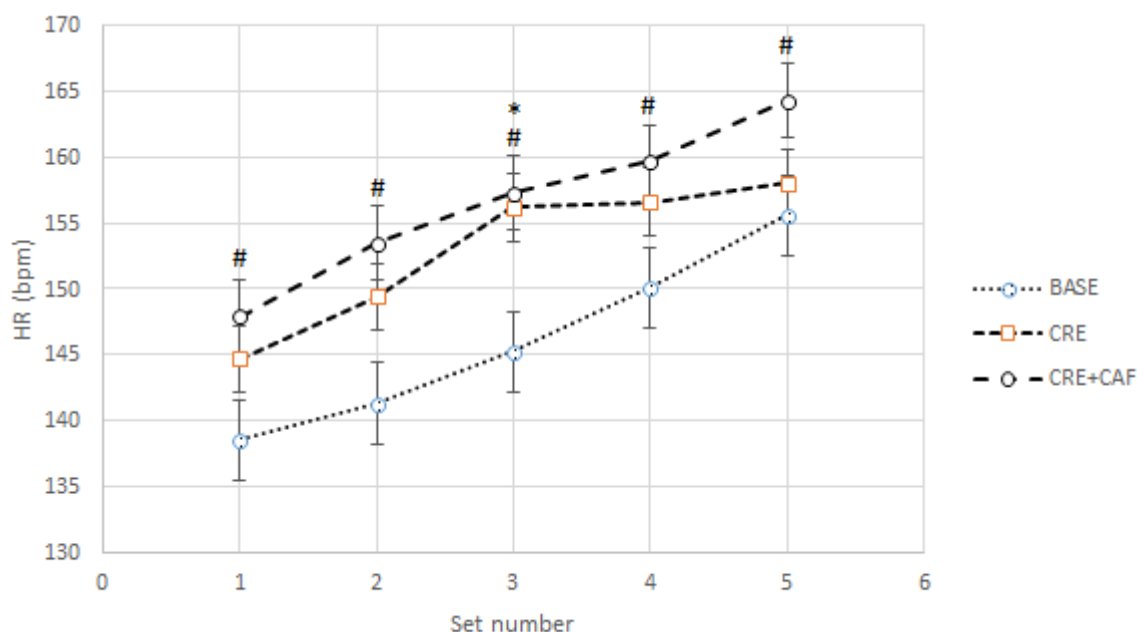

Figure 5. HR responses in BASE, CRE and CRE+CAF conditions. *CRE is significantly different from BASE (p<0.05). #CRE+CAF is significantly different from BASE (p<0.05). Values are presented as mean ± SE

DISCUSSION

This was the first study to investigate the acute effects of caffeine following short-term creatine supplementation on torque production. The main finding of the present study was that after 6 days of creatine supplementation, isokinetic peak torque significantly improved in the first bout of unilateral knee extensions. However, a single caffeine dose following creatine loading did not offer an additive ergogenic effect on isokinetic performance.

In contrast to the present study, previous reports investigating the acute effects of caffeine following short-term creatine supplementation and caffeine abstinence have consistently reported significant performance improvements. Doherty et al. (2002) found that ingesting 5 mg.kg^{-1} of caffeine after 6 days of creatine loading improved running time to exhaustion at an exercise intensity equivalent to 125% VO$_2$max. Consistent with this, Lee et al. (2011) and Lee et al. (2012) found the same supplemental protocol, albeit using 6 mg.kg^{-1} of caffeine, to augment intermittent sprint performance and lengthen time to exhaustion during an incremental maximum exercise test, respectively. However, data from the present study indicate that this supplemental regime is unable to enhance torque production during an isokinetic-strength protocol**.** It is plausible that the comparably lower dose of caffeine administered in this study (3 mg.kg^{-1}) following creatine loading was inadequate to elicit significant improvements in peak torque. In agreement with this notion, Astorino et al. (2008) found that 5 mg.kg^{-1} of caffeine increased torque production during a series of isokinetic knee extensions, whereas a lower 2 mg.kg^{-1} dose had no effect. More recently, Pallarés et al. (2013) reported an 8.9% mean propulsive velocity (MPV) improvement in squat and bench press exercises after ingestion of 6 mg.kg^{-1} of caffeine against loads of 75% 1 repetition-maximum. Nonetheless, 3 mg.kg^{-1} of caffeine was inefficacious during the bench press at this load. This suggests that a high caffeine dose of $\geqslant$5 mg.kg^{-1} may be required to enhance neuromuscular performance, in particular isokinetic torque production. This concept seems to agree with reports suggesting that high caffeine doses are needed to activate the proposed mechanism that underpins caffeine-induced strength improvements (Rousseau et al., 1988; Pallarés et al., 2013). Caffeine may augment muscle excitation-contraction coupling (Mohr et al., 1998; Meyers & Cafarelli, 2005) via enhanced sarcoplasmic reticulum Ca^{2+} kinetics (Weber, 1968; Tarnopolsky, 2008), specifically in the ryanodine receptor (Penner et al., 1989). However, *in vitro* studies have demonstrated that increased intracellular Ca^{2+} concentration only occurs at supraphysiological caffeine doses that are impractical for humans (Kalmar & Cafarelli, 2004). Thus, the dose needed to stimulate this cellular action may even be too large to administer to humans *in vivo* (Magkos & Kavouras, 2005; Kalmar, 2005).

It is also admissible to note that there was a marked individual variation in the response to caffeine. Although 4 of the 12 participants increased isokinetic torque with caffeine ingestion (range, 8.2 to 12.4 N·m), 8 participants failed to show any substantial change from the creatine-only condition (range, -20.6 to 4 N·m). This notable variation is compounded by the 95% confidence intervals spanning zero (-4.0 N·m−7.4 N·m). Likewise, all other studies reporting individual data do not show improved performance with caffeine ingestion for every participant (e.g., Doherty, 1998; Doherty et al., 2004; Wiles et al., 2006; Hudson et al., 2008). While caffeine concentrations were not measured in this study, the discrepancy in performance improvements indicates a

large inter-individual variation in the kinetics of caffeine metabolism. This may be related to caffeine habituation; although evidence exists suggesting caffeine tolerance does not affect subsequent endurance performance (Graham, 2001; Glaister et al., 2008). In addition, participants in this study were all accustomed to ingesting caffeine at >50 mg.day^{-1}. Recent data propose that variations in genotype may alter caffeine metabolism and potentially mediate the magnitude of caffeine-induced performance improvements. Caffeine is metabolized in the liver by cytochrome P450 1A2 (Butler et al., 1989), which shows distinct variability between individuals (Gu et al., 1992). A single substitution in the gene causes some people to be slow caffeine metabolizers, whereas those who are homozygous for the allele metabolize caffeine more rapidly (Sökmen et al., 2008). Cornelis et al. (2007) demonstrated that habitual caffeine consumption is related to these genotypes, which may explain the disparity in individual responses to caffeine's ergogenic effects. Further research is required to simultaneously examine the effect of differences in genotype on high-intensity exercise performance after caffeine ingestion.

Supplementation with creatine enhanced isokinetic peak torque in set 1 of the knee extensions by 6% (2.0%−10.6%; 95% confidence intervals), but did not significantly impact subsequent sets. The improvement in initial peak torque may partially be explained by osmotic changes caused by the increase in intracellular creatine content, which may result in an increase in maximal tension induced by Ca^{2+} activation and in an augmented Ca^{2+} sensitivity (same level of force produced with a lower Ca^{2+} concentration) (Murphy et al., 2004). However, the inefficacy of creatine during sets 2, 3, 4 and 5 of isokinetic knee extensions is more difficult to explain. Previous studies (Vandenberghe et al., 1996; Hespel et al., 2002) have suggested that chronic caffeine ingestion may attenuate the ergogenic benefits of creatine supplementation by counteracting the creatine-induced shortening of muscular relaxation time (Wakatsuki et al., 1993; van Leemputte et al., 1999). Slowing of relaxation in fatigued muscle can be explained by inhibition of sarcoplasmic reticulum Ca^{2+}ATPase activity because of falling pH and ATP affinity (Gillis, 1985). Therefore, it is probable that caffeine reduces the functional capacity of sarcoplasmic reticulum Ca^{2+}ATPase during rapid muscular contractions (Hespel et al., 2002). However, in this study, participants ingested a single dose of caffeine after abstaining from caffeine during the duration of the creatine loading. Importantly, Hespel et al. (2002) noted that acute caffeine ingestion does not impair muscle relaxation. Consistent with this, this study reported no statistical difference ($p > 0.05$) in peak torque between CRE and CRE+CAF conditions, thus caffeine did not appear to negate the potential ergogenic value of creatine ingestion. Since creatine supplementation significantly enhanced peak torque and total work in set 1 compared to BASE, it is probable that participants fatigued more quickly in the first set of knee extensions and therefore the increased capacity for ATP resynthesis due to an augmented availability of PCr was negligible in subsequent sets.

Alternatively, participants' previous supplementation history and dietary creatine content may partly explain the inability of creatine to facilitate sustained high force outputs in this study. Participants were instructed to stop supplementing with creatine four weeks prior to the commencement of the present study. However, despite some evidence suggesting that muscle creatine stores return to baseline by four weeks post supplementation (Febbraio et al., 1995; Vandenberghe et al., 1997), magnetic resonance data with meat-eaters indicate that muscle PCr may stay elevated for at least five weeks (Lemon et al., 1995). This would only occur if dietary intake of creatine and endogenous production matches the daily excretion from the body. Thus,

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variability in dietary creatine content following cessation of supplementation could lead to substantial inter-individual differences in the duration of this residual ergogenic effect (Lemon, 2002). This, in turn, may have influenced the BASE trial for some participants in the current study. Different results may have been observed if a longer washout period had been implemented before the start of the BASE trial. This should be considered in future research and perhaps stricter inclusion/exclusion criteria in regards to previous supplementation and dietary creatine intake should be implemented.

A limitation of the present study is that the baseline condition was not randomized along with the CRE and CRE+CAF conditions. It was contemplated that another washout period (4 weeks) was not feasible given the possibility that fitness levels of the participants could change over a longer experimental period. Another aspect of the study that should be considered when interpreting these data is that the test-retest error of the exercise protocol is currently unknown. Thus, the type II error rate was not controlled and it is difficult to determine whether the creatine-induced performance improvements are 'worthwhile' (Wilkinson, 2014). Nevertheless, it is tempting to suggest that the 6% improvement in initial peak torque is meaningful for sports performance, particularly when considering the positive confidence intervals (2.0%−10.6%; 95%).

CONCLUSIONS

Notwithstanding the debate between statistical and practical significance, this study provides evidence that short-term creatine supplementation can augment initial torque production in the first bout of exercise. However, a single caffeine dose following creatine loading may not provide an additive ergogenic effect on isokinetic performance beyond creatine alone. Future research should administer a larger dose of caffeine (≥ 5 mg.kg^{-1}) when assessing the effect of caffeine on torque production. Additional research should also include a caffeine-only condition to better determine the potential synergistic effects of creatine and caffeine on high-intensity performance. In practice, these data suggest that athletes engaged in high-intensity exercise should supplement with creatine for an improvement in initial force production. Furthermore, considering the wide individual variation of torque production responses observed in this study, athletes should perform individual assessments of caffeine before use and not rely solely on generalizations from the scientific literature.

FIRST AUTHOR'S BIOGRAPHY

Sam Orange completed his undergraduate BSc Sports Science degree at York St John University in 2014. He is now studying an MRes in Exercise Science at Northumbria University.

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