

**THE EFFECTS OF TEACRINE AND CAFFEINE ON ENDURANCE AND COGNITIVE
PERFORMANCE DURING A SIMULATED MATCH IN HIGH-LEVEL SOCCER
PLAYERS**

By

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ABSTRACT OF THE THESIS

The Effects of Teacrine and Caffeine on Endurance and Cognitive Performance During a
Simulated Match in High-Level Soccer Players

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Theacrine (1,3,7,9-tetramethyluric-acid) is a pure alkaloid with a similar structure to caffeine and acts comparably as an adenosine receptor antagonist. Early studies have shown non-habituating effects, including increases in energy, focus, and concentration in Teacrine®, the compound containing pure theacrine. **PURPOSE:** to determine and compare the effects of Teacrine® and caffeine on cognitive performance and time-to-exhaustion during a simulated soccer game in high-level male and female athletes. **METHODS:** Elite male and female soccer players (N=24; $M_{Age}=20.96\pm 2.05y$, $M_{Male}VO_{2max}=55.31\pm 3.39mL/O_2/kg$, $M_{Female}VO_{2max}=50.97\pm 3.90mL/O_2/kg$) completed a simulated 90-min soccer match protocol on a treadmill, with cognitive testing including simple reaction time (SRT); choice-RT during a go/no-go task (CRT); and complex-RT during a dual task of go/no go (COGRT) with distraction questions at halftime, and end-of-game. End-of-game testing was followed by a run to exhaustion at 85% VO_{2max} . Participants completed four sessions in randomized order ingesting either 275mg teacrine (TCr), 275mg caffeine (Caf), 125/150mg teacrine+caffeine (TCr+Caf), or placebo(P) 30 min prior to the match. Time of day and pre-exercise nutrition were controlled. RM-MANOVAs with univariate follow-ups were conducted and significance was set at $P<0.05$. **RESULTS:** Time-to-exhaustion trended toward improvements in all conditions when compared to placebo ($ES_{TCr}=0.43$, $ES_{Caf}=0.41$,

$ES_{TCr+Caf}=0.51$). There was a condition main effect ($P<0.05$) in which Caf ($0.595\pm 0.054s$) and TCr+Caf ($0.590\pm 0.059s$) improved CRT compared to P ($0.608\pm 0.067s$). There was a significant Time main effect for $COGRT_{Wrong}$, with improved accuracy at post compared to mid (16.46 ± 2.02 vs. 19.20 ± 2.13). A Time main effect also occurred for SRT, with better RT at mid compared to post ($0.639\pm 0.054s$ vs. $0.646\pm 0.054s$). However, a Time x Condition interaction ($P<0.05$) revealed that P improved from mid to post instead ($0.646\pm 0.064s$ vs. $0.632\pm 0.049s$). **CONCLUSION:** The 27-38% improvements in time-to-exhaustion reflect an increased performance capacity with these supplements that may have important implications for “added time” scenarios. The larger improvement in choice-RT from TCr+Caf may be due to overlapping peak times for the supplements, leading athletes to sustain greater focus under fatigue for longer periods compared to the other conditions. Peak times may also play a role as the largest SRT improvements occurred at mid compared to post-game; perhaps a higher dosage would cause less of a decline during the transition between Caf and TCr. The improvement seen in accuracy post-game may indicate a training effect for allocation of resources toward the end of a game when players need greater concentration.

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Chapter 1: Stimulatory Supplements and Athletic Performance

By

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Introduction

For centuries, humans have been consuming tea and coffee for their ability to increase energy levels and alertness. The active ingredient in these beverages that provides such benefits, caffeine (1,3,7-trimethylxanthine), was first discovered and isolated in the 15th century (Weinberg & Bealer, 2001). Since then, caffeine has become the most thoroughly researched and widely consumed energy-improving compound in the world (Goldstein et al., 2010), and has Generally Recognized as Safe (GRAS) status when consumed in doses of 1-9 mg/kg BW (Graham, 2001). However, caffeine consumption potentially has some undesirable side effects including, but not limited to, increased heart rate and habituation from chronic use (Brunye, Mahoney, Lieberman, & Taylor, 2010; Geethavani, Rameswarudu, & Rameshwari, 2014). Caffeine is often consumed in the form of coffee, although it is also common in pre-workout supplements due to its function as an ergogenic aid. It has been shown to enhance both aerobic and anaerobic performance, although the research has been inconsistent for the latter (Brunye et al., 2010; Graham, 2001; Meeusen, Roelands, & Spriet, 2013). Caffeine supplementation's positive effect on performance was first thought to be caused by an epinephrine-induced increase in fatty acid oxidation, thus sparing glycogen (Graham & Spriet, 1991). However, it is now known that caffeine ingestion increases plasma free fatty acids and the rate of lipid metabolism and decreases the reliance on muscle glycogen for energy. This shift in substrate utilization has been shown to increase endurance in cycling time trials (Costill, Dalsky, & Fink, 1978; Hargreaves, Kiens, & Richter, 1991).

Additionally, caffeine operates on the autonomic nervous system and blood vessels through the inhibition of adenosine receptors. Adenosine is a known vasodilator and contributes to the regulation of skeletal muscle blood flow during exercise by stimulating prostaglandin and nitric oxide synthesis (Mortenson, Nyberg, Thaning, Saltin, & Hellsten, 2009). Inhibition of the adenosine receptors initiates vasoconstriction, which subsequently provides an increase in systolic blood pressure and heart rate. When tested in comparison to the response of exercise alone, an acute dose of caffeine stimulated mild increases in both resting blood pressure and heart rate and contributed to a greater blood pressure response from exercise when used in conjunction (Casiglia et al., 1991; Daniels, Mole, Shaffrath, & Stebbins, 1998; Geethavani et al., 2014).

Caffeine's effects are also likely mediated through the central nervous system (CNS) via adenosine receptor antagonism, which potentially translates into improvements in high intensity exercise performance (Davis et al., 2003). Adenosine acts as a CNS depressant, promoting sleep and suppressing energy through adenosine receptor mechanisms. When bound, it initiates decreases in the concentration of neurotransmitters that enhance vigilance and alertness such as dopamine and serotonin (Meeusen et al., 2013). Dopamine mediates feelings of reward and manages motor control, with increases in dopamine improving concentration and mood (Volkow et al., 2015). Dopamine's pathway is thought to be linked with the serotonin pathway and influence serotonin release through inhibition of the 5-HT receptor (Daw, Kakade, & Dayan, 2002), and these interactions play a key role in signal transduction and increases in central fatigue (Chaouloff et al., 1987; Meeusen et al., 2013; Meeusen, Watson, Hasegawa, Roelands, & Piacentini, 2006). As an adenosine receptor antagonist, caffeine blocks these depressive

effects by binding to the receptors, increasing the concentration of dopamine and serotonin (Meeusen et al., 2013).

The receptors that play a significant role in mediating the effects of caffeine are the D₂ receptor (D₂R) in the dopamine pathway and A_{2A} receptor (A_{2A}R) in the adenosine pathway (Meeusen et al., 2013). These receptors interact within the striatum, one of the principal components of the basal ganglia, which serves to facilitate voluntary movement (Sheth, Brito, Mukherjea, Rybak, & Ramkumar, 2014). Through allosteric inhibition and second-messenger interactions, adenosine blocks D₂R signaling. A_{2A}R agonists decrease D₂R agonist binding, increasing dopamine signaling and thereby increasing arousal and alertness (Volkow et al., 2015). This interaction is a probable mechanism in the regulation of instrumental response output (conditioning stimuli signaling reinforcement or punishment) and effort-related choice behavior (response of the subject to choose a high effort response with a more highly preferred reinforcer versus a low effort response that results in a less valued reinforcer) (Salamone et al., 2016). This is potentially an important factor in sport performance due to the influence of fatigue, effort, and motivation that are related to this specific brain area (Meeusen et al., 2013).

Operating as an adenosine receptor antagonist, there is a clear ergogenic effect of caffeine (Davis et al., 2003; Karcz-Kubicha et al., 2003). After consumption, caffeine reaches its peak concentration within one hour; therefore, intake timing can influence the magnitude of its provided benefits on cognition (Conway, Orr, & Stannard, 2003; Ryan et al., 2013). While the research investigating the effects of dosing in athletes has been inconsistent in the prescribed amount, there appears to be a clear range for optimal performance between 3 and 6 mg/kg bodyweight (BW) or ~200-400 mg (Clarkson,

1993). Caffeine is also hypothesized to operate on three attention networks in particular: alerting, orienting, and executive attention (Brunye et al., 2010). Alerting achieves and maintains a state of vigilance during task performance, activating the thalamus and the right and left frontal and parietal brain regions. Orienting involves selectively attending to regions of space by directing attention to cued areas, activating the superior parietal lobe. Executive attention includes resolving conflict amongst potential responses to a presented stimulus and generally activates the anterior cingulate and lateral prefrontal cortices (Brunye et al., 2010). These networks play a role in an individual's ability to perform at a higher level and may give insight as to how caffeine plays a role in improving response to stimuli.

The effect of caffeine on one's ability to efficiently use alerting cues and executive attention was examined in doses of 0, 100, 200, and 400 mg (Brunye et al., 2010). The 200 mg dose was shown to induce a performance improvement, while no additional improvement was shown at 400 mg (Brunye et al., 2010). These findings were supported in a review by Lorist *et al.* that noted enhanced fundamental aspects of cognitive performance with doses of 3 and 5 mg/kg BW (Lorist, Snel, & Tieges, 2004). Positive effects with 6 mg/kg BW have also been shown in elite youth soccer players using a reactive agility test that tested reaction time in the dominant and non-dominant side. Although improvements were primarily seen in the non-dominant side in the caffeine condition, these findings only trended towards significance (Jordan, Korgaokar, Farley, Coons, & Caputo, 2014).

When consumed in lower doses (<3 mg/kg BW), caffeine is proposed to not alter the peripheral, whole-body response to exercise, but does improve vigilance, alertness

and mood, and cognitive processes during and after exercise (Spriet, 2014). However, research by Doyle *et al.* has contradicted this statement, testing reaction time and accuracy in collegiate fencers using caffeine doses ranging from 1.5 to 7.5 mg/kg BW in 1.5 mg/kg intervals compared to a placebo (Doyle, Lutz, Pellegrino, Sanders, & Arent, 2016). The investigators expected caffeine to follow a curvilinear relationship consistent with the inverted-U hypothesis that the more optimal arousal and a subsequent improvement in performance would be seen at low-to-moderate dosing and performance decrements at the higher doses (Arent & Landers, 2003; Doyle et al., 2016). The results showed fencers followed the expected trend, with the most significant increases in performance at consumption of between 3 and 6 mg/kg BW, and overall performance deteriorating at 7.5 mg/kg BW (Doyle et al., 2016). The dose-response relationship for motor control seen in the fencers supports the hypothesis that caffeine has an ergogenic effect on executive attention and motor coordination and may point to an optimal dosage. Additionally, the inverted-U relationship indicates there may be a threshold for performance benefits with caffeine consumption, as seen by performance decrements at the higher doses (Doyle et al., 2016).

In addition to effects on reaction time and motor performance, caffeine's impact on aerobic endurance has received considerable research attention. Early research in male high school distance runners saw no increase in run time-to-exhaustion at 80% $\text{VO}_{2\text{max}}$ with a dose of approximately 400 mg caffeine; however, the sample size was small ($n=5$) and may have reduced the power of the study (Sasaki, Maeda, Usui, & Ishiko, 1987). With a similar design, a randomized, double-blind trial by Graham and Spriet, well-trained distance runners completed four sessions, two cycling and two running, each at

85% $\text{VO}_{2\text{max}}$, with a dose of 9 mg/kg caffeine or placebo (Graham & Spriet, 1991). The ingestion of 9 mg/kg caffeine showed a 44% increase in run time-to-exhaustion compared to placebo, and a 51% increase in cycle time-to-exhaustion (Graham & Spriet, 1991). Later research by these investigators in a population of elite runners found that 3 and 6 mg/kg doses of caffeine increased run time-to-exhaustion at 85% $\text{VO}_{2\text{max}}$ by 22 ± 9 and $21.9 \pm 7.2\%$ respectively, adding to the growing body of evidence that caffeine improves endurance (Graham & Spriet, 1995).

Similarly, in trained cyclists, doses of 2 and 3 mg/kg of caffeine produced a moderate effect on 15 minutes of maximal cycling performance that was designed to simulate an extended effort at the end of a race (Jenkins, Trilk, Singhal, O'Connor, & Cureton, 2008). Subjects were asked to cycle for 15-min at 80% $\text{VO}_{2\text{peak}}$, and then completed their performance ride, which was recorded as work produced relative to BW (kJ/kg) (Jenkins et al., 2008). Doses of 2 and 3 mg/kg showed improvements in work completed of 4 and 3%, respectively (Jenkins et al., 2008). These results coincide with prior cycling research that displayed a 3% improvement in time-trial performance with a 6 mg/kg BW dose of caffeine (Cox et al., 2002). An additional study in well-trained cyclists demonstrated a significant average increase of 27% in time-to-exhaustion trials at 80% maximal power output across all experimental conditions of 3, 9, and 13 mg/kg BW of caffeine when compared to placebo (Pasman, van Baak, Jeukendrup, & de Haan, 1995). The observed improvements in cycling performance reiterates the findings in runners and trained athletes that 3 and 6 mg/kg BW doses of caffeine increases endurance performance (Graham & Spriet, 1995).

Sports requiring both aerobic and anaerobic components have also shown benefits from caffeine when given in the form of “energy drinks” (Del Coso et al., 2012; Lara et al., 2014). A dose of 3 mg/kg BW of caffeine was provided in the form of a sugar-free Red Bull® and its effects on measures of sprint repeatability and a simulated soccer match (intra-squad match with two 40-min halves and a 15-min halftime on artificial turf) were examined in semi-professional male players. Those players who consumed the energy drink demonstrated an increase in the number of sprints in the match and an increase in total distance covered (Del Coso et al., 2012). A similar study in females using the same protocol for both match simulation and dosing described by Del Coso *et al.* showed an increase in total running distance and the number of sprints performed, with caffeine improving CMJ height and average peak running speed during a sprint test in a team of experienced soccer players (Lara et al., 2014). While these studies demonstrated several improvements that warrant the use of caffeine in sports with similar demands, a major limitation comes from caffeine being given in the form of an energy drink. Although the ingredients in the drink remained the same in the control with the absence of caffeine, there may be interactions between compounds with the addition of caffeine that affected the improvements in performance (Franks, Schmidt, McCain, & Fraer, 2012). Additionally, the opponents were not standardized, and the varying degrees of skill and performance may have had an influence.

The proposed dose range for improvements in endurance performance and cognitive function has varied between studies from 100 to 400 mg in absolute doses and up to 9 mg/kg BW in relative doses (Clarkson, 1993; Graham, 2001; Spriet, 2014). In a variety of sports, the research indicates that at least a moderate dose of caffeine (3-6

mg/kg BW or ~200-400 mg) is necessary to produce an ergogenic effect on endurance performance (Goldstein et al., 2010; MacIntosh & Wright, 1995). When deciding what dose of caffeine should be consumed, the demands of the sport or activity, as well as the desired benefits, should be considered. Through the mechanisms outlined, caffeine has been shown to improve several aspects of performance, particularly when used in an athletic population. While these effects from caffeine appear to be beneficial, an alternative supplement called theacrine shares the characteristic improvements but may avoid the timing effects, habituation or adverse effects associated with caffeine.

Theacrine

Theacrine (1,3,7,9-tetramethyluric acid) is a purine alkaloid extracted from the cultivated tea plant *Cameilla kucha* (Zheng, Ye, Kato, Crozier, & Ashihara, 2002), and similar to caffeine has a GRAS status. TeaCrine® is a nature-identical, chemically equivalent, and commercial version of theacrine, and has shown to be safe in doses ranging from 200-300 mg/day for an eight-week period with no adverse side effects or differences in hemodynamic variables between the two doses (Taylor et al., 2016). The median lethal dose was found to be 810.6 mg/kg in mice, which is well above the dose being used in any safety study that has been performed (Wang et al., 2010). An acute oral administration of 200 or 400 mg appears to be safe with the absence of any adverse effects on systemic hemodynamics, and no significant interactions for oxygen consumption, carbon dioxide production, or the respiratory exchange ratio (Ziegenfuss et al., 2016).

Research has shown acute supplementation to enhance mood state, mental focus, and energy production (Taylor et al., 2016; Zheng et al., 2002). The proposed analgesic

and anti-inflammatory effects of theacrine are similar to caffeine and may be related to the composition of the compound (Wang et al., 2010; Ward, Whitney, Avery, & Dunner, 1991). Structurally, there is an extra methyl group and ketone group on the pyridine ring of the purine backbone compared to caffeine, which may be responsible for its pharmacokinetic and pharmacodynamic properties (Ziegenfuss et al., 2016).

Mechanistically, theacrine produced significant increases in activity levels when administered in acute doses to rats, and pre-treatment with theacrine before administration of adenosine A₁ and A_{2A} receptor agonists attenuated the motor-depressant effects (Feduccia et al., 2012). Activity was defined as ambulatory distance observed over a three-hour session. Rats were injected with 24 or 48 mg/kg BW of theacrine over the course of one-week and were monitored in an activity chamber. There was an observed dose-dependent response of higher activity levels compared to the control group, supporting the hypothesis that theacrine acts as an adenosine antagonist. There also appeared to be no habituation effect following chronic exposure, as shown by the lack of decreases in activity over a seven-day period (Feduccia et al., 2012).

Due to its recent discovery, few studies examining the use of TeaCrine® with measures of performance exist. The research has generally studied the effect of a combination of theacrine with caffeine, in order to detect the prospective added effect of theacrine in conjunction with caffeine on cognitive function (Kuhman, Joyner, & Bloomer, 2015). The proposed peak time of theacrine is approximately two hours with a half-life of approximately four times that of caffeine (Graham, 2001; He et al., 2017). This prolonged half-life and peak time can potentially allow for continued maintenance of amplified arousal, which suggests the use of TeaCrine® may be more beneficial in an

athletic population with larger cognitive demands due to the potential improvements in concentration and energy.

Research on the effects of TeaCrine® independently are needed to determine the influence of the supplement on measures of cognitive performance. The hemodynamic responses, along with oxygen consumption and subjective measures of cognitive performance using the 150-mm anchored visual analog scale (VAS), were tested by Ziegenfuss *et al.* (2016) in a two-part design. The first segment of the study utilized a randomized, open-label, dose-response investigation and participants were given either 200 or 400 mg TeaCrine® per day over a seven-day period, with no placebo comparison group. The results of this part of the study showed greater improvements in mood in the 200 mg compared to the 400 mg dose. While the dose-response results are significant, the lack of a placebo group reduces the interpretability of these findings.

The second segment of the study included a crossover investigation with a single dose of 200 mg TeaCrine® compared to placebo, using the 100-mm anchored VAS for subjective measures of cognitive function and other psychometric parameters (Ziegenfuss *et al.*, 2016). There were no significant differences for oxygen consumption or hemodynamic variables, demonstrating no adverse effects to acute administration of theacrine. The significant positive effects found in psychometric measures of energy with a trend for improved concentration suggest there may be cognitive improvements with supplementation of TeaCrine® (Ziegenfuss *et al.*, 2016). While the VAS is a validated tool, a main limitation in this study was the use of subjective ratings to measure cognition. Consequently, the use of objective methods of testing to assess TeaCrine® and its potential effects are preferential.

The only study to test physical performance using TeaCrine® utilized resistance exercise and measures of muscular strength and endurance and found no acute effects with supplementation (Snyder, 2016). The results may be insignificant due to a failure to use the peak timing of TeaCrine® appropriately, since the performance measures were tested 30 minutes following ingestion and concluding approximately 90 minutes later, making it possible that the researchers missed the window where improvements could have potentially occurred. Therefore, further research testing different modes of exercise and objective measures of cognitive performance with TeaCrine® supplementation is necessitated.

While TeaCrine® has shown promise improving mood and cognitive function, it may be more beneficial to investigate in combination with caffeine. The combination may provide additional improvements in cognition and performance compared to either supplement alone. When compared to each supplement independently, caffeine and TeaCrine® together displayed no adverse effects on heart rate or blood pressure. (Kuhman et al., 2015). The effects of caffeine and another branded form of theacrine (TheaTrim™) were studied using a combination condition with an undisclosed dose (Kuhman et al., 2015). The combination of TheaTrim™ and caffeine was hypothesized to increase the effects on cognitive performance and subjective feelings of energy and mood when compared to a 150 mg caffeine-control condition. Reaction time was tested using different light cues and subject response time as an average of five trials. In addition to reaction time, cognitive flexibility and working memory were also assessed via matching and connecting patterns as quickly as possible from beginning to end (low to high; 1-13; A-L) (Kuhman et al., 2015). No significant differences were found between the

combination and the caffeine-only conditions for reaction time or cognitive performance. Consistent with prior research of theacrine supplementation, the subjective feelings and mood questionnaires indicated increased attentiveness, focus, and energy with a decrease in measures related to fatigue in the combination condition (Kuhman et al., 2015). A main limitation to this study was ambiguity in the dosing of the combination condition, so it is uncertain how much impact the added theacrine had on the outcome measures or how the ingredients interacted within the combination condition.

The most recent research on the potential compound interactions between theacrine and caffeine investigated the pharmacodynamics and pharmacokinetics of the supplements. Using four treatment arms of 25 mg theacrine, 125 mg theacrine, 150 mg caffeine, and 125 mg theacrine + 150 mg caffeine, the researchers measured pharmacodynamic response markers via blood samples, heart rate and blood pressure (He et al., 2017). Pharmacokinetic or exposure parameters were determined as maximum plasma concentration, time to reach maximum concentration, oral clearance rate, and area under the curve. The co-administration of 125 mg theacrine and 150 mg caffeine showed no differences in hemodynamic parameters compared to all other treatment arms, suggesting the combination is safe to be administered at these doses (He et al., 2017). The findings also demonstrate a significant pharmacokinetic interaction expressed as increased theacrine exposure. Theacrine's absorption rate and half-life was unaffected by co-administration but displayed an increase in area under the curve, suggesting that caffeine may enhance the bioavailability of theacrine (He et al., 2017).

Although the exact mechanism for the interaction between caffeine and theacrine is unknown, clarifying the compound interaction may provide better insight into dosing

and timing strategies. Furthermore, investigating objective measures of cognitive function, as well as incorporating exercise, may further distinguish the improvements noted in energy and concentration. The observed increases in energy and concentration could be particularly beneficial in longer duration sports such as soccer where athletes are under high physiological and cognitive demands. Specifically, at more advanced levels of play, increases in mood, decreases in fatigue, and improvements in cognitive function could be pivotal to performance for these players.

Physiology of Soccer

The average distance covered in a 90-minute soccer match has been reported to be between 8-13 km (Bangsbo, 1994; Drust, Reilly, & Cable, 2000; Stolen, Chamari, Castagna, & Wisloff, 2005). The intermittent nature of a match includes standing, walking, jogging, running, and sprinting, with a reported change in speed every six seconds on average (Bangsbo, 1994; Stolen et al., 2005). Soccer is considered a power endurance sport, utilizing both aerobic and anaerobic metabolism with a reported average and peak heart rate (HR) between 80-90% and above 95% of maximal values, respectively (Gupta & Goswami, 2017; Stolen et al., 2005). Monitoring HR can be useful in assessing the physiological stresses on players throughout matches and has become more popular in the last decade as it is non-invasive, inexpensive, and time-efficient to use in a team setting (Achten & Jeukendrup, 2003; Ali & Farrally, 1991; Aslan et al., 2012; Djaoui, Haddad, Chamari, & Dellal, 2017). In order to quantify the physical demands that a soccer match places on the athlete, several physical (distance, number of sprints etc.) and physiological (oxygen uptake, HR, etc.) measures have been tested. These factors can be used to quantify training load to reflect an accurate representation of

the actions of athletes, and this information can be used to develop protocols designed to assess the sport in a lab setting that controls for extraneous variables (environment, opponent, travel, etc.) and mimics the demands of a match (Achten & Jeukendrup, 2003).

The most decisive movements in a match utilize anaerobic metabolism, with an observation that elite soccer players perform 150-250 brief, intense actions during a game and there is a linear increase with level of play (Mohr, Krstrup, & Bangsbo, 2003). These actions involve decision-making, speed changes, and anticipatory movement patterns to maintain possession, switch between defense and offense quickly, and attack strategically. Physical capacity alone cannot predict success in team sports such as soccer and research has shifted its focus towards cognitive function, with a particular interest in executive function (EF). EF operates from the frontal lobes and is responsible for attentional control, processing speed, cognitive flexibility, goal setting, response inhibition, and working memory (Alvarez & Emory, 2006; Vestberg, Reinebo, Maurex, Ingvar, & Petrovic, 2017). It has been suggested that a higher performance on EF is important for success in soccer (Verburgh, Scherder, van Lange, & Oosterlaan, 2014) and as such the measures of EF that were of interest to this study included response inhibition and executive control.

A soccer match can require a player to shift quickly from a narrow to a broad focus, known as local and global attending, respectively. Soccer players have demonstrated switching from local to global attending more rapidly than non-athletes, suggesting attentional flexibility and the reallocation of resources under temporal and spatial constraints (Pesce, Tessitore, Casella, Pirritano, & Capranica, 2007). In longer duration sports, cognitive function may be impaired under exhaustive conditions as a

result of a number of physiological factors within a soccer match (Sudo et al., 2017). Visuomotor processing (tested as response inhibition and reaction time) was shown to decrease with a progressively increasing physical load (Zwierko & Lesiakowski, 2014), supporting the hypothesis that cognitive function can be impaired in situations that induce fatigue, such as overtime in a soccer match.

While the cognitive component of a soccer match can play a large role in determining a match's outcome, there is also the physiological component of a soccer match, reflected in the player's ability to sustain their level of play. Although the standard time of a match is 90 minutes split into two 45-minute halves, match length can be extended up to 120 minutes in the event of a tie (Harper et al., 2016). Reductions in performance capacity have been shown following match play (Drust et al., 2000; Mohr et al., 2003), and these reductions are exacerbated further in extra-time (ET). Previous research indicated that a 120-minute simulated soccer exercise induced neuromuscular fatigue and negatively impacted technical and physical performance measures (Harper et al., 2016; Russell, Sparkes, Northeast, & Kilduff, 2015). When compared to a regular 90-minute match, players on average covered ~5 km more total distance and ~400 m more high-intensity distance, and performed ~170 more accelerations and ~90 more decelerations (Winder, Russell, Naughton, & Harper, 2018).

These increases in physical demand during ET have been shown to result in deterioration of performance (Goodall et al., 2017; Harper et al., 2016; Winder et al., 2018) and as such new focus has been on strategies to cope with the increased demands of ET. Recent research has focused on supplementation with carbohydrate solutions, and has shown attenuations in the decline of performance (Harper et al., 2016; Russell,

Benton, & Kingsley, 2012). However, it may be worth examining the caffeine and theacrine and the potential impact on performance during ET, which may provide more concrete evidence supporting the use of supplementation. A few studies have investigated caffeine's effects on soccer players during match play (Del Coso et al., 2012; Lara et al., 2014); however, the researchers did not examine these effects during ET where it could prove the most beneficial. Additionally, strategies of dosing and timing in both caffeine and theacrine supplements may aide in increasing both cognitive and physical performance when physical demands are increased further in ET and players are required to maintain a high level of play.

Summary

The safety of the combination of caffeine and theacrine, as well as the conclusions drawn from studies utilizing these supplements, advocate a potential synergistic effect in physical and cognitive performance measures with their combination. Preliminary research supports the hypothesis that theacrine improves measures of physical performance as demonstrated by increased voluntary activity levels, enhanced subjective measures of mood, and decreased fatigue without any adverse effects or habituation to the compound. It has been hypothesized that when used in a protocol designed to fatigue the body through exercise, TeaCrine® may aid in maintaining or improving cognitive function. The combination of TeaCrine® and caffeine may enhance these outcomes further and amplify the effects of both supplements.

Athletes may benefit from using caffeine and theacrine to enhance performance, yet previous research has not implemented any physical exercise intervention. Therefore, the current study aims to determine and compare the effects of TeaCrine® and caffeine

on various measures of cognitive performance under fatiguing conditions of a simulated match in high-level male and female soccer players. Secondary purposes are to determine whether there is a synergistic effect of TeaCrine® and caffeine in combination as well as the impact on time-to-exhaustion in an “added time” scenario.

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Chapter 2: The Effects of Teacrine And Caffeine on Endurance and Cognitive
Performance During a Simulated Match in High-Level Soccer Players

By

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Introduction

Caffeine (1,3,7-trimethylxanthine) is one of the most commonly researched supplements and has considerable support for ergogenic effects over a wide range of sports (MacIntosh & Wright, 1995; Spriet, 2014) with moderate-to-large doses (3-6 mg/kg BW or ~200-400 mg). Caffeine functions as a competitive inhibitor of adenosine, regulating sleep/wake cycles by binding to adenosine receptors to block their actions while increasing concentrations of neurotransmitters such as dopamine and serotonin to mediate concentration, mood, and fatigue (Chaouloff et al., 1987; Graham, 2001; Meeusen et al., 2013; Sheth et al., 2014; Volkow et al., 2015). Additionally, this inhibition of adenosine receptors alters the autonomic nervous system, subsequently increasing systolic blood pressure and heart rate, with further augmentation of this response under exercise conditions (Casiglia et al., 1991; Daniels et al., 1998; Geethavani et al., 2014). Furthermore, there are effects on cognitive function via the three networks of executive attention, orienting, and alerting, with moderate doses shown to induce cognitive performance improvements in soccer players and fencers (Brunye et al., 2010; Doyle et al., 2016; Jordan et al., 2014; Lorist et al., 2004). In addition to psychophysiological effects, caffeine has been shown to produce notable ergogenic effects on aerobic capacity. Much of the evidence for this effect pertains to improved time trial performance in cyclists and run time-to-exhaustion in distance runners when consumed in doses from 2-6 and 3-9 mg/kg BW, respectively (Butts & Crowell, 1985; Cox et al., 2002; Pasma et al., 1995). While similar benefits have been suggested for anaerobic performance, the results are mixed with some studies showing improvements in mean sprint times in swimmers and increases in peak power measured via Wingate

tests, while other studies have shown no significant changes in 1RM strength (Astorino, Rohmann, & Firth, 2009; Goods, Landers, & Fulton, 2017; Grgic, Trexler, Lazinica, & Pedisic, 2018; Hahn, Jagim, Camic, & Andre, 2018; Woolf, Bidwell, & Carlson, 2008). However, it is important to note that the research investigating sports with both aerobic and anaerobic components has primarily used energy drinks to explore the effects of caffeine on performance outcomes, which may confound interpretations of caffeine's ergogenic effects due to potential compound interactions of ingredients within the energy drinks (Del Coso et al., 2012; Franks et al., 2012; Lara et al., 2014).

Although caffeine has been shown to improve several aspects of performance, there are several undesirable side effects potentially associated with it, including the increases in cardiovascular responses, habituation from chronic use, and timing effects that should be taken into consideration (Brunye et al., 2010; Conway et al., 2003; Graham, 2001). A recently developed compound similar to caffeine, theacrine (1,3,7,9-tetramethyluric acid), may hold some promise given that it has a purportedly prolonged peak time of approximately two hours and has been shown to increase mood and subjective measures of cognitive function with no adverse side effects or habituation (He et al., 2017; Taylor et al., 2016; Ziegenfuss et al., 2016). Theacrine appears to operate similarly to caffeine as an adenosine receptor antagonist, so it can be hypothesized that the use of TeaCrine® would mimic and provide longer-lasting effects than caffeine, without the sharp decline that usually occurs as the concentration of caffeine decreases in the body (Feduccia et al., 2012; Kuhman et al., 2015; Snyder, 2016; Taylor et al., 2016). Currently, there is only one study that has investigated the effects of theacrine independently on subjective measures of mental status including such things as energy,

focus, concentration, and fatigue, with significant improvements noted (Ziegenfuss et al., 2016). An additional study has tested the effects on muscular strength and endurance in resistance trained males, though there appeared to be few significant effects (Snyder, 2016).

Due to their similar mechanisms and varying half-lives, a majority of the research has focused on the combination of caffeine and theacrine to produce fast, yet prolonged ergogenic effects. Studies have shown no adverse effects on heart rate or blood pressure compared to either supplement independently, suggesting this combination is safe to be administered in doses of 125 mg theacrine/150 mg caffeine (He et al., 2017; Kuhman et al., 2015). A proprietary blend of caffeine and theacrine (TheaTrim) was shown to have no significant impact on subjective measures of cognitive function, although there were significant effects noted for increases in subjective feelings of focus and energy (Kuhman et al., 2015). However, due to the fact that the quantity of theacrine in this combination was undisclosed, the results should be interpreted with caution. Given the limited information and mixed results, further investigation into its potential benefits in sports performance is warranted.

The efficacy these supplements in athletic settings may largely depend on the physical and cognitive demands of the sport. For example, soccer is highly aerobic but also includes a mix of anaerobic power and cognitive load, with all three contributing to predict a player's performance and success (Pesce et al., 2007; Stolen et al., 2005; Verburgh et al., 2014; Vestberg, Gustafson, Maurex, Ingvar, & Petrovic, 2012). Reported ranges for total distance in a match is between 8-13 km, with changes in speed every six seconds on average, creating a match of intermittent nature and varying demands

(Bangsbo, 1994; Drust et al., 2000; Stolen et al., 2005). Furthermore, the utilization of executive function has been found to be a predictor of success in soccer (Alvarez & Emory, 2006; Verburgh et al., 2014; Vestberg et al., 2012; Vestberg et al., 2017) and soccer players have demonstrated the ability to reallocate resources under temporal and spatial constraints. Therefore, executive function may play a large role in match play, particularly under fatiguing conditions (Pesce et al., 2007; Sudo et al., 2017; Zwierko & Lesiakowski, 2014). Supplements such as caffeine and/or theacrine may enhance athletic performance by improving a player's work capacity, as well as by mitigating the effects of fatigue on cognitive function. Limited research exists observing the differences between caffeine and theacrine in the area of sport performance, as well as the effects of the combination of the two supplements. Use of an ergogenic aid that could enhance cognitive ability and reduce fatigue without a "crash" afterwards or habituation effect could improve a player's ability to sustain a higher level of play for a longer period of time. The purpose of this study was to determine and compare the effects of TeaCrine® and caffeine on various measures of cognitive performance under fatiguing conditions of a simulated athletic contest in high level male and female soccer players. Secondary purposes were to determine whether TeaCrine® and caffeine in combination have a synergistic effect, as well as the impact on time-to-exhaustion in an "added time" scenario.

Materials & Methods

Experimental Approach to the Problem. A within-subjects, placebo-controlled, double-blind design was used to determine the effects of caffeine and TeaCrine® on performance. Subjects completed four test sessions in randomized order after ingesting

either 275 mg placebo (PL), 275 mg TeaCrine® (TCr), 275 mg caffeine (Caf), or a 125/150 mg combination of TeaCrine® and caffeine (TCr+Caf) 30 minutes prior to exercise. This time-frame was used in order to allow the caffeine and Teacrine® to be absorbed and achieve peak concentration through the middle of the test session. Absolute dosing was chosen due to previous research that used similar absolute amounts of TeaCrine® in addition to practical supplementation strategies. All supplements were consumed as a single capsule. Placebo capsules were filled with 275 mg of cellulose. Participants completed all sessions at the same time of day (within 1 hour) and were instructed to abstain from vigorous exercise for 24 hours prior to each session. Experimental sessions were separated by a minimum of at least 48 hours.

Subjects. Male (n=12) and female (n=15) Division I and professional soccer players were recruited for this study. All subjects were highly trained, participating in soccer-related activities a minimum of 5 days per week at the time of the study. Subjects were excluded if they had any injuries that would prevent them from completing the protocol, had a history of caffeine sensitivity, drank more than four cups of percolated coffee per day, or currently took OTC products containing pseudoephedrine or other stimulants. All subjects read and signed an informed consent form and the study was approved by the Rutgers University Institutional Review Board. Three subjects (two males, 1 female) were excluded from the statistical analysis due to noncompliance. Subject characteristics are presented in Table 1.

Performance Testing. Subjects were instructed to arrive at the Rutgers Center for Health and Human Performance (CHHP) euhydrated, 2 hours fasted, and having refrained from exercise 24 hours prior to testing. Body weight (BW), body composition

(%BF), lean body mass (LBM), and fat mass (FM) were measured using air-displacement plethysmography (BodPod, COSMED, Concord, CA, USA) according to manufacturer's guidelines.

Participants completed a dynamic warm-up prior to performing a $\text{VO}_{2\text{max}}$ test. $\text{VO}_{2\text{max}}$ was assessed using a treadmill graded exercise test. The test consisted of a constant 2.0% incline grade with speed increases every two minutes until exhaustion (Jones & Doust, 1996). Males began the protocol at 7.9 km/h, while females began at 6.4 km/h. Speed increases for subsequent stages of the protocol were 10.0, 11.7, 13.7, 15.6, 17.1, 18.2, 19.8, and 21.1 km/h. Rating of perceived exertion (RPE) was obtained at the end of each stage (Borg, 1982).

Ventilatory, metabolic, and cardiovascular responses were continuously monitored using direct gas exchange with breath by breath sampling using a Quark CPET metabolic cart (COSMED, Concord, CA, USA). The gas analyzers and spirometer were calibrated prior to each test according to manufacturer's guidelines (Nieman, Austin, Dew, & Utter, 2013). Heart rate (HR) was continuously monitored using the Polar H7 HR transmitter (Polar Electro Co., Woodbury, NY, USA).

Participants were familiarized with the tasks to be performed in each session. The Dynavision D2™ Reaction Board was used for cognitive testing (Dynavision International LLC, Chester, OH, USA). Three familiarization rounds were performed on the reaction board prior to $\text{VO}_{2\text{max}}$ testing (Wells et al., 2014). Three 1-minute trials, with 1-minute rest periods, were used to test reaction time. The first trial tested simple reaction time (SRT) via the pressing of a light stimulus that appeared in a random location on the board. SRT was scored as time for participants to press the stimulus averaged over the

course of the minute. The second trial tested choice reaction time and score (CRT and CRT_{Score}), using a red and green stimulus to designate “go/no-go”, where the red stimulus was “go” and the green stimulus was “no-go”. Similarly to SRT, CRT was scored using average RT of pressing the red stimulus, and CRT_{Score} was scaled based on number of red targets pressed and green targets avoided, calculated as “total number of red hit plus green avoided” divided by the total number of targets that appeared during the minute.

The last trial tested cognitive load (i.e., complex) reaction time (COGRT) and score ($COGRT_{Score}$) with the “go/no-go” light stimulus and cognitive load tasks ($COGRT_{Wrong}$), which consisted of a series of either four letters, which the subject had to repeat aloud in the correct order, or a simple math problem, which the subject had to answer correctly. COGRT was scored similarly to SRT and CRT for average RT of pressing the red stimulus. $COGRT_{Score}$ was scaled based on number of red targets pressed and green targets avoided, calculated as “total number of red hit plus green avoided” divided by the total number of targets that appeared during the minute. The “load” tasks were shown every 3 seconds and remained on the screen for 1 second for a total of 20 questions per block. Subjects were able to answer until the next question appeared, and answers were recorded as correct, incorrect, or no answer. These questions were scored as a percentage answered wrong (CRT_{Wrong}), with incorrect and no answers contributing to this score. One full round of testing included one trial of each reaction time task separated with 1 min recovery.

Supplement Trials. Participants recorded their 24h dietary intake prior to their initial performance testing session and were asked to repeat this diet for each day of the experimental trials. Participants wore a Polar H7 HR transmitter synced to a Polar V800

watch to monitor HR throughout the session. Upon arriving at the CHHP, participants consumed the session-assigned capsule with water, and after a 15-minute period of quiet rest, began a 15-minute warm-up. A general warm-up of five minutes of aerobic activity was performed on a treadmill at a self-selected pace. Next, a dynamic warm-up was performed consisting of high knees, butt kicks, lunges, lunge with a rotation of the upper body, power skips, power skips into a lunge, straight leg kicks, hamstring walk-outs, and lateral squats.

Following the warm-up, subjects proceeded into the testing room to complete a 90-minute simulated soccer game protocol on a high-speed treadmill (HPCosmos T170, COSMED, Concord, CA, USA). The simulated game also included a 15-minute half-time. The protocol was comprised of the varying exercise intensities characteristic of match-play (e.g., stationary, walking, jogging, running, and sprinting; see Table 2). The speed zones and relative time spent in each were based on research in high-level male players (Mohr et al., 2003). The higher speeds for the female players were scaled accordingly (~5-12% slower) based on GPS game data obtained from Division I college players at Rutgers University (Figure 1). The first and second half were identical (Figure 1), with a total game distance of 12.86 and 12.7 km for males and females, respectively. RPE was assessed at each 15-minute interval of the protocol. Testing environment was controlled for each session.

Following the conclusion of the first half, subjects performed one full round of cognitive testing using the same procedure as explained in performance testing, and the remaining time was used as a rest period for a total time of 15 minutes. After completion of the second half, subjects performed a third full round of cognitive testing. Following

completion of the final cognitive test, which was consistent with the 5-minute rest period between the end of regulation and beginning of overtime in an actual match, subjects were placed back on the treadmill and ran at a speed corresponding to 85% of their VO_{2max} . Subjects were instructed to run at this speed until volitional fatigue, with no motivation from the testers. This was recorded as their run time-to-exhaustion (RTE). The treadmill clock and speed were covered so the participants were blinded to time and distance.

Statistics. Descriptive statistics (mean \pm SD) were used to quantify subjects' physical characteristics. RM MANOVAs with univariate follow-ups were used to determine differences among conditions for SRT, CRT, and COGRT. Univariate follow-ups and simple contrasts were performed following significant multivariate effects. Separate RM ANOVAs were used to determine differences in RTE for the "overtime" runs, RPE, and HR for each session. Results were considered statistically significant when the probability of a type I error was less than 0.05 ($P < 0.05$).

For each univariate analysis, the assumption of sphericity was tested using an examination of the Huynh–Feldt (H–F) epsilon for the general model. If this statistic was greater than 0.75, sphericity was considered to have been met, and the unadjusted univariate statistic was used. If epsilon was less than 0.75, a violation of the assumption of sphericity was considered to have occurred, and the H–F adjusted statistic was used to determine significance. Effect sizes (ES) were calculated in order to compare magnitude of changes for each experimental condition compared to PL using Hedges' g formula for ES computation. This ES computation was used for all variables, with a positive ES

representing better or faster results, and a negative ES representing slower or worse results.

Results

There were no main effects or interactions for Sex for any of the variables ($P > 0.20$). Because of this, data were collapsed across Sex for all remaining analyses. There was a significant multivariate effect for the conditions ($P = 0.025$), therefore univariate follow-ups for each variable were conducted.

Simple Reaction Time. There was a significant Time main effect ($P = 0.031$) for SRT from halftime to end-of-game. However, a Condition x Time interaction ($P = 0.022$) revealed that PL improved from halftime to end-of-game for SRT while all other conditions showed slower RT from halftime to end-of-game (0.647 ± 0.059 vs 0.631 ± 0.047 s, $ES_{PL} = 0.27$; 0.645 ± 0.053 s vs 0.659 ± 0.070 s, $ES_{TCr} = -0.09$; 0.629 ± 0.057 s vs 0.647 ± 0.054 s, $ES_{Caf} = -0.30$; 0.635 ± 0.058 s vs 0.647 ± 0.066 s, $ES_{TCr+Caf} = -0.22$). These data are shown in figure 2.

Simple contrasts at halftime showed significant differences between conditions. Caf and TCr were faster at halftime when compared to PL (0.629 ± 0.057 s vs 0.647 ± 0.059 , $P = 0.032$, $ES_{Caf} = 0.31$; 0.629 ± 0.057 s vs 0.645 ± 0.054 s, $P = 0.052$, $ES_{TCr} = 0.29$). There were also significant differences between conditions at end-of-game. TCr had slower SRT compared to PL (0.659 ± 0.069 vs 0.631 ± 0.047 s, $P = 0.017$, $ES = 0.47$), with no other condition differences.

Choice Reaction Time & Score. There was a significant Condition main effect for CRT ($P = 0.003$). Follow-ups indicated Caf and TCr+Caf were faster compared to PL ($P = 0.034$ and $P = 0.000$, respectively). These data are shown in figure 3A. There was a

Condition effect for CRT_{score} (P=0.008). Follow-ups showed worse scores for TCr compared with PL (P=0.014). These data are shown in figure 3B.

Simple contrasts at halftime showed significant condition differences. Caf produced faster CRT compared to PL ($0.593 \pm 0.054s$ vs $0.614 \pm 0.069s$, P=0.012, ES=0.34), and showed better CRT_{score} compared to TCr (98.848 ± 0.272 vs 97.383 ± 0.512 , P=0.009, ES=3.57). TCr+Caf produced faster CRT compared to PL ($0.592 \pm 0.057s$ vs $0.614 \pm 0.069s$, P=0.001, ES =0.35), and compared to TCr ($0.592 \pm 0.012s$ vs $0.607 \pm 0.014s$, P=0.061, ES=0.35), but was no different than Caf. TCr+Caf also showed better CRT_{score} when compared to TCr (98.548 ± 0.335 vs 97.383 ± 0.512 , P=0.029, ES=2.69). TCr had worse CRT_{score} when compared to PL (97.383 ± 0.512 vs 98.652 ± 0.394 , P=0.005, ES=-2.78). There were also significant differences between conditions at end-of-game for CRT_{score}, with worse scores in TCr+Caf compared to PL, though this effect was small (98.370 ± 1.748 vs 98.678 ± 1.612 , P=0.029, ES=0.18). No other condition differences were noted. These data are shown in figure 3B.

Cognitive Load Reaction Time, Score, & Wrong Answers. No main effects were seen for COGRT (P>0.13) or COGRT_{score} (P>0.2), but there was a significant time main effect for COGRT_{wrong} (P=0.042). Follow-ups indicated greater accuracy at end-of-game compared to halftime across conditions (P=0.037). These data are shown in figure 4.

Planned simple contrasts at halftime revealed significant differences between conditions for COGRT, with no other condition effects for any other measures. Caf presented faster COGRT when compared to PL ($0.658 \pm 0.047s$ vs $0.676 \pm 0.059s$, P=0.049, ES=0.34) and was also faster compared to TCr ($0.658 \pm 0.047s$ vs $0.678 \pm 0.062s$, P=0.029,

ES=0.36). TCr+Caf showed faster COGRT when compared to TCr ($0.665\pm 0.054s$ vs $0.678\pm 0.062s$, $P=0.042$, $ES_{TCr+Caf}=0.22$). There were no differences between conditions at end-of-game for any measures ($P>0.1$).

Run Time to Exhaustion. There was a trend toward improvements in RTE in all conditions when compared to placebo ($RTE_{PL}=194.1\pm 96.9s$). TCr presented an average increase of 27% ($RTE_{TCr}=245.9\pm 142.3s$, $ES_{TCr}=0.43$, $P=0.52$). Caf increased on average 32% ($RTE_{Caf}=255.4\pm 189.1s$, $ES_{Caf}=0.41$, $P=0.139$), while TCr+Caf showed an average increase of 38% ($RTE_{TCr+Caf}=267.0\pm 175.7s$, $ES_{TCr+Caf}=0.51$, $P=0.051$). These data are shown in figure 5. Over 70% of subjects had their longest RTE in the TCr+Caf (45.8%) or Caf (25%) conditions, with 12.5% having the longest RTE in the PL.

Heart Rate. HR was measured as a mean of first half (H1), second half (H2), and overtime (OT; reflected as RTE). There was a significant Time main effect across the simulated soccer protocol, with an increase in HR across all time points (H1 to OT 160.642 ± 9.447 vs 178.260 ± 11.462 bpm, $P=0.000$, $ES=1.86$). These data are shown in figure 6.

Rating of Perceived Exertion. Taken in 15-minute intervals, there were six time points for RPE. There was a significant Time main effect, with an increase in RPE from T1 to T6 (11.174 ± 1.573 vs 14.690 ± 1.981 , $P=0.000$, $ES=2.23$). While there were no significant differences shown between conditions, average RPE across all time points showed a small-to-moderate effect of lower RPE in Caf and TCr+Caf and a trivial effect in TCr compared to PL ($ES_{Caf}=-0.44$, $P=0.004$; $ES_{TCr+Caf}=-0.33$, $P=0.194$; $ES_{TCr}=-0.12$, $P=0.282$). These data are shown in figure 7.

Discussion

The primary results of this study indicate the combination of 150 mg Caf with 125 mg TCr produces a larger beneficial effect on both cognitive function and endurance than Caf or TCr independently, supporting the hypothesis that the supplements may work synergistically. Additional findings suggest the consumption of 275 mg Caf showed greater improvements in cognitive function compared to 275 mg TCr, which produces small improvements when compared with PL. It should be noted that while previous research has focused on relative dosing strategies, an absolute dose is more consistent with administration methods in this population, and the lack of sex effects supports this notion that an absolute dose elicits similar results.

Contrary to our hypotheses, TCr did not significantly improve cognitive measures of performance when compared to Caf, which could be due to a variety of influences, particularly the timing of testing, as previous research has shown supplement uptake and usage to be influenced by time of day (Burke et al., 2015). Although time of day for testing was matched for all visits for each subject, standardizing the session times of different subjects was not logistically feasible. While diet was not controlled between subjects, the within-subject design allowed for controlling diet within each participant between their respective sessions. In addition to the timing of testing, the timing of each supplement may also play an important role in the RT results. These results revealed that all conditions containing Caf showed better cognitive performance at halftime when compared to end-of-game. This aligns with the literature on timing for peak concentration of Caf at 1-hour following consumption (Lorist et al., 2004). Additionally, while the proposed peak concentration of TCr is approximately two hours, it may occur later than

anticipated which potentially explains the lack of significance in the RT results in all measures with the exception of COGRT_{Wrong}.

The training background of players may also contribute to these cognitive benefits by enhancing processing speed and attention, which may potentially explain the faster SRT in PL. It has been suggested that well-trained individuals can mitigate decreases in cognitive performance under fatiguing conditions (Brisswalter, Collardeau, & Rene, 2002; Tomporowski & Ellis, 1986; Verburch et al., 2014; Vestberg et al., 2012).

Considering the high level of skill in the athlete used, it is likely that this phenomenon may have contributed to the improvements seen in the PL condition. However, the level of athlete recruited for this study should be noted as a strength in comparison to previous research on Caf or TCr in recreationally-trained participants. The aforementioned training effect may also explain the fewer incorrect responses across all conditions at end-of-game compared to halftime. While there were no significant main effects for COGRT in the current study, the improvements in COGRT_{Wrong} agrees with previous research showing a significant increase in the number of correct responses in a visual vigilance task (Fine et al., 1994). Players may allocate resources to maintain performance toward the end of the match when skill and quick decision-making are critical determinants to the outcome.

The increases in RTE across all conditions compared to PL suggests that players can maintain a higher level of performance at later stages in a match with consumption of Caf and TCr. These results trended towards statistical significance, but given the magnitude of differences, the clinical significance may be reflected in implications for late game or added time scenarios in matches. Although TCr and Caf each had similar magnitudes of positive effects on RTE compared to PL (27% and 32%, respectively), it

appears that the combination may have been even more impactful given the 38% increase in RTE. These improvements are supported by previous studies that demonstrated an ergogenic effect of Caf for producing increases in endurance performance in trained cyclists and distance runners following consumption of 2 and 3 mg/kg BW (Graham, 2001; Jenkins et al., 2008; Pasman et al., 1995). With over 50% of subjects having their longest or second longest RTE in TCr+Caf, the effect of the combination condition appears fairly robust. It should be noted that a simulated protocol in a lab setting cannot replicate the competitive aspect of a match, particularly during RTE which would coincide with an overtime period. No additional motivation was provided to any subject during this part of the protocol to ensure consistency across conditions, and all subjects were blinded to the treadmill display. This allowed for adequate control of extrinsic factors similarly across conditions, and the results demonstrate consistency in the conditions that produced longer RTE. The RTE results may also be partially explained by a subjective decrease in perceived exertion in these conditions. A meta-analysis on the ergogenic effects of Caf support this notion, showing up to 29% of variance in performance improvements being accounted for by decreases in RPE (Doherty & Smith, 2005; Jenkins et al., 2008). Additionally, Caf's effect on endurance has also been shown to have a glycogen sparing effect through increasing plasma free fatty acids and the rate of lipid metabolism (Costill et al., 1978; Hargreaves et al., 1991). Though, more research needs to be conducted on the mechanistic side of TCr to determine if there is a similar influence on the mobilization of substrates.

It is worth noting that a main strength of this study was the simulated soccer protocol, which appears to be a valid tool in replicating the physical demands of a soccer

match. With this intermittent protocol, the HR response was consistent with that of previous studies that demonstrated an average HR ranging from 155-172 bpm during a 90-minute match, with expected increases in HR between each 45-min half (Aslan et al., 2012; Bangsbo, 1994). This may have future application for laboratory-based studies using soccer players. Though it is impossible to fully mimic the competitive demands of a soccer match, it would appear that this protocol was able to simulate the physiological load. Additional strengths of this study protocol were the varying levels of difficulty in assessing RT using CRT and COGRT in addition to SRT. Using different tests allowed testing for several aspects of cognitive ability and increase the practicality of the protocol.

When taking the overall cognition and endurance effects into account, it appears that the combination of TCr+Caf was the most beneficial in terms of increasing and maintaining energy, concentration, and level of performance. These results would also suggest the benefits of the combined TCr+Caf supplementation may be due to the differences in timing of peak concentration. Additionally, the overall improvements in RT and RTE from TCr+Caf may mitigate impaired CNS activity that has been demonstrated in previous research following a 90-minute match (Brownstein et al., 2017; Verburgh et al., 2014; Vestberg et al., 2012). A potential mechanism to explain the observed improvements may be TCr exposure, as a co-ingestion of Caf and TCr has been shown to significantly alter TCr disposition (He et al., 2017). This results in increased bioavailability and enhanced TCr exposure parameters, including area under the curve plasma concentration and maximum concentration (He et al., 2017). These parameters have been shown to have a maintenance effect in plasma concentration without affecting the half-life of TCr, with no effects on Caf parameters (He et al., 2017), supporting the

notion that timing of supplements may have played a role in the RT data. Furthermore, this may provide a possible explanation for the almost 40% increase in RTE with TCr+Caf. Further research is needed to determine appropriate dosing strategies to optimize the potential benefits of combined Caf and TCr.

Conclusion

This is the first study to our knowledge that has investigated the cognitive and physical performance effects of TCr and TCr+Caf in power-endurance athletes. The combination of TCr+Caf may provide the greatest cognitive benefits during complex decision making, potentially due to overlapping peak concentrations or enhanced bioavailability. Similar benefits were also seen for RTE, which may have clinical importance for extra-time scenarios during matches. Interestingly, the improved cognitive accuracy at end-of-game in all conditions may indicate a training effect in highly skilled players for allocation of resources.

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Tables & Figures

Table 1. Subject Demographics

Variable	Males (n=10)	Females (n=14)
Age (yrs)	21.8±2.53	19.65±3.62
Height (cm)	178.6±6.88	165.2±9.78
Weight (kg)	74.7±8.67	65.76±8.01
Body Fat (%)	10.76±3.48	19.64±3.62
VO _{2max} (mL/kg/min)	55.31±3.39	50.97±3.90

Table 1. Subject demographics. Displays differences between males and females. No significant differences between age, height, weight, body composition (%BF), or aerobic capacity (VO_{2max}) between groups.

Table 2. Activity Profile

Mode	Km/h	% Time	Grade
Standing	0	10.11%	2.00%
Walking	<8.05	33.30%	2.00%
Jogging	8.06-9.66	32.59%	2.00%
Running	9.67-14.48	16.11%	2.00%
Intense Running	14.49-22.53	6.67%	2.00%
Sprinting	>22.53	1.22%	2.00%

Table 2. Activity profile of the game simulation separated into percentage of time spent in each speed zone. The protocol only differed in speeds between males and females at the top two speeds. Females ran at 18.5 and 24.1 km/h and males at 19.31 and 27.35 km/h. The time in these zones did not differ.

Figure 1

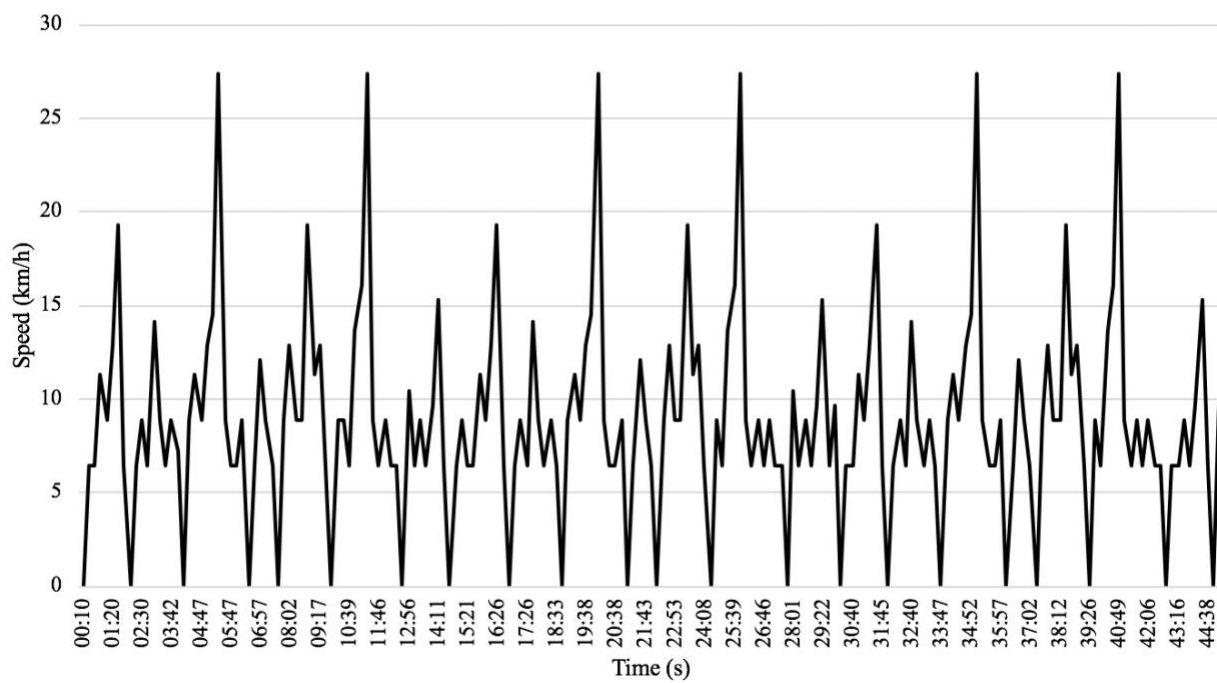


Figure 1. Schematic representation of the male's speeds for the soccer-specific game protocol.

Figure 2

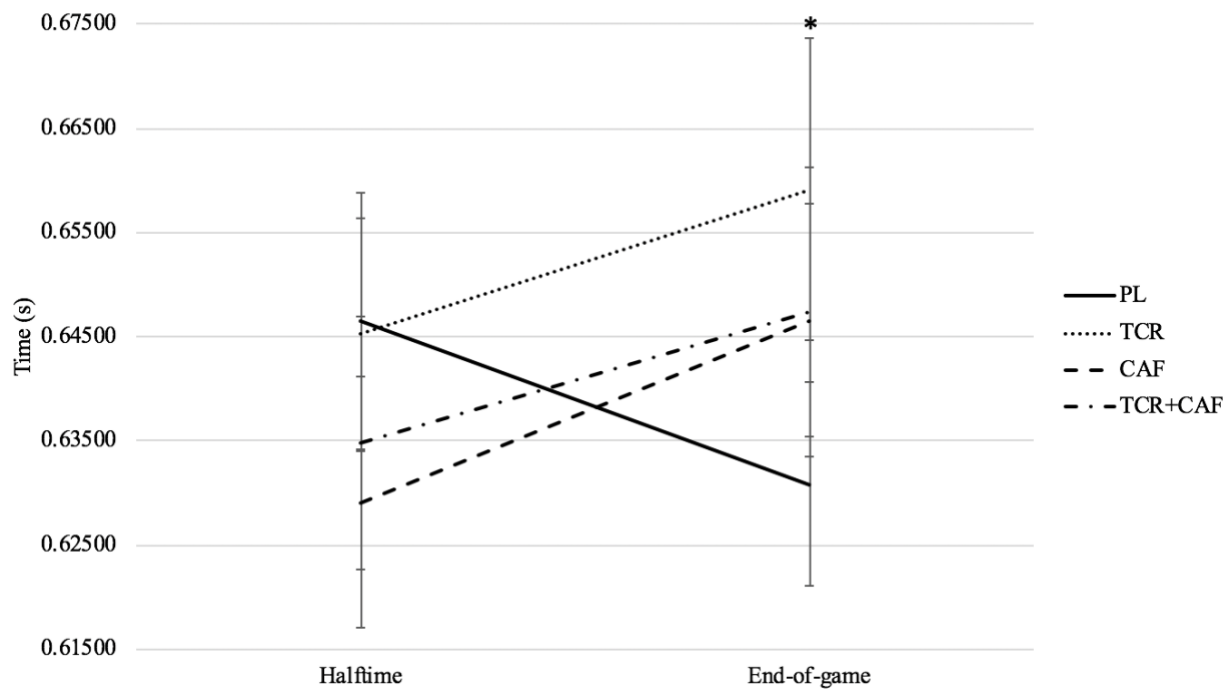


Figure 2. Simple Reaction Time

* Denotes different from halftime values

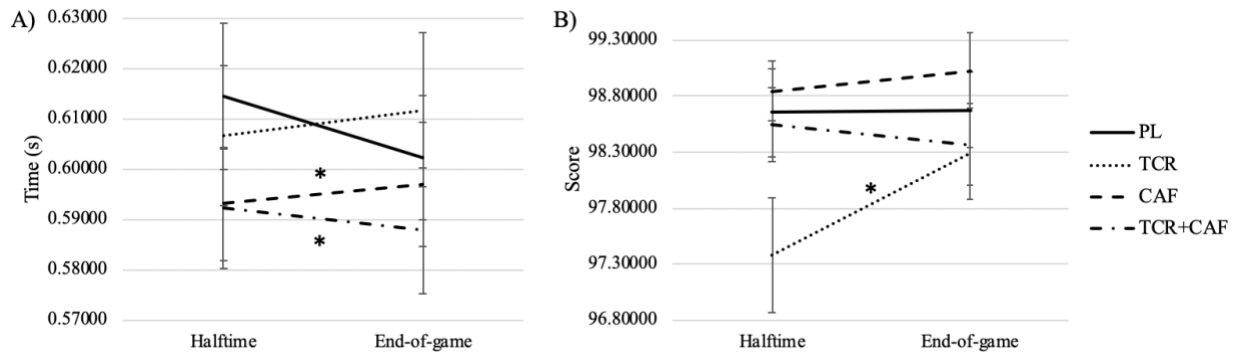


Figure 3A. Choice Reaction Time

* Denotes different from PL

Figure 3B. Choice Reaction Time Score

* Denotes different from PL

Figure 4

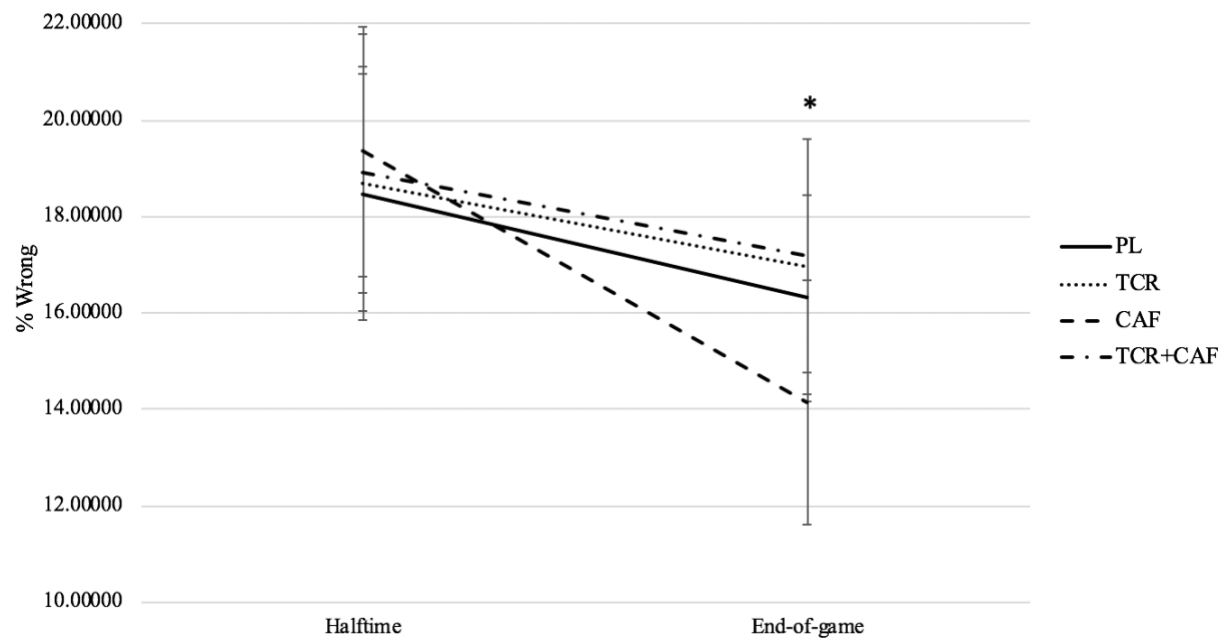


Figure 4. Cognitive Load Reaction Time % Wrong Answers

* Denotes different from halftime

Figure 5

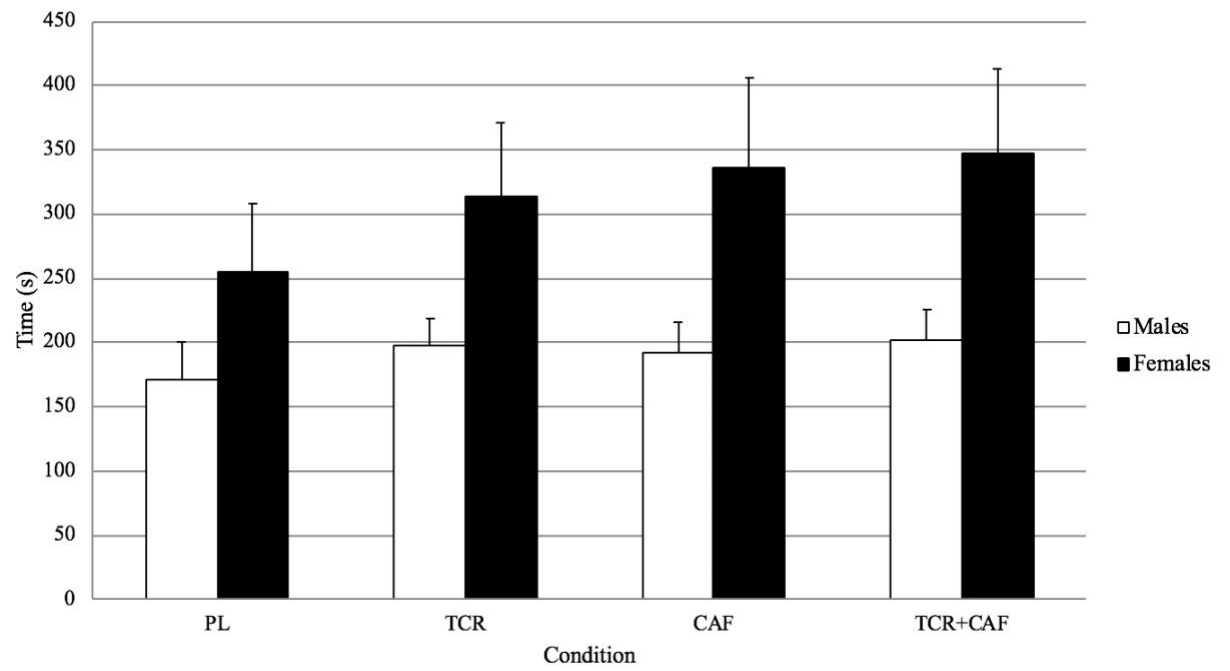


Figure 5. Run to Exhaustion

Figure 6

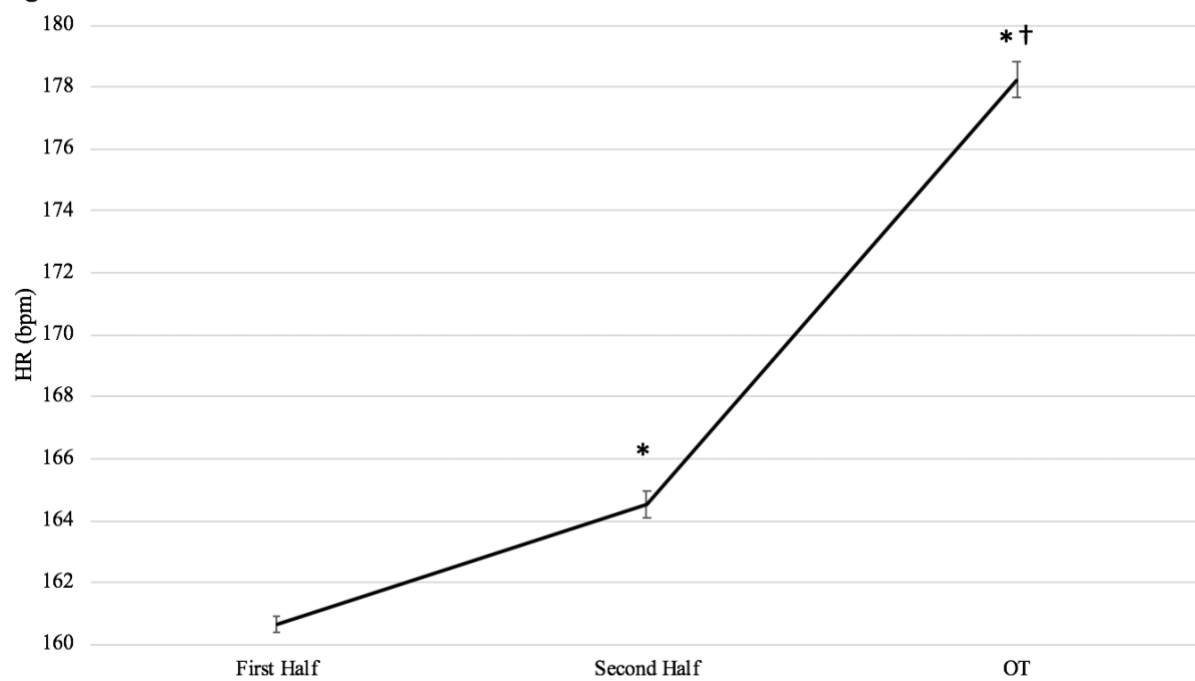


Figure 6. Heart Rate Response

* Denotes different from first half

† Denotes different from second half

Figure 7

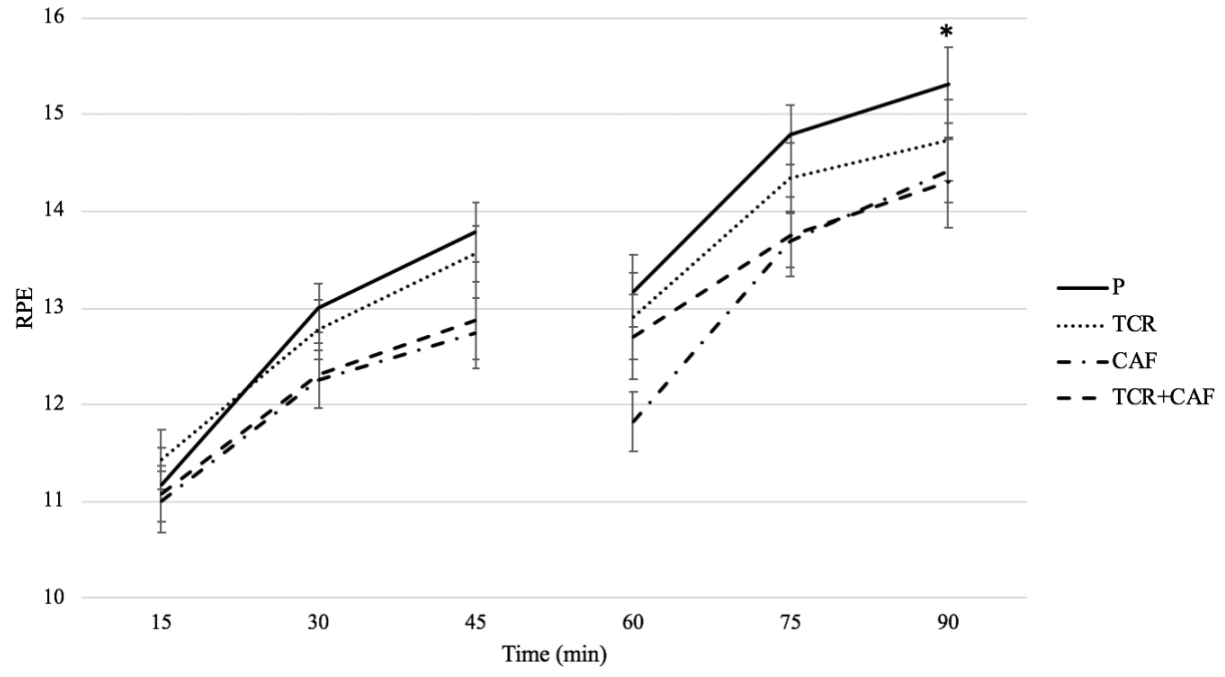


Figure 7. Rating of Perceived Exertion Response

* Denotes different from T1