The effect of mouth rinsing with different concentrations of caffeine solutions on reaction time

ABSTRACT

Caffeine mouth rinsing (CAF-MR) has been shown to improve reaction time (RT). CAF-MR studies have generally used 1.2% CAF concentrations, but the effect of using different concentrations is unknown. Therefore, we compared the effect of different concentrations of CAF-MR on RT. Forty-five trained male athletes (age: 18±3 y) volunteered to participate in this double-blind, randomized controlled crossover study. Participants completed five testing sessions (Control, Placebo (water)-MR, and 1.2%, 1.8%, and 2.4% CAF-MR), with hand and foot RTs assessed immediately after MR. All CAF-MR conditions resulted in significantly faster hand and foot RT compared to Control and Placebo (all $p<0.001$, except for foot RT with 1.8% CAF-MR vs. Placebo: NS). For both hand and foot RT, 1.2% and 1.8% CAF-MR did not significantly differ, but RT for 2.4% CAF-MR was significantly faster than both ($p<0.001$). Improvements in RT for 2.4% CAF-MR vs. Placebo were 22% for hand RT and 21% for foot RT. In conclusions, these findings demonstrate that higher CAF-MR concentrations than those typically used can result in greater improvements in RT. This has implications for the practical use of CAF-MR to enhance performance in sports in which optimal RT is a factor to success.

KEYWORDS: Caffeine; Mouth Rinsing; Reaction Time; Cognitive Function

Introduction

Caffeine (1,3,7 trimethylxanthine; CAF) is a dietary supplement commonly used by athletes to improve sports performance (Pickering, 2019). CAF exerts a pleiotropic effect on cells through a variety of mechanisms, including intracellular calcium mobilization, adenosine antagonism, and phosphodiesterase inhibition (Chung, 2021). The resulting physiological effects, such as glycogen-sparing secondary to adrenaline-induced mobilization of free fatty acids, and enhanced excitation-contraction coupling caused by increased Na^+/K^+ pump activity, may contribute to improvements in endurance and anaerobic performance (Davis & Green, 2009). However, CAF is also known to be associated with cognitive effects, including changes in arousal, mood, concentration (McLellan et al., 2016), attention, and vigilance (Guest et al, 2021). One of the most common and consistent cognitive effects of CAF is improved reaction time (RT) (Saville et al., 2018~~;~~ Torres & Kim, 2019; Santos et al., 2014; McLellan et al., 2016). RT is the total time required to identify a stimulus, choose the appropriate response, transform this response into a motor plan, and apply the motor plan, and it is an important factor in sports where motor control, decision making, coordination and other cognitive functions are factors in success (Meeusen & Decroix, 2018). CAF shortens both simple and/or visual RT and complex RT in snipers and taekwondo athletes (Torres & Kim, 2019; Santos et al., 2014). Consumption of CAF at doses between 12.5-600 mg (~0.2-5.5 mg/kg) improves RT in both rested and sleep-deprived individuals (McLellan et al., 2016).

A leading hypothesis for the cognitive effects of CAF involves the antagonism of adenosine receptors (McLellan et al., 2016). After absorption into the bloodstream, CAF crosses the blood-brain barrier and inhibits adenosine activity by binding to adenosine receptors (Guest et al., 2021). Thus, it inhibits the negative effects of adenosine on neurotransmission, arousal and pain perception (Davis & Green, 2009). In this way, the feeling of muscle pain decreases, alertness increases, and fatigue is delayed (Ehlert et al., 2020).

CAF is generally ingested in the form of energy drinks, sports gels, and beverages and foods such as tea, coffee, cola, and chocolate (Davis & Green, 2009), with subsequent absorption in the gut. However, CAF can also be rapidly absorbed through the buccal mucosa, which can lead to similar plasma CAF levels but with a faster time to peak CAF levels (Kamimori et al., 2002; Pomportes et al., 2017). This has implications for athletes, as undesirable side-effects of CAF consumption that may adversely affect sports performance (e.g., nausea, lower abdominal cramps, bloating, urge to defecate, gastroesophageal reflux, and heartburn), can be prevented by utilizing mouth rinsing (MR) as a means of administering CAF (Boekema et al., 1999; Wilson, 2016; Van Cutsem et al., 2018; De Pauw et al., 2015).

In support of MR as a means of administering CAF, CAF-MR has been confirmed to improve RT (De Pauw et al., 2015). Interestingly, however, it has been demonstrated that CAF-MR in the buccal cavity for a limited time (5-10 s) is too short to increase plasma CAF concentration (Doering et al., 2014, Pickering, 2019; Ehlert et al., 2020). Thus, the ergogenic effect of CAF-MR on RT is unlikely to rely on mechanisms involving increased plasma CAF levels. Other possible mechanisms involve stimulation of nerves with direct links to the brain (Wickham & Spriet, 2018), potentially related to the bitter taste of CAF (Matsumoto, 2013; Poole & Tordoff, 2017; Best et al., 2021). In support of this, De Pauw et al. (2015) demonstrated that the improvement in RT with CAF-MR was associated with activation of both the orbitofrontal cortex and the dorsolateral prefrontal cortex, which are the attention and reward areas in the brain.

As ingestion of ~300 mg of CAF is generally recommended for achieving optimal ergogenic effects, most CAF-MR studies have been performed with 25 mL liquids containing 300 mg (i.e., 25 mL liquid equals ~25 g and 300 mg / 25 g = 1.2%) CAF (McLellan et al., 2016). However, regardless of the precise mechanism of the ergogenic effect of CAF-MR on RT, if the mechanism relies on direct effects of CAF on the brain rather than plasma levels, then higher CAF-MR concentrations may be expected to enhance the ergogenic effect through increased nerve stimulation. To our knowledge, no studies have directly compared the effects of different concentrations of CAF-MR on RT. Therefore, the aim of the present study was to investigate the effect of MR of three different concentrations of CAF solutions (1.2%, 1.8% and 2.4%) on RT.

Materials & methods

Participants

Forty-five healthy male athletes, trained in volleyball or football, volunteered to participate in the study (mean±SD (range) for age: 18±3 y (15-33 y); weekly training duration: 14±3 h (9-20 h); training experience: 9±3 y (5-20 y)). Inclusion criteria for the study were having a regular training experience of at least 5 years, training frequency of at least 3 days a week for at least 60 minutes per session, participating in national or international competitions, not having a serious injury in the preceding 6 months, and not using alcohol or drugs regularly. Participants had no previous experience of any type of MR intervention. All participants were asked to fill out a questionnaire about their training experiences, training frequencies, injury history, and nutrition habits to be controlled the inclusion and exclusion criteria for the study. Participants were informed of the nature of the study and signed an informed consent form in accordance with the principles of the Declaration of Helsinki and those of the local ethical committee (21-1.1T/58).

Experimental Design

The study was a double-blind, randomized controlled crossover study. Participants attended six sessions over a 30-day period, with a minimum of 3 days in between sessions. Following an initial familiarization session, the five testing sessions involved three different doses of CAF-

MR (1.2%, 1.8% and 2.4%), Placebo (water)-MR, and Control (no MR application). These were administered to the participants in a randomized order. Hand and foot RT tests were performed immediately after MR application as in methods of the other studies, because of the claim that CAF administrations directly to the mouth may affect the brain more quickly through several proposed mechanisms (Wickham & Spriet, 2018; Guest et al., 2021).

Participants were asked to avoid vigorous activity and CAF consumption in the 24 hours before testing. They recorded their diet for the day before the first session and were asked to repeat a similar diet before the subsequent sessions. Participants were asked to drink ~0.5 L of water in the morning before testing. All testing sessions were conducted at the same time of day, between 11:00 and 13:00 **(**to minimize potential circadian rhythm effects), at an ambient temperature of 22-24°C and a relative humidity of ~70%, in a noise-free and light-filled test environment.

Familiarization session

This session aimed to familiarize the participants with the test device, RT test protocol, test environment, and researchers.

To evaluate habitual CAF intake, a questionnaire developed for this study was applied face to face by a nutritionist involved in the study. The athletes were asked about the type of caffeinated foods or beverages and frequency of consumption to estimate the participants' daily CAF consumption.

Subsequently, participants rinsed their mouth with 25 mL of water, and were asked to spit the water back into a graduated bowl at the same amount without swallowing it. Following this practice, the athletes practiced the RT test trials.

Testing sessions

To standardize the procedures in this study, athletes were required to participate in the tests at least 2 hours after breakfast and not to consume CAF products the morning before the test. CAF consumption was checked from the diet lists before the tests. The previous night's sleep duration (SD), and mood level (ML; using the Brief Mood Introspection Scale, -10 to +10; Kavcioglu, 2011) before the test were recorded to determine the effect of the participants' regular training period. Participants were asked to rinse their mouth with one of the four 25-mL solutions (1.2%, 1.8%, 2.4% CAF, or water) for 10 s in a double-blinded fashion, or perform the control condition (no MR), directly before the hand RT test. Solutions were prepared in a nontransparent graduated cylinder (Falkon Isolap Sterile Tube). After rinsing, the solution was spit out into the graduated tube, and it was checked whether the solution was swallowed.

Simple reaction time test for hand

The visual RTs of the subjects were determined using a Newtest 1000 (Finland) test device. The device consists of two separate parts: the warning sign, which is placed on the table with the selected time, and the stimulus piece, allowing the participant to receive the stimulus. Participants were asked to sit in a chair with their dominant hand on the table and respond to 5 light stimuli given at unequal intervals by touching the button on the device as fast as possible. Response times of the participant to these stimuli were recorded in milliseconds. The mean of the five measurements was used as the outcome measure.

Simple reaction time test for foot

A purpose-built device with high validity ($R^2 = 0.994$) and reliability (ICC: 0.99, CV: 0.4%), based on results from an unpublished study performed in our laboratory, was used for foot RT measurement. Stimuli were given manually by the researcher from a place the athlete could not see. To determine foot RT, the participants were asked to stand with their chosen foot on the receiver connected to the device on the floor without wearing shoes, and to respond to the light stimuli given at unequal intervals by lifting their foot from the receiver as quickly as possible. The other foot was positioned in a balanced way as determined by the participant. Response times of the participants to these warnings were recorded in milliseconds, and the mean of the five measurements was recorded.

Side effect and blinding effectiveness evaluation

After each test session, participants were asked to guess what the solution they had rinsed in their mouth was. Participants were also asked if CAF-MR was associated with any side effects (Wikoff et al., 2017).

Statistical analysis

The required sample size was calculated using G∗Power software (version 3.1.9.2, Franz Faul, Universitat Kiel, Dusseldorf, Germany) for repeated measures ANOVA for detecting a large effect size (1.2) with $\alpha = 0.05$, and a $1 - \beta$ error probability of 0.8, which revealed that a sample size of 13 participants was required.

All data are presented as mean±SD. Study data were analyzed using SPSS Statistics for Windows version 25 (IBM, Armonk, NY; 2015). The Shapiro-Wilk test was performed to assess normality of data, and skewness and kurtosis values were checked. Inter-intervention comparison of related variables was performed using repeated measures ANOVA, with Bonferroni *post hoc* tests to perform pairwise comparisons. The potential modifying effects of age, training experience, training time, and habitual CAF consumption was assessed by creating dummy variables and including these as independent variables (age: <18 y vs. ≥18 y; training time: <16 h/week vs. ≥16 h/week; training experience: <10 y vs. ≥10 y; habitual CAF consumption: <125 mg/day vs. ≥125 mg/day). Alpha was set at 0.05.

Results

Repeated measures ANOVA revealed significant differences in RT between the experimental conditions for both hand ($F(4, 152) = 42.616$, $p<0.001$, $\eta p^2 = 0.529$; **Figure 1A**) and foot ($F(4, 152) = 39.502$, $p<0.001$, $\eta p^2 = 0.510$; **Figure 1B**). For hand RT, *post hoc* analysis revealed that there was no significant difference between the Control and Placebo conditions, but that all CAF-MR conditions resulted in significantly faster RT (all $p<0.001$). RT for concentrations of 1.2% and 1.8% CAF-MR did not significantly differ, but RT for 2.4% CAF-MR was significantly faster than all four other conditions ($p<0.001$). Hand RT for 2.4% CAF-MR was 22% faster than for Placebo, compared to 15% and 11% for 1.2% CAF-MR and 1.8% CAF-MR respectively.

Results for foot RT followed a similar pattern. RT following CAF-MR was significantly faster than Control for all three CAF concentrations ($p<0.001$), but only 1.2% and 2.4% CAF-MR were significantly faster than Placebo ($p<0.001$). Again, RT for concentrations of 1.2% and 1.8% CAF did not significantly differ, but RT for 2.4% CAF was significantly faster than all four other conditions ($p<0.001$). Foot RT for 2.4% CAF was 21% faster than for Placebo, whereas 1.2% CAF-MR and 1.8% CAF-MR resulted in 9% and 6% faster RT compared to Placebo respectively.

Figure 1

Inclusion of age (<18 y *vs*. ≥18 y), weekly training hours (<16 h/week *vs*. ≥16 h/week), training experiences (<10 y *vs*. ≥10 y), and daily CAF consumption (<125 mg/day *vs*. ≥125 mg/day) as between-subjects factors in the model did not result in significant interaction effects, suggesting that these parameters did not influence the effect of CAF-MR on RT. According to the results of the habitual CAF intake questionnaire, the average daily CAF consumption of the participants was 163 mg/day, and only 15% of them had 300 mg/day. In addition, when the consumption of energy drinks was examined due to the intense caffeine content, it has been determined that only 4 athletes are regular but rarely (once a month) consumers. Only the main effect of daily CAF consumption on hand RT was significant (p=0.045), demonstrating faster hand RT for participants consuming <125 mg/day (407±41 ms) compared to participants consuming ≥125 mg/day (438±46 ms).

Mood level (ML) and sleep duration (SD) taken before the RT test of the participants in each session are shown in **Table 1**. No significant differences were observed between the five conditions.

Table 1

No CAF-related side effects were reported after rinsing. All participants were able to distinguish between Placebo and CAF-MR conditions, but on average participants were unable to correctly identify the 3 different concentrations.

Discussion

In this study, the effect of mouth rinsing CAF solutions prepared at different concentrations on hand and foot RT was investigated. In support of a previous study (De Pauw et al., 2015) it was found that CAF-MR has a significant positive effect on both hand and foot RT. A novel finding of the present study is that the effect of CAF-MR can be enhanced by using greater CAF concentrations: the effect of the 2.4% CAF-MR concentration was significantly greater than that for concentrations of 1.2% and 1.8%. This has implications for the practical use of CAF-MR to enhance performance in sports in which optimal RT is a factor to success.

On a practical level, CAF-MR is considered to be a valuable alternative strategy for athletes who wish to obtain some of the performance benefits of CAF, or who do not want to consume CAF, while minimizing the side effects (e.g. anxiety, tremors, gastrointestinal distress) resulting from consuming ergogenic doses of CAF (McLellan et al., 2016; Pallarés et al., 2013; Van Cutsem et al., 2018). Based on the recommended dose for ingesting CAF (~300 mg), most studies investigating the effects of CAF-MR have been carried out with 25 mL solutions containing 1.2% CAF (i.e., 300 mg), but there is no clear reason why the total CAF dose in a MR solution should be the same as that used in studies in which CAF is ingested. In the present study we provide initial evidence that the ergogenic effect of CAF-MR on RT is dose dependent, and concentrations higher than those typically used provide a greater ergogenic effect. Although taste buds are present in all parts of the oral cavity, there is evidence that bitter taste is most strongly felt on the back of the tongue (Gam et al., 2014). Based on this information, although there is the opinion that bitter tastes such as CAF may not have an effect in MR applications that do not include swallowing, the improvement observed in hand and foot RTs in MR applications at all three CAF concentrations we used in our study suggests that the bitter taste may have a stimulating effect.

Caffeine has consistently been shown to improve exercise performance when consumed in doses of 3–6 mg/kg body mass (Guest et al., 2021). However, when the primary studies are examined, it is seen that the ergogenic effect of caffeine is generally determined by using a dose of 6mg/kg (Grgic et al., 2021). Minimal effective doses of caffeine currently remain unclear, but they may be as low as 2 mg/kg body mass. Very high doses of caffeine (e.g. 9 mg/kg) are associated with a high incidence of side-effects and do not seem to be required to elicit an ergogenic effect (Guest et al., 2021). In a study examining the ergogenic effect of caffeine dose on muscular endurance performance, it was reported that with a 1mg/kg increase in caffeine dose, the effect size on muscular endurance increased by 0.10 and the dosage explained only 16% of the variance between the studies (Warren et al., 2010). In another dose study, resistance-trained athletes showed significantly increased muscular endurance performances only after high dose (750 mg: 3% vs 250 and 500 mg) caffeine mouth rinse when they performed bench press movement 60% of 1-RM repetitions to failure performance (Karayigit et al., 2021). However, consuming low-dose (100 mg) caffeine increased the simple reaction time positively, while high-dose (400mg) did not in middle-aged women (Waer et al., 2021). The dose-dependence of caffeine's effects has been interpreted as, in parallel with the occupancy hypothesis, the higher the caffeine dosages, the more adenosine and taste receptors within the mouth can be stimulated, thus helping to improve muscular performance (Karayigit et al., 2021). Additionally, application of CAF-MR at both low (Bottoms et al, 2014; Sinclair, 2014) and high (Beaven et al, 2013; Kizzi et al, 2016) concentrations have been shown to improve both short-term (Beaven et al, 2013; Kizzi et al, 2016) and long-term (Bottoms et al, 2014; Sinclair, 2014) exercise performances. The result of the few studies available suggest that optimal doses should be considered depending on the source of caffeine, exercise testing, type of muscle movement, and may differ between individuals (Grgic et al., 2021).

The use of high concentrations of CAF in MR solutions may have potential limitations, especially in terms of flavor (Pickering, 2019). Thus, the acceptability of high concentrations of CAF-MR solutions should be established in future studies. From a research perspective, the bitter taste of CAF-MR makes effective blinding more difficult, and this may create the potential for bias through expectancy effects (Chan & Maglio, 2019; Pickering, 2019; Saunders et al., 2017). For these reasons, no CAF concentrations higher than 2.4% were investigated in this study, but future studies should attempt to achieve effective blinding of higher concentration CAF-MR solutions, to determine if RT can be improved further.

There are a number of limitations to this study that warrant a mention. Firstly, although participants were asked to maintain their usual training and diet, and replicate the diet consumed before the first test in all other tests, it would be appropriate to keep the diet of the athletes under greater control. Secondly, we were only able to compare 3 concentrations of CAF-MR, so the 'optimal' dose for the average athlete remains unknown. Future studies should examine the ergogenic effects of CAF-MR with a concentration greater than 2.4% (or smaller than 1.2%), alongside investigations into interindividual differences in response and the reasons for these. And thirdly, the present study was not designed to provide information on possible mechanisms of the ergogenic effects of CAF-MR. We did not determine plasma CAF levels following MR, but as RT was measured directly following just 10 s of rinsing, we can be confident that improvements in RT following CAF-MR were not dependent on increased plasma CAF levels and subsequent crossing of the blood-brain barrier by CAF.

In conclusion, the present study is the first to demonstrate that CAF-MR with a higher concentration than what is typically used (2.4% vs. 1.2% respectively) results in significantly greater improvements in both hand and foot RT. This information provides athletes competing in sports in which a faster RT may improve performance with an opportunity to enhance their performance.

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Table 1. Mood level (ML), and sleep duration (SD) at the start of testing session.

	Control	Placebo	1.2% CAF-MR	1.8% CAF-MR	2.4% CAF-MR
Mood level	5.3±4.0	5.7±3.9	5.7±4.1	5.3±4.0	5.7±3.8
SD (h)	7.9±0.9	8.3±1.6	8.0±1.2	8.3±1.4	8.1±5.1

Figure 1. Differences in hand (A) and foot (B) RT for the five experimental conditions. All conditions were significantly different from each other (p<0.001), except for the conditions with a letter above their columns; these were not significantly different from **a**: Control, **b**: Placebo, **c**: 1.2% CAF-MR, and **d**: 1.8% CAF-MR.