

A Project Project (Honours Project / ~~Capstone Project~~) entitled
“THE EFFECT OF MOUTH RINSING WITH HIGH CONCENTRATIONS OF CAFFEINE
SOLUTIONS ON LOWER BODY MUSCULAR ENDURANCE AMONG HONG KONG
COLLEGE ATHLETES.”

Submitted by

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Submitted to the Education University of Hong Kong
for the Bachelor Degree of Education (Physical Education)

in *“April, 2024”*

Declaration

I, Yiu Fan Sun declare that this research / project report represents my own work under the supervision of by Dr. Bob Fenghua SUN, and that it has not been submitted previously for examination to any tertiary institution.

Signed Yiu Fan Sun

Student Name

Date 25th April 2024

Abstract

Introduction: The use of caffeine mouth rinse (CMR) solutions to enhance athletic and workout performance while mitigating the possible adverse effects of caffeine is increasingly gaining attention. Although one previous study investigated the effect of different dosages of CMR on upper-body muscular performance (Karayigit et al., 2021), the possible benefits of a very high concentration of CMR on lower-body strength were not explored. **Purpose:** This study aimed to investigate whether high-concentration CMR impacted lower-body muscular endurance in male college athletes who had engaged in resistance training. **Methodology:** In a randomized, single-blind, and cross-over design, thirteen college athletes with prior experience in resistance training completed three conditions. The conditions involved a 5-second mouth rinse with 25 mL solutions containing 4% CAF solution (VHCMR), 3% CAF solution (HCMR), or sweetened water as a placebo (PLA) prior to a test measuring leg press muscular endurance. The blinding method was to add sucralose, which contains no glucose. In each condition, muscular endurance (repetitions), heart rate, muscle soreness, and ratings of perceived exertion (RPE) were measured. These metrics were recorded for all conditions. The data collected were analyzed using IBM SPSS. Repeated measures ANOVA was used to examine the main effects for (1) condition, (2) time or trial, and (3) condition x time or trial interaction. **Results:** VHCMR significantly increased muscular endurance compared to PLA ($p=0.006$), but there was no difference between VHCMR and HCMR ($p=0.109$). In addition, no significant difference in muscular strength was detected between HCMR and PLA ($p=0.062$). Moreover, there was no significant difference in heart rate, RPE, and muscle soreness among the three conditions. **Conclusion:** The VHCMR, but not the HCMR adopted in the present study, seems to improve lower-body muscular endurance.

1. Introduction

Caffeine (CAF) is a dietary supplement that enhances sports performance in various forms of exercise (Grgic et al., 2019) and has stood out as one of the most popular dietary ergogenic aids utilized by athletes (Knapik et al., 2016). During the 1970s, it was suggested that caffeine could improve performance by boosting fat metabolism, consequently conserving glycogen use. In the following three decades, caffeine was identified as an adenosine blocker and recognized for its direct impact on the sarcoplasmic reticulum, which aids in sustaining force output (Goldstein, 2009). Scholarly evidence suggested that caffeine consumption has been linked to improvements in a range of physical performance measures, including cardiorespiratory endurance (Southward et al., 2018), movement velocity (Raya-González et al., 2020), power output (Grgic et al., 2018), and muscular endurance during resistance exercises (Palito et al., 2016; Karayigit et al., 2021). For instance, Grgic et al. (2020) found that taking 4-6 mg/kg of caffeine boosted bench press strength and had an additive effect on caffeine's ergogenicity. According to Goldstein et al. (2010), caffeine was typically consumed at dosages of 3-6 mg/kg of body weight approximately 60 minutes prior to engaging in exercise. Caffeine acts as an adenosine antagonist and is believed to be the reason for its performance-enhancing benefits (Graham, 2001). Once consumed and absorbed into the bloodstream, caffeine passes through the blood-brain barrier, where it acts as an inhibitor of adenosine at its receptor sites (Graham, 2001; Ribeiro & Sebastiao, 2010). When adenosine activity is inhibited by caffeine, alertness, and motivation are enhanced while the perception of physical exertion and muscle pain are reduced (Graham, 2001; Meeusen et al., 2013). Caffeine's positive effects on neurotransmission, perceived exertion, and arousal are believed to be the result of complex and varied mechanisms, which are likely multifactorial in nature. However, the underlying mechanism for these effects is thought to involve antagonism of adenosine receptors (specifically A1, A2A, and A2B) (Grgic,2021). However, consuming

caffeine before or during exercise may lead to certain adverse effects, including but not limited to anxiety, headaches, gastrointestinal discomfort, and insomnia. These potential side effects should be taken into consideration when determining whether or not to consume caffeine as a supplement prior to exercise (Pallares et al., 2013). A 5-ounce cup of coffee typically contains around 100 mg of caffeine, while the same serving size of tea contains approximately 50 mg. Additionally, a 12-ounce serving of cola typically contains between 35 and 55 mg of caffeine (Goldstein, 2009). If high concentration of caffeine solution is used to enhance sports performance by ingesting, safety issue may be a concern. Note that 25 ml of 3% caffeine concentration solution contains 750mg of caffeine that is equal to around 7.5 cups of coffee. It is not safe to investigate high concentration of caffeine solution if the solution will be ingested. In addition, recent research suggested that caffeine's ergogenic effects may not solely result from its ingestion and absorption, and some have proposed that the activation of bitter taste receptors may also play a role (Pickering, 2019). Therefore, an alternative usage of caffeine, Caffeine mouth Rinsing (CMR), has been investigated recently.

Caffeine mouth rinsing (CMR) has been put forward as a viable strategy for enhancing exercise performance without the potential adverse effects associated with caffeine ingestion. This approach warrants further investigation as a potential alternative or adjunct to traditional caffeine supplementation protocols (Wickham & Spriet, 2018; Ferraz et al., 2021). The CMR technique involves rinsing the mouth with caffeine for a period of 5-20 seconds without actually swallowing or ingesting the caffeine solution (Karayigit et al., 2021). Research has suggested that the mechanism underlying the effectiveness of CMR may be related to the activation of bitter taste receptors in the oral cavity upon exposure to caffeine (Matsumoto, 2013; Meyerhof et al., 2010). This implies that the effects of caffeine mouth rinsing may be similar to those observed with carbohydrate mouth rinsing (Chambers et al., 2009; Ehlert et al., 2020). To be precise,

the activation of bitter taste receptors by caffeine has been found to stimulate the brain regions responsible for information processing and reward, resulting in increased mental alertness through the transmission of dopamine (Gam et al., 2014). This mechanism may contribute to the cognitive-enhancing effects of caffeine (Pickering, 2019). An additional theory explaining the performance-enhancing effects of CMR is the potential binding of caffeine with adenosine receptors located in the mouth. This action may facilitate the release of neurotransmitters and increase the firing rates of motor units (Kamimori et al., 2002). The effect of CMR on sports performance has been investigated in much research. For instance, it was proven that a 10-second CMR protocol improved fatigue tolerance, as indicated by a decrease in EMG activity and wavebands, as well as an increase in RPE (Melo et al., 2018). Rinsing with caffeine has been shown to enhance 30-minute cycling performance by enabling participants to elevate cadence, power, and velocity without experiencing a simultaneous rise in perceived exertion or heart rate (Bottoms et al., 2014). Furthermore, high-concentration CAF mouth rinsing (HCMR) effectively enhances upper-body muscular endurance (Karayigit et al., 2021). These findings suggested that CAF mouth rinse may be a practical and effective approach for improving exercise performance, and further investigation into optimal dosages and protocols is warranted.

Studies have indicated that the main cause of fatigue during maximal resistance exercise is linked to a decrease in neural drive, as stated by Walker et al. (2012). Furthermore, Smilios et al. (2010) have proposed that during a moderate load muscular endurance session, there may be an increase in muscle electrical activity that could counteract the onset of fatigue. Hence, CMR has the capacity to enhance central drive and, therefore, represents a viable strategy for improving strength and muscular endurance performance (Karayigit et al., 2021). For instance, an investigation into the effects of CMR on muscular performance found that there were no discernible benefits on one repetition maximum (1-RM) or 60% of 1-RM repetitions to failure

performance (Clarke et al., 2015). However, a similar experiment was done using HCMR, which showed the benefit of CMR to muscular endurance (Karayigit et al., 2021). This showed the potential of HCMR in enhancing sports performance compared to traditional studies. The results of this study also support Clark's (1926) occupancy hypothesis, which suggests that higher doses of caffeine can stimulate more adenosine and taste receptors in the mouth, leading to improved muscular performance. However, most studies used 2% or below caffeine concentration in the experiment, and only one study investigated high concentration (3% CAF) CMR (Karayigit et al., 2021). There is a lack of investigation into the high concentration (4% or above CAF) of CMR in sports performance. According to Caffeineinformer, the highest CAF concentration found in commercial coffee is around 4%. Therefore, the highest CAF concentration being investigated will be 4% since athletes can hardly obtain a 5% or higher CAF concentration solution for daily practice. Since CMR has been shown to have no impact on upper body strength, it is highly likely that it will also have no effect on lower body strength. However, it has been noted that moderate concentration CMR (MCMR) has been effective in improving upper body endurance, with higher concentration CMR (HCMR) yielding even better results (Karayigit et al., 2021). Based on this, there is a strong possibility that MCMR will have a positive effect on lower body endurance and that the effect will be comparably weaker than that of HCMR. Thus, only HCMR and a very high concentration of CMR (VHCMR) will be investigated in this study. This study aims to find out the effect of HCMR and VHCMR on lower body endurance.

2. Methodology

2.1 Participants:

Thirteen healthy, resistance-training experienced college athletes were recruited for this study. All study participants were Hong Kong-based athletes who possessed at least two years of experience in resistance training and engaged in lower-body resistance exercises at least twice per week. The eligibility requirements for participant inclusion in this study were carefully defined. In order to participate, individuals had to meet the following criteria: (a) not a smoker, (b) a very low consumer of caffeine, (c) free from neuromuscular and musculoskeletal disorders, and (d) capable of successfully executing leg press exercises with a load equivalent to 60% of their current body mass. Before providing written informed consent, participants were fully briefed on the study's procedures, including any potential risks associated with their participation. Furthermore, all experimental protocols were conducted in accordance with the guidelines of the Human Research Ethics Committee, which approved the study.

2.2 Experimental Design:

Following a single familiarization session, participants were randomly assigned to complete three conditions in a randomized, single-blind, and cross-over design. The three experiment conditions are mouth rinsing with 4%(1000 mg) CAF solution (very high concentration), 3%(750mg)CAF solution (high concentration), or water as a placebo for five seconds prior to a leg press muscular endurance test. After rinsing the area surrounding the buccal cavity for five seconds, solutions were expectorated into a plastic cup. To mask the solutions, 300mg of sucralose will be added to both solutions. Sucralose is a sweetener that does not contain glucose. To eliminate any potential impact of circadian rhythm and fatigue

accumulation on the test results, the testing sessions will be scheduled at the same time slot, more than 72 hours apart, on different days. Additionally, participants were given instructions to refrain from consuming any foods or beverages containing caffeine or alcohol and participate in vigorous physical activity for a complete 24 hours before each visit.

2.3 Exercise Protocol:

The correct form and exercise skills were taught and confirmed in the familiarization section before the experimental session. Depth (starting concentric position ≤ 90 degrees of knee flexion) on the leg press machine was strictly required (Jones et al., 2021), and requirements were guided to ensure participants performed the correct form during the test. The experimental setup was referred to in a previous study that investigated the effects of caffeine ingestion on lower body strength and endurance (Jones et al., 2021). The protocol used in that study was well supported as effective for measuring lower body endurance. Throughout the experimental trials, participants performed a single warm-up set consisting of 15 repetitions at 30% of 1RM, followed by 12 repetitions at 50% of 1RM. After warming up, participants rested for 2 minutes. Participants then mouth rinsed with a solution provided by the investigator. After mouth rinsing for five seconds, participants immediately performed repetitions to failure (RF) at 60% of their 1RM. The RF test was performed twice, and a two-minute break was given between each set of leg presses. Heart rate, muscle soreness, and rating of perceived exertion (RPE) were recorded after the first attempt and after the second attempt. Only heart rate was recorded before the warm-up session to serve as a control. Muscle soreness was measured using a 10-point scale ranging from 1 to 10 (Dierking et al., 2000), while RPE was measured using the simplified Borg scale ranging from 1 to 10 (Borg, 1973). The number of repetitions was noted to represent participants'

lower body muscular endurance. To ensure consistency, all measurements will be taken by the same investigator. A schematic representation of the exercise protocol can be seen in Figure 1.

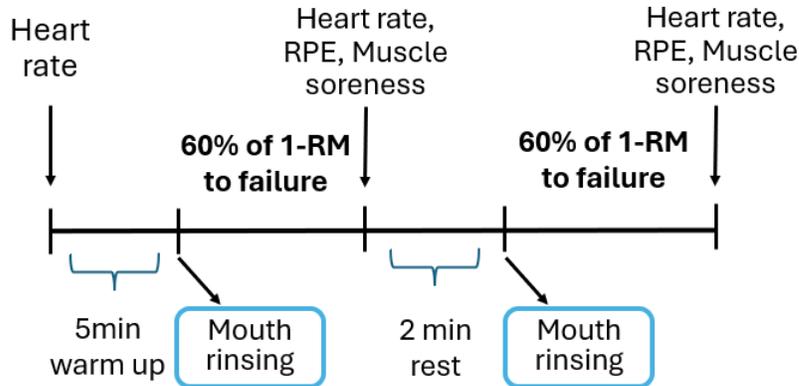


Figure 1: illustration of study procedure

2.4 Statistical Analysis:

The data collected was analyzed using the IBM SPSS. Repeated measures ANOVA was used to examine the main effects for (1) condition, (2) time or trial and (3) condition x time or trial interaction. Statistical significance was found when $p < 0.05$.

3. Results

3.1 60% of 1-RM Muscular Endurance Performance

There was a main effect for condition($p=0.016$) and time($p=0.005$). However, no condition x time interaction was detected($p=0.102$). VHCMR showed a significant difference in repetition numbers compared to PLA($p=0.006$), but there was no difference between VHCMR and HCMR($p=0.109$). In addition, no difference was found between HCMR and PLA ($p=0.062$). Analyzing the first and second trials separately using repeated measures, a similar result was generated, indicating no significant difference between HCMR and PLA in both the

first trial ($p=0.058$) and the second trial ($p=0.098$). In contrast, there was a significant difference between VHCMR and PLA in the first ($p=0.038$) and the second trial ($p=0.001$). Moreover, there was no significant change between VHCMR and HCMR in the first trial ($p=0.365$). However, there was a significant difference between VHCMR and HCMR in the second trial ($p=0.025$).

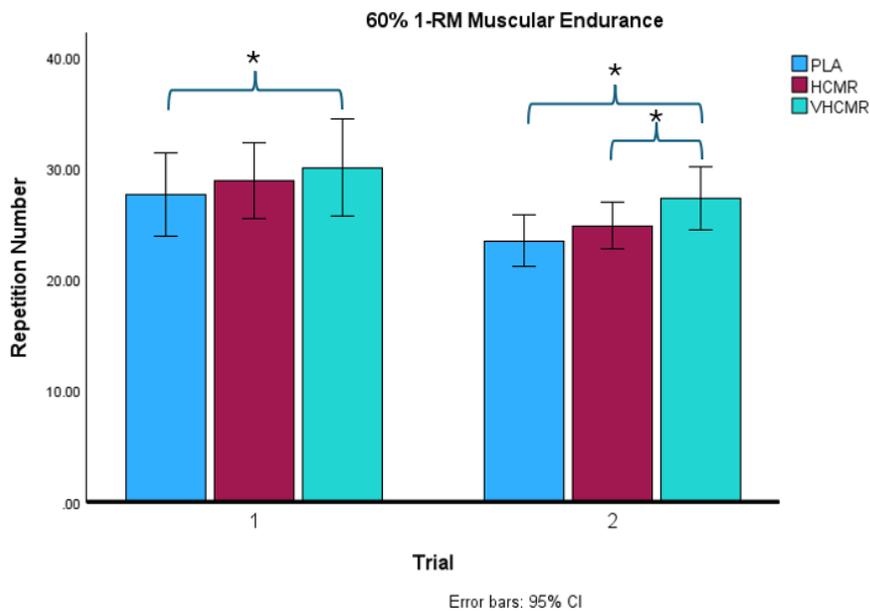


Figure 2. Mean (SD) 60% of 1-RM endurance performance. PLA: placebo; HCMR: high concentration of caffeine mouth rinsing; VHCMR: very high concentration of caffeine mouth rinsing. * : Significantly different from PLA and VHCMR; HCMR and VHCMR in 2nd trial

3.2 Heart Rate (HR)

HR did not differ between conditions ($p=0.625$), and no condition x time interaction was detected ($p=0.541$). However, there was a main effect for time ($p<0.001$).

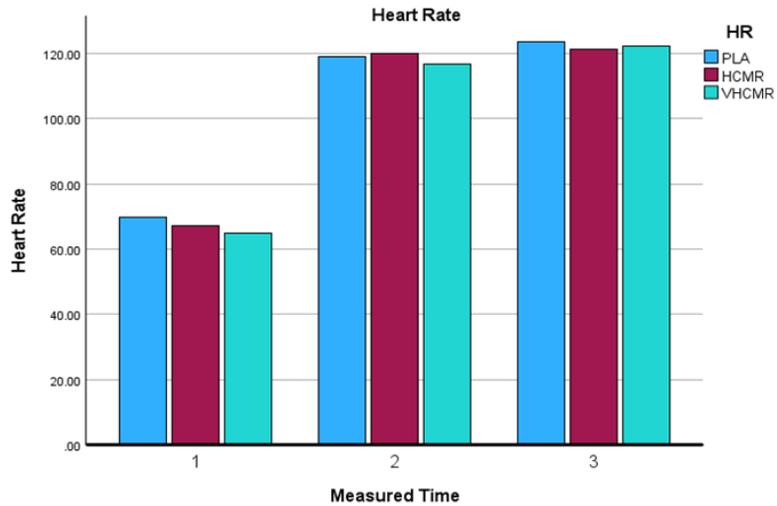


Figure 3. Mean Heart Rate (beat / minute)

3.3 Perceived Exertion (RPE)

RPE did not differ between conditions ($p=0.234$), and no condition x time interaction ($p=0.588$) existed. However, there was a main effect for time ($p=0.004$).

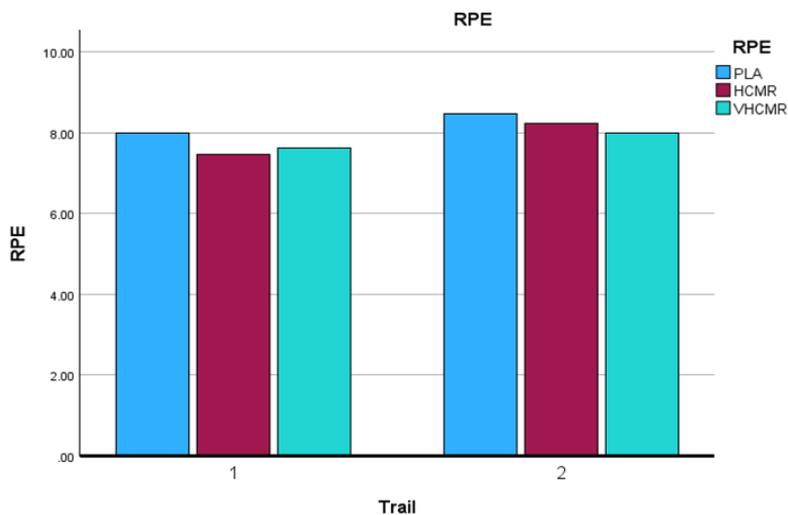


Figure 4. Mean RPE (rating: 1 - 10)

3.4 Muscle Soreness

Muscle soreness did not differ between conditions($p=0.828$), and there was no condition x time interaction($p=0.896$). However, there was a main effect for time ($p<0.001$).

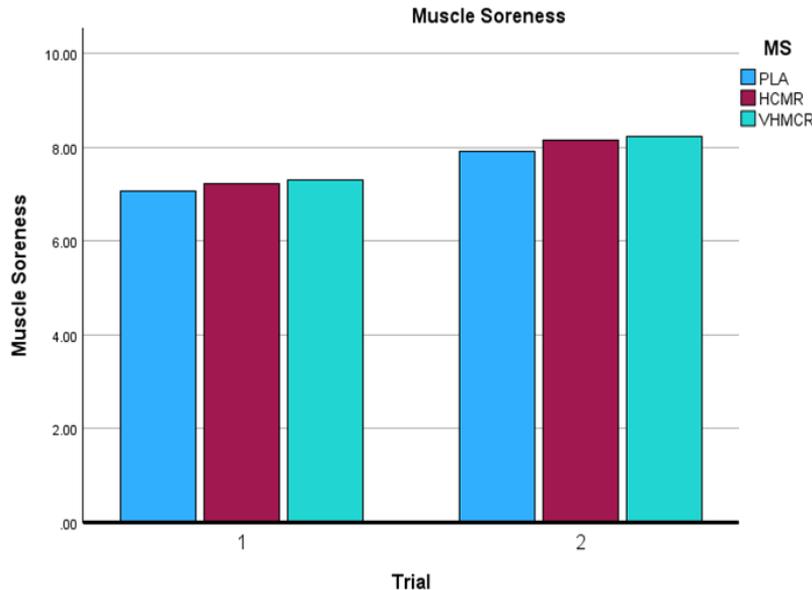


Figure 5. Mean muscle soreness (rating: 1 - 10)

4. Discussion

4.1 Muscular endurance

The current study shows that mouth rinsing with a very high concentration(4%) of caffeine for five seconds significantly enhances the leg press endurance performance. However, high concentration(3%) CMR and PLA have no benefit to leg press endurance performance.

Moreover, a significant difference was found between VHCMR and HCMR in the second trial.

The result supports Clark's (1926) occupancy hypothesis, which suggests that higher doses of caffeine can stimulate more adenosine and taste receptors in the mouth, leading to improved muscular performance. VHCMR may be a practical and effective way to improve resistance training endurance performance.

In both trials of the RF test, VHCMR significantly enhanced the mean repetitions. The result is as expected since it is proven that HCMR has a significant benefit to bench press endurance performance (Karayigit et al., 2021). However, HCMR showed no benefit to leg press endurance in the current study. Furthermore, in Karayigit's (2021) study, HCMR lowered RPE values compared to PLA, while both HCMR and VHCMR showed no effect on RPE. These contrasts may result in the following reasons: 1. The effect of different concentrations of CMR has different effects on the upper body and lower body; 2. There are differences in the experimental setup; 3. There were some factors affecting the result. For the first hypothesis, in an analysis of recent studies (Grgic, 2021), an investigation was conducted to ascertain whether there exists a variance in the impact of caffeine on 1-RM strength between upper and lower body exercises. The findings concluded that caffeine demonstrates ergogenic effects in both lower and upper-body strength exercises. However, this finding is about the effect of caffeine ingestion on strength. The concentration of caffeine was not investigated. Therefore, it can not be concluded that there was no significant difference in the effect of CMR between upper and lower body endurance. The current research is the first study to investigate the effect of VHCMR and HCMR on lower body endurance. Therefore, there was a lack of evidence to support the hypothesis. The second hypothesis has a high chance of being the correct answer. In Karayigit's (2021) setup, both 1-RM and 60% of 1RM RF tests were done in the same session, while only 60% of 1-RM RF test was done in the current study; only 48 hours resting period was given between sessions, but at least 72 hours of resting period was given in current study; 10 hours of fasting was required while fasting was not required in current study. The differences in the experimental setup may have an unknown impact on the result. On the other hand, hypothesis 3 is also a reasonable assumption. Despite the limitations in both studies of small participant size (<15) and lack of measurement of EEG, EMG, blood caffeine, or neurotransmitter levels in both

studies, the participants' differences may also contribute to the contrasting results. Literature suggests that inter-individual differences in bitter-tasting ability, which could influence the responses to caffeine mouth rinse (CMR), may be linked to the participants' genotypes (Pickering, 2019). Karayigit (2021) posits in their study that a majority of the participants possessed the C allele, potentially diminishing their perception of bitter taste (Pickering, 2019). As a result, they may not have responded to the low (250 mg) and moderate (500 mg) doses of CMR. The participants' difference in bitter-tasting ability, which can hardly be measured, may significantly affect the effect of CMR. It is possible that the participants in the current studies may have a weaker bitter-tasting ability compared to participants in other studies. This may also be a research direction. On the other hand, Clarke et al. proposed that during resistance exercise at an intensity of 60% of 1-RM, when the RPE approaches near maximal levels, it results in a "ceiling effect." This effect makes it exceedingly difficult to discern significant differences between the effects of 300 mg of CMR and PLA. This may also help explain why there is no significant difference in RPE in current research.

There is only one research investigating the impact of caffeine mouth rinse (CMR) in a dose-dependent manner on muscular endurance performance (Karayigit et al., 2021). However, that research did not investigate VHCMR, and only one previous study can hardly support the hypothesis that CMR is dose-dependent. Since there is no previous research investigating the dosage of CMR higher than 1000mg(4%) or above, the ceiling effect of concentration is unknown. Note that there may be inter-differences in bitter-tasting ability and weak, bitter-tasting ability will require a higher concentration of CMR to reach the enhancing effect on muscular endurance (Pickering, 2019); using VHCMR or a concentration above 4% of CMR is recommended, especially when training lower body muscular endurance. Furthermore, the solution is only used for mouth rinsing and will not be swallowed; therefore, it is safe to use a

higher concentration of CMR. However, it is noteworthy that, as reported by Caffeine Informer, the highest caffeine concentration observed in commercial coffee is approximately 4%. Mixing solutions by users themselves is required if a solution with CAF concentration above 4% is used. Although mixing the solution is not difficult, the process may be inconvenient and time-consuming. Also, the extreme bitter taste may be unacceptable for some people. Therefore, the usage of a higher concentration of CMR may be mainly applied to professional sports players who have caffeine intolerance.

Unlike Karayigit's (2021) setup, fasting is not required in the current study. Caffeine mouth rinse (CMR) seems to offer more significant benefits in enhancing exercise performance when administered in a fasted state. This observation is supported by the majority of studies indicating that CMR does not confer any advantages when conducted in a fed state (Dolan et al., 2017; Doering et al., 2014). Therefore, fasting overnight before the test is required in Karayigit's (2021) CMR test. However, Karayigit(2021) claimed that fasting is not typical for conducting muscular endurance training, which is one of the limitations of his study. The majority of ingested carbohydrate forms have shown performance benefits compared to noncaloric placebos (Jeukendrup, 2004). During prolonged endurance exercise, carbohydrate sports beverages offer several benefits. They provide fluid to counteract dehydration and electrolytes to help mitigate hypernatremia (Hamilton et al., 1991; Coyle et al., 1986). In most situations, athletes train in a fed state, especially when training for muscular endurance. Therefore, participants in the current research do not require fasting to simulate normal training conditions athletes may face. This observation also helps explain the discrepancy in the lack of effect when using HCMR on lower-body muscular endurance, contrasted with the significant effect observed on upper-body muscular endurance in Karayigit's (2021) CMR test. It is possible that HCMR would show a significantly enhanced effect on the leg press endurance test if participants were fasted for more

than 10 hours before the test. Since fasting is uncommon in endurance training and it is proven that HCMR is effective in enhancing upper body muscular endurance in a fasted state, it may be meaningless to investigate HCMR in a fasted state. Fortunately, VHCMR showed a significant effect in enhancing muscular endurance in the fed state in current research. According to Clark's (1926) occupancy hypothesis, which suggests that higher concentrations of CMR can stimulate more adenosine and taste receptors in the mouth, leading to improved muscular performance, the hindering influence of the fed state on muscular endurance may be reduced or overcome as the concentration of CMR increases. This result provides a more executable CMR practice for athletes who would want to enhance their muscular endurance performance by using CMR.

4.2 Heart Rate

It is expected that HR did not differ between conditions and that no condition x time interaction was detected ($p=0.541$) since previous research has shown similar results. In two studies, CMR led to comparable heart rates and ratings of perceived exertion across conditions despite a significantly greater distance covered during the caffeine conditions (Ehlert et al., 2020). Consistent reports of no effect of CMR on heart rate values have been documented, aligning with the current findings indicating that none of the CMR doses had an influence (Karayigit et al., 2021). In addition, it is not surprising that there was a main effect for time since athletes' heart rate increased after they completed the RF test.

4.3 Perceived Exertion (RPE) and Muscle Soreness

RPE and muscle soreness show no significant change between conditions, and no condition x time interaction was detected. By inhibiting adenosine action, caffeine can boost alertness and motivation during exercise while decreasing perceptions of exertion and skeletal muscle pain (Graham, 2001; Meeusen et al., 2013). Kizzi et al. (2016) discovered that participants perceived

less pain during the last two of five sprints in the caffeine condition despite generating greater power. HCMR significantly decreased RPE values (Karayigit et al., 2021). There is a contrast between Graham's (2001) hypothesis and some previous research results in the current study. After conducting in-depth research, it was discovered that previous studies have presented contrasting results, with some indicating no change in ratings of perceived exertion (RPE) following caffeine mouth rinse (CMR). These discrepancies have been thoroughly discussed in the literature. Both caffeine mouth rinse (CAF-MR) and carbohydrate mouth rinse (CHO-MR) interventions showed no significant difference in ratings of perceived exertion (RPE) (Beaven et al., 2013; Clarke et al., 2015; Karuk et al., 2022). The absence of variance in RPE between interventions was attributed to the phenomenon where performances with maximal effort typically result in RPE levels nearing maximal thresholds (Beaven et al., 2013). Beaven et al. (2013) reasoned the lack of disparity in RPE observed during sprints between interventions to the ceiling effect. Additionally, Clarke et al. (2015) proposed the hypothesis that during resistance exercise at an intensity of 60% of 1-RM, when the ratings of perceived exertion (RPE) approach near maximal levels, it results in a "ceiling effect." This effect makes it exceedingly difficult to discern significant differences between the effects of 300 mg of caffeine mouth rinse (CMR) and a placebo, which aligns with the idea shared by Beaven et al. (2013). Although it is reasonable for the current to have no significant change in both RPE and muscle soreness, the decreased value in RPE in Karayigit's (2021) CMR test was not explained. Karayigit (2021) explained that the decrease in RPE is due to the high dosage of HCMR, which exceeded the ceiling of 300mg of CMR, as proposed by Clarke(2015). However, both HCMR and VHCMR showed no significant change in both RPE and muscle soreness. The reason for the occurrence of the contrast can be explained by reasons that have been discussed in 4.1. The effects of different concentrations of caffeine mouth rinse (CMR) may vary between the upper body and lower body. Moreover,

variations in experimental setups, including differences in fasting states, contribute to these discrepancies. Additionally, factors such as individual participant differences (bitter-tasting ability) may also influence the outcomes (Pickering, 2019). On the other hand, both RPE and muscle soreness show a main effect over time. This is as expected since participants accumulated fatigue after doing the RF test in the first trial. It is reasonable that the RPE and muscle soreness value increased from the first trial to the second trial. The result also aligns with Karayigit's (2021) experimental result.

4.4 Implication

The current research is the first research investigating the effect of CMR on muscular endurance in a dose-dependent manner with participants in the fed state. Additionally, this research is the initial examination of the influence of extremely elevated levels of CMR concentration (4%). As mentioned, consuming caffeine before or during exercise may lead to certain side effects, including but not limited to anxiety, headaches, gastrointestinal discomfort, and insomnia (Pallares et al., 2013). Some athletes may have caffeine tolerance, so they are not able to ingest caffeine solutions. Note that 25ml of 4% caffeine concentration solution contains 1000mg of caffeine, that is, approximately 10 cups of 5oz of coffee (Goldstein, 2009). There may also be safety concerns for normal athletes to ingest solutions with high concentrations of caffeine. Therefore, the current research provided an alternative and safe way for athletes to enjoy the enhancing effect provided by high caffeine concentration solution in a side-effect-free approach. Combined with a similar experiment done by Karayigit (2021), which showed that HCMR has a boost effect on upper-body muscular endurance in a fasted state, it is confirmed that VHCMR will be effective in enhancing both upper and lower-body muscular endurance in a fasted state. Although it is unknown if VHCMR is more effective than HCMR in a fasted state to enhance upper body muscular endurance, VHCMR will at least share a similar

boost effect with HCMR due to the occupancy hypothesis (Clarke, 1926) that higher concentration will result in better-enhancing effect. If athletes are not willing to fast before their training session, rinsing with a very high concentration (4%) of caffeine solution for five seconds prior to each set of exercises will help enhance lower muscular endurance performance in the fed state. Practically, coaches and athletes may utilize the results of current research in training programs and competition strategies. Trainers may consider integrating VHCMR protocols into exercise routines, especially for athletes engaged in resistance training or sports requiring sustained lower-body efforts. Moreover, the use of a very high concentration of caffeine solution for mouth rinsing before competitions that demand short bursts of muscular endurance, such as indoor rowing events, can also enhance athletes' performance during the competition.

On the other hand, the current research also pioneers in investigating very high concentrations of CMR. Future research may take current research results as a reference and focus on investigating mouth rinsing with at least a 3% caffeine concentration solution since it is proven that solution with caffeine concentration lower than 3% will show no enhancing effect on both upper and lower body muscular endurance (Karayigit, 2021). Especially when investigating the effect of CMR under a fed state, at least 4% of CMR should be investigated according to current research results.

4.5 Limitation

There are limitations in current research. 1. The sample size is small. Only thirteen participants were investigated, and the result may not be representative. Some people with weak, bitter-tasting abilities may not show similar experimental results to participants in the current study (Pickering, 2019). However, this seems to be a common difficulty for researchers when recruiting large sample sizes of participants. According to a systematic review of the effects of

CMR on exercise performance (Grgic et al., 2018), most previous research investigating caffeine mouth rinsing has similar sample sizes (participants < 15) to current research. There is a lack of sizeable sample research on the effects of CMR. 2. The test is only single-blind. The investigator prepared the solution for the current research. Additionally, 1000mg of caffeine mixed in 25ml of the solution resulted in a strong bitter taste that may not be effectively masked by 300 mg of sucralose solution. Participants may guess the concentration of caffeine in solutions by tasting. This may affect the blinding effect. 3. Participants in the current research are all very low consumers of caffeine and non-smokers. Athletes who consume caffeine frequently or smoke may not show a similar enhancing effect in muscular endurance as participants in the current study. 4. The current experiment only investigated two sets of exercises. However, athletes typically train for much more than two sets of exercises in one training session. The effect of CMR after doing two sets of exercises is not confirmed. 5. There is a lack of measurement of EEG, EMG, blood caffeine, or neurotransmitter levels. The introduction of caffeine to the oral cavity enhances brain activity in the dorsolateral and orbitofrontal cortex, areas linked with reward processing and cognition (De Pauw, 2015), potentially playing a central role in regulating the feeling of muscle soreness and RPE. Although there is no significant change in PRE and muscle soreness among the three conditions in the current study, measuring more indicators may provide extra data for analysis that may explain the constant value in RPE and muscle soreness value. Furthermore, testing for blood caffeine levels may also be helpful to determine if participants followed instructions to refrain from consuming any foods or beverages containing caffeine. 6. The effect of CMR on lower body muscular endurance in a fasted state is not conducted. Although there was no significant change in muscular endurance among HCMR groups in the current study, HCMR may have been effective if participants had experimented in a fast state. 7. Solutions with a very high concentration of caffeine could not be found in

commercial drinks. Athletes must mix the solution by themselves. In addition, athletes must mouth rinse with the solution prior to each set of exercises. This may cause inconvenience to execute.

4.6 Future research

4% of caffeine solution is the highest concentration solution to be investigated. Given the evidence presented in both the Karayigit (2021) study and the current research, which aligns with Clark's (1926) hypothesis proposing that elevated doses of caffeine can activate additional adenosine and taste receptors in the oral cavity, resulting in enhanced muscular endurance performance, there arises an opportunity to explore solutions with even higher concentrations of caffeine. Subsequent studies could investigate whether a CMR saturation point exists concerning caffeine concentration and determine the optimal concentration for maximizing CMR effectiveness. On the other hand, future research should be conducted in both fast and fed states. Although a previous search claimed that CMR does not confer any advantages when conducted in a fed state (Dolan et al., 2017; Doering et al., 2014), the current study showed that VHCMR can help enhance lower body muscular endurance. Additionally, note that there are many potential benefits to training under fed state (Hamilton et al., 1991; Coyle et al., 1986); it is not common for most athletes to train under fast conditions, especially when training for endurance. Therefore, investigating the effect of a high concentration of CMR under a fed state may be more practical and meaningful than under a fast state. Another direction to investigate is the effect of different dosages of CMR on other sports performance. Research has proposed that the bitter taste of CMR has been found to enhance mental alertness by stimulating the brain regions responsible for information processing and reward (Gam et al., 2014). Future research may investigate if a higher concentration of CMR will better enhance mental alertness. On the other hand, only the short-term effect of a high concentration of CMR is investigated in current

research. The effect of high or very high concentrations of CMR in long-term athletic performance adaptations may also be a possible research direction.

5. Conclusion

The VHCMR, but not the HCMR adopted in the present study, seems to improve lower-body muscular endurance. Heart Rate, Perceived Exertion (RPE), and muscle soreness did not differ between conditions, and no condition x time interaction existed. Practically, the results of the current study can be utilized by resistance-trained individuals who perform resistance training in the fed state to enhance their muscular endurance performance. Athletes may consider using very high concentrations of CMR instead of caffeine ingestion, which may cause side effects and require time to metabolize.

(7025 words including reference)

(Note that AI was used in the essay for paraphrasing.)

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