

# Acute caffeine mouth rinse does not affect attention and hand-eye coordination in recreationally active adults

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## Abstract

*Study aim:* The purpose of this study was to evaluate the acute effect of different four caffeine mouth rinse intervention (caffeinated coffee, decaffeinated coffee, placebo, control) on attention and hand-eye coordination.

*Material and methods:* Sixty-five healthy, recreationally active female ( $n = 41$ ) (age  $22.89 \pm 3.94$  years; body mass index  $20.87 \pm 2.63$  kg/m<sup>2</sup>) and male ( $n = 24$ ) (age  $29.91 \pm 12.06$  years; body mass index  $22.56 \pm 2.21$  kg/m<sup>2</sup>) volunteered to participate in this randomized, single-blind, placebo-controlled, crossover study. The Stroop Color-Word Test (SCWT) and Mirror-Tracing Test (MTT) was used. Participants first completed a SCWT or MTT, then rinsed and expectorated 25 ml of caffeinated coffee (containing 0.13% caffeine) or decaffeinated coffee or placebo (water) or control that did not rinse for 10 s, followed by SCWT or MTT again. Data were analyzed using a 4 (mouth rinse interventions)  $\times$  2 (pre-test and post-test) repeated measures ANOVA.

*Results:* SCWT time, MTT draw time and MTT number of error measures were not significantly different between four mouth rinse interventions ( $p > 0.05$ ).

*Conclusions:* Caffeinated coffee or decaffeinated coffee mouth rinse for 10 s provided immediately prior to SCWT or MTT did not affect attention and hand-eye coordination.

**Keywords:** Caffeine – Decaffeinated coffee – Mouth rinse – Attention – Hand-eye coordination

## Introduction

Caffeine, which is known to increase human vigilance and mental alertness, is one of the most used nutritional supplements in the world [29] and also the most consumed psychoactive stimulant [34]. It is reported that the intake of caffeine (3-6 mg/kg) reveals the optimal ergogenic effect, lower doses have the potential of being ergogenic, and there are significant differences in the “optimal” caffeine dose for the athlete [31]. Caffeine, a strong adenosine receptor antagonist, is a stimulant of the central nervous system crossing the blood-brain barrier easily [10, 17]. Proposed mechanisms that may explain the ergogenic effects of caffeine is improved neuromuscular function, increased endorphin release, improved vigilance and alertness, and reduced perception of exertion during exercise [28]. There are studies indicating its positive effects on endurance

performance [8, 22], muscular endurance [14, 35], muscular strength and power [3, 12, 19], and sprint performance [7, 26].

Together with the ergogenic effects of carbohydrate mouth rinse (MR), caffeine MR has created an emerging interest [36]. It was reported by Kamimori et al. that caffeine was absorbed via the buccal mucosa and the time to reach the maximal caffeine concentration in the blood accelerated in the caffeinated chewing gum application when compared to the intake of caffeine capsules [24]. The mechanism related to the effect of MR was reported as the inhibition of adenosine by caffeine through binding to the adenosine receptors in the mouth, the increase in the permeability of the buccal mucosa via this interaction, and triggered caffeine absorption into the blood [36]. Nevertheless, Doering et al. found no change in plasma caffeine concentration following eight repeated 35 mg caffeine for 10 seconds each time mouth rinses during endurance

cycling time-trial performance [13]. Accordingly, a different mechanism was discussed regarding caffeine MR. In a study conducted with 58 natural and 46 synthetic bitter compounds, caffeine was shown to activate the bitter taste receptors in the oral cavity [30]. While the underlying mechanisms have not yet been clearly defined, it has been proposed that the activation of these bitter taste receptors might activate the gustatory neural pathways, and, as a result, stimulate the regions of the brain (dorsolateral prefrontal cortex and orbitofrontal cortex) related to information-processing and reward [2, 21, 36].

There are few data on the effect of caffeine MR for improvements in cognitive control and reaction time. De Pauw et al. investigated that caffeine MR increased activity in the dorsolateral prefrontal cortex and the orbitofrontal cortex and improved reaction time of the incongruent stimuli of the Stroop task [11]. It is the first study that evaluating the effect of different (0.3 g/25 ml caffeine, 1.6 g/25 ml maltodextrin or placebo) MR solutions on cognitive performance and brain activity using electroencephalography (EEG). Pomportes et al. have similarly demonstrated improvements in cognitive function during 40-min submaximal exercise following a caffeine MR [32].

MR is a strategy including the rinse of the solution in the mouth without swallowing it for 5–20 seconds and thus enabling to avoid the adverse side effects of the substance intake [34]. Caffeine ingestion may cause side effects such as tremor, nausea, irritability, anxiety, or gastrointestinal problems [29]; particularly, it is not recommended for young athletes due to those adverse effects on health [4], in some cases such as Ramadan, it is not preferred also due to different reasons [34]. Caffeine ingestion may not be recommended in some sports like archery. A study showed that caffeine intake led to mild headaches, anxiety and tremor in archers and tremor may affect the performance adversely by causing a decrease in the hand stability [27]. In sports requiring attention such as archery, marksmanship etc., MR with caffeine can be considered as an alternative to improve attention. Also, MR may also be beneficial in sports where quick decision-making is important (e.g., soccer and basketball). Another advantage of

MR may be that it offers repetitive use in cognitive tasks or competitions [9]. Therefore, the aim of this study was to evaluate the acute effect of different four MR intervention (caffeinated coffee, decaffeinated coffee, placebo, control) on attention and hand-eye coordination in healthy and recreationally active adults. The Stroop Color-Word Test (SCWT) was used to measure attention and the Mirror-Tracing Test (MTT) was performed for hand-eye coordination. We used coffee because it is the most widely used caffeinated beverage in the world. We hypothesized that caffeinated coffee and decaffeinated coffee MR would improve attention and hand-eye coordination compare to placebo and control condition. Additionally, caffeinated coffee MR would have a higher effect than decaffeinated coffee MR.

## Material and methods

### Participants

Sixty-five healthy, recreationally active, non-smoking male ( $n = 24$ ) and female ( $n = 41$ ) participants volunteered to participate in this randomized, single-blind, placebo-controlled crossover study. Characteristics of the participants are provided in Table 1.

Exclusion criteria included be of any physical and cognitive limitations (chronic illness, somatic and mental disorders and color-blind – SCWT contains colors-); and currently using any other medication, nutritional ergogenic aids or performance enhancing substances. Caffeine rich food and beverage recall was completed by each participant to determine habitual caffeine intake. The range of caffeine intake was 50–300 mg/day. Womack et al. described that subjects were classified as low (0–150 mg/day), moderate (150–300 mg/day), and high (>300 mg/day) habitual caffeine users. The participants had modest caffeine habits [37].

Participants were informed of the experimental procedures and risks prior to giving written consent. The investigation was approved by the Akdeniz University Clinical Research Ethics Committee. All procedures were realized in according to Declaration of Helsinki.

**Table 1.** Descriptive characteristics of participants

	Female ( $n = 41$ ) (Mean $\pm$ SD)	Male ( $n = 24$ ) (Mean $\pm$ SD)	Total ( $n = 65$ ) (Mean $\pm$ SD)
Age [years]	22.89 $\pm$ 3.94	29.91 $\pm$ 12.06	25.48 $\pm$ 8.58
Height [cm]	164.73 $\pm$ 6.22	177.46 $\pm$ 9.30	169.43 $\pm$ 9.67
Weight [kg]	56.75 $\pm$ 8.81	74.41 $\pm$ 10.83	63.27 $\pm$ 12.82
BMI [kg/m <sup>2</sup> ]	20.87 $\pm$ 2.63	22.56 $\pm$ 2.21	21.86 $\pm$ 2.79

**Experimental design**

Experimental trials were conducted in standard laboratory conditions (22–25 °C, 25–45 % relative humidity), at the same time of the day (morning hours), in a quiet and well-lit room. Participants were instructed to avoid strenuous exercise for the 24 h preceding each test session. They were also required to refrain from alcohol, caffeine containing drinks and foods or supplement, medication during the 24 h (12 hours for caffeine) prior to each test session. Moreover, 2 hours before the tests, participants consumed light breakfast. At the beginning of the first test session, food consumption record at breakfast was obtained from participants. Also, participants were instructed to maintain the same food intake for each test session.

SCWT was used for measuring to focus of attention [5] and MTT was used for measuring to hand-eye coordination [16]. These tests were applied in different days. There were at least 7 days between the same tests. A maximum of two sessions was performed in a week and each participant performed eight experimental sessions (4xSCWT, 4xMTT). SCWT or MTT were performed consisting of two familiarization trials to minimize learning effects for each session. Tests were applied pre and post 10 s the MR.

Four MR intervention were performed in a single-blind, randomized, crossover protocol. Ninety-five participants

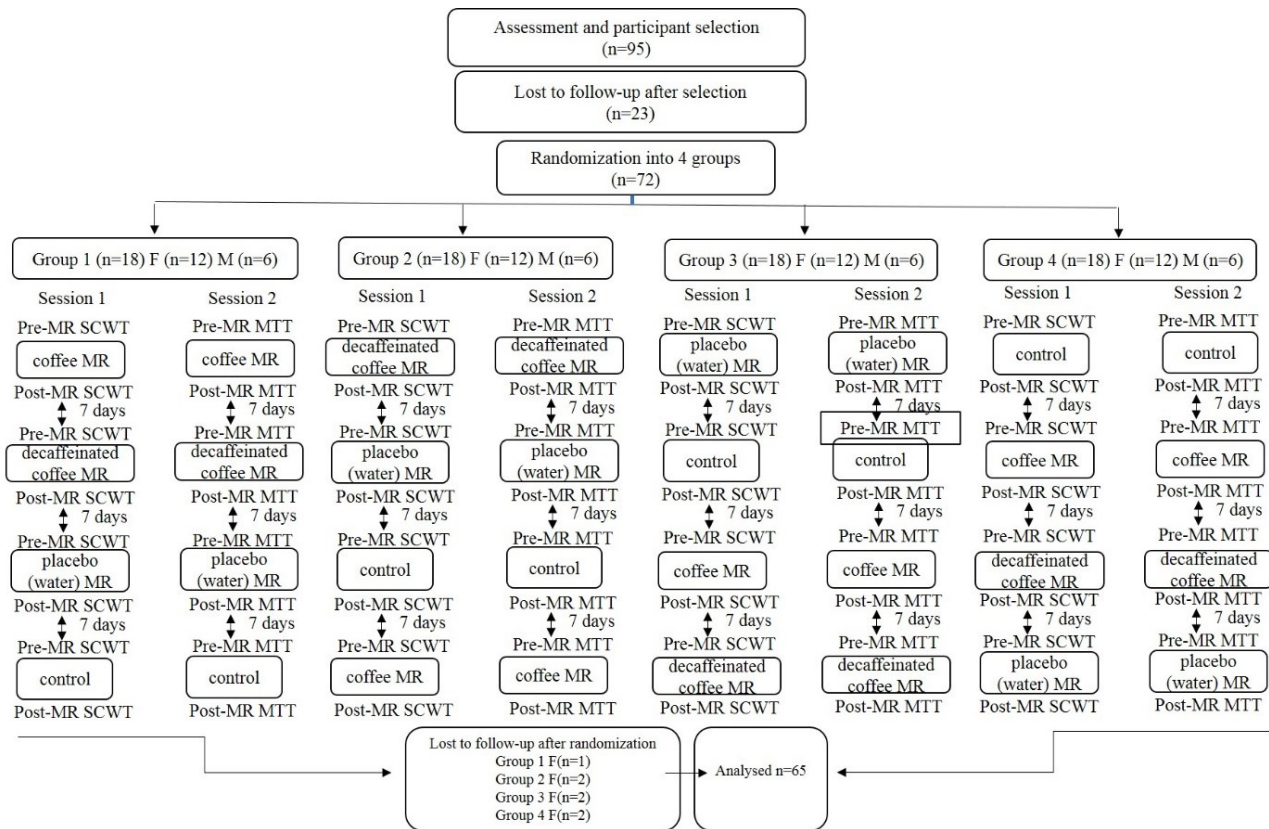
were initially enrolled in this study. Participants of 23 lost to follow up after selection. Seventy two participants were randomized into four groups. The first group completed a rinsing protocol as caffeinated coffee, decaffeinated coffee, water and control, respectively. For the second group was as follows, respectively decaffeinated coffee, water, control and caffeinated coffee. For the third group was as follows, respectively water, control, caffeinated coffee and decaffeinated coffee. The fourth group completed a rinsing protocol as control, coffee, decaffeinated coffee and water respectively. However, 65 participants completed the study and were included in analyses. The study design and timeline are shown in Figure 1.

**Eligibility check**

Alcohol consumption, food consumption and sleep status were checked by asking participants before each test session. All were also checked to refrain from exercise the 24 h and from caffeine containing foods and drinks 12 h leading into the test visits. The participants were tested on another day if they did not meet all of these criteria.

**The Stroop Color-Word Test**

There is no standard version of the SCWT available for test materials, application, or scoring [23]. In the most



**Figure 1.** Study design and timeline

MR – mouth rinse; SCWT – The Stroop Color-Word Test; MTT – Mirror-Tracing Test; F – female; M – male.

common version of the SCWT, participants are requested to read three different tables as quickly as possible. Two of them are comprised of the congruent situations which require participants to read the color names written in black ink and to name the color squares in different colors. In the third table, colors and words are written in incongruent colors (for example, the word “red” is written in green), and participants are asked to tell the names of the ink without reading the names of the colors. In this incongruent situation, participants need to tell the color of the ink instead of reading the word. In other words, participants switch to naming the ink color, which is less automatically done, from reading the color name, which is

more automatically done [33]. In the inaccurate reading, the individuals were warned by the researcher by tapping the pen on the table, and the participant corrected the error and resumed the test. SCWT time (in seconds) were recorded. The SCWT was applied to the participants four times in total, at one-week intervals.

### The Mirror-Tracing Test

The participants completed the test in a quiet room, in front of the Automatic Mirror Tracer (Lafayette, Model 58024A), and on a comfortable chair that could be adjusted to height. The participants were requested to track the pen-like metallic stylus connected to a device only in the

**Table 2.** Test scores before and after four MR interventions in SCWT and MTT

(n = 65)		SCWT-time [sec]	MTT-draw time [sec]	MTT-number of errors
<b>Caffeinated Coffee</b>				
Pre MR	Male	24.92 ± 14.20	46.87 ± 26.96	12.83 ± 20.16
	Female	21.02 ± 6.49	34.12 ± 19.92	6.20 ± 10.33
	Total	22.46 ± 10.12	38.83 ± 23.40	8.65 ± 14.94
Post MR	Male	21.00 ± 6.02	36.79 ± 21.72	10.04 ± 21.08
	Female	20.93 ± 6.22	28.49 ± 12.33	4.49 ± 6.33
	Total	20.95 ± 6.09	31.55 ± 16.76	6.54 ± 13.86
<b>Decaffeinated Coffee</b>				
Pre MR	Male	22.67 ± 7.60	43.04 ± 23.40	4.58 ± 4.51
	Female	21.66 ± 7.02	30.15 ± 11.19	4.88 ± 9.83
	Total	22.03 ± 7.19	34.91 ± 17.73	4.77 ± 8.23
Post MR	Male	22.58 ± 8.17	37.37 ± 23.98	4.29 ± 3.30
	Female	20.32 ± 6.61	25.46 ± 9.47	4.61 ± 9.12
	Total	21.15 ± 7.19	29.86 ± 17.21	4.49 ± 7.48
<b>Placebo (water)</b>				
Pre MR	Male	23.00 ± 7.83	53.29 ± 36.51	9.88 ± 19.41
	Female	19.90 ± 6.83	32.17 ± 24.09	6.41 ± 9.48
	Total	21.05 ± 7.31	39.97 ± 30.77	7.69 ± 13.94
Post MR	Male	21.92 ± 5.39	40.67 ± 21.40	5.88 ± 9.11
	Female	19.76 ± 6.34	29.56 ± 20.92	6.54 ± 10.81
	Total	20.55 ± 6.06	33.66 ± 21.62	6.29 ± 10.15
<b>Control</b>				
Pre MR	Male	22.33 ± 7.78	48.08 ± 36.48	8.96 ± 18.56
	Female	20.39 ± 6.26	34.46 ± 23.21	6.83 ± 13.01
	Total	21.11 ± 6.87	39.49 ± 29.31	7.62 ± 15.19
Post MR	Male	21.79 ± 7.13	36.67 ± 21.47	5.13 ± 8.06
	Female	21.66 ± 7.93	28.24 ± 13.87	5.71 ± 9.43
	Total	21.71 ± 7.56	31.35 ± 17.40	5.49 ± 8.89

MR – mouth rinse; SCWT – Stroop Color and Word Test; MTT – Mirror-Tracing Test.

mirror (a barrier was used to prevent a direct look) and complete a set clockwise by making as fewer mistakes as possible inside the determined area of the dark-colored hexagram and as quickly as possible in their preferred hand. The number of errors was automatically recorded by the device when the stylus went outside the certain areas of the hexagram [25]. Also the draw time (in seconds) were recorded.

### Rinsing protocol

All participants were instructed to rinse with water to clean their mouth prior to each test. After the SCWT or MTT, participants were given 25 mL mouth rinse solutions at room temperature containing either: a) caffeinated coffee (containing 0.13% caffeine/25 ml – this dose was used for the concentration of caffeine found in commercially available 2g instant coffee) or b) decaffeinated coffee or c) water (placebo) or d) control that did not rinse. The duration of each mouth rinse period was 10 s. Participants then expectorated the solution back into the plastic cup. Cups were then checked to determine if any of the solutions had been ingested. The SCWT or MTT was performed immediately after to MR.

### Statistical analyses

Data are presented as means  $\pm$  standard deviation. Normality of data distribution was confirmed by Shapiro-Wilk test. All data were not normally distributed, were positively skewed and therefore data were log-transformed. Raw data were presented in tables and figures. The effects of MR interventions on SCWT and MTT were analysed using a 4 (MR interventions)  $\times$  2 (pre-test and post-test) repeated measures ANOVA. Sphericity was verified by Mauchly's test. When the assumption of sphericity was not met, the significance of F ratios were adjusted with the Greenhouse-Geisser procedure. Effect sizes were calculated using partial eta squared ( $\eta^2$ ; 0.01 – small effect, 0.06 – medium effect and 0.14 – large effect [15]. A significance level of  $p < 0.05$  was set.

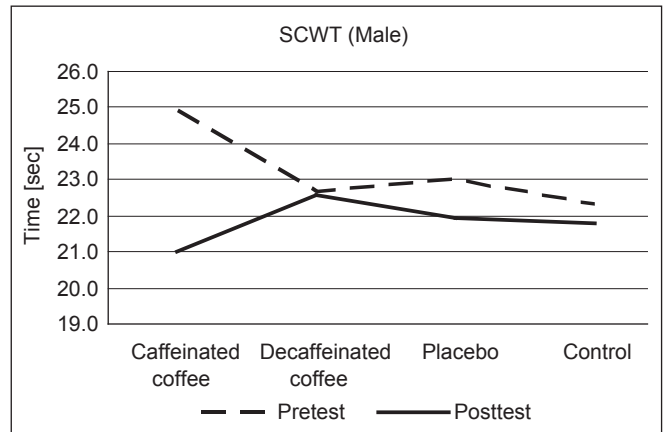
## Results

There was no significant difference between the interventions in pre and post-tests in all test scores (intervention  $\times$  time) in total and female and male. Test scores for the SCWT and MTT before and after four different MR interventions were shown in Table 2.

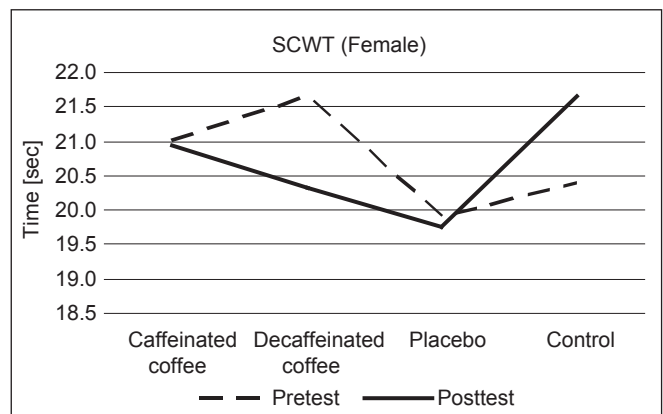
### SCWT time

There was not main effect for time ( $F_{1, 64} = 1.135$ ,  $p = 0.291$ ,  $\eta^2 = 0.017$ ) and for an interaction effect ( $F_{3, 192} = 0.918$ ,  $p = 0.433$ ,  $\eta^2 = 0.014$ ) at SCWT time in total (Figure 4). There was not main effect for time

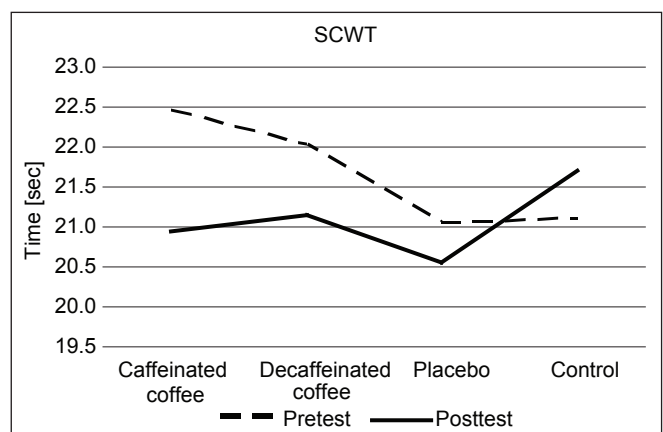
( $F_{1, 23} = 1.962$ ,  $p = 0.181$ ,  $\eta^2 = 0.078$ ;  $F_{1, 40} = 0.031$ ,  $p = 0.863$ ,  $\eta^2 = 0.001$ ) and for an interaction effect ( $F_{3, 69} = 0.883$ ,  $p = 0.462$ ,  $\eta^2 = 0.037$ ;  $F_{3, 120} = 1.199$ ,  $p = 0.235$ ,  $\eta^2 = 0.029$ ) at SCWT time in male and female respectively (Figure 2 and 3).



**Figure 2.** Total test duration of SCWT in male



**Figure 3.** Total test duration of SCWT in female



**Figure 4.** Total test duration of SCWT in total

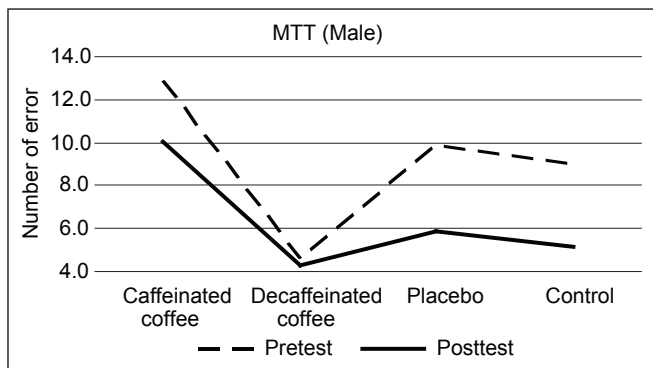
**Mirror-Tracing Test**

*Number of error.* There was significant difference ( $F_{1,64} = 7.755, p = 0.03$ ), and medium effect size ( $\eta^2 = 0.108$ ) for time. There was no significant difference for an interaction effect ( $F_{3,192} = 0.827, p = 0.481; \eta^2 = 0.013$ ) on MTT number of error in total (Figure 7).

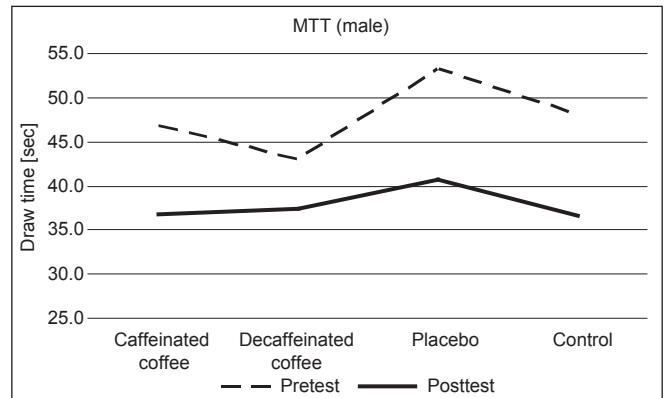
There was significant difference ( $F_{1,23} = 11.032, p = 0.02$ ), and large effect size ( $\eta^2 = 0.342$ ) for time. There was no significant difference for an interaction effect ( $F_{3,69} = 1.015, p = 0.302, \eta^2 = 0.042$ ) on MTT number

of error in male (Figure 5). And there was not main effect for time ( $F_{1,40} = 1.229, p = 0.274, \eta^2 = 0.030$ ) and for an interaction effect ( $F_{3,120} = 0.426, p = 0.735, \eta^2 = 0.011$ ) on MTT number of error in female (Figure 6).

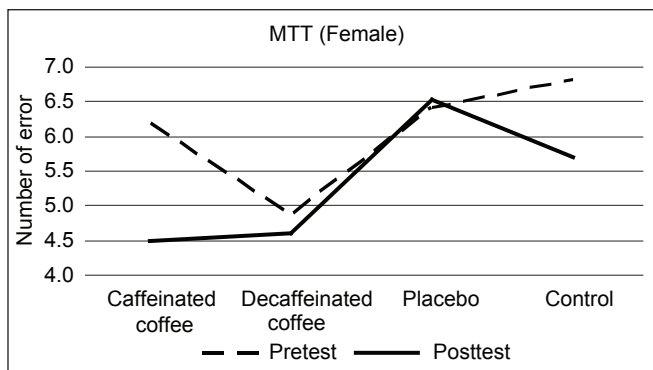
*Draw time.* There was significant main effect ( $F_{1,64} = 110.927, p=0.02$ ), and large effect size ( $\eta^2 = 0.634$ ) for time but there was no interaction effect ( $F_{3,192} = 0.919, p = 0.433; \eta^2 = 0.014$ ) at MTT draw time in total (Figure 10).



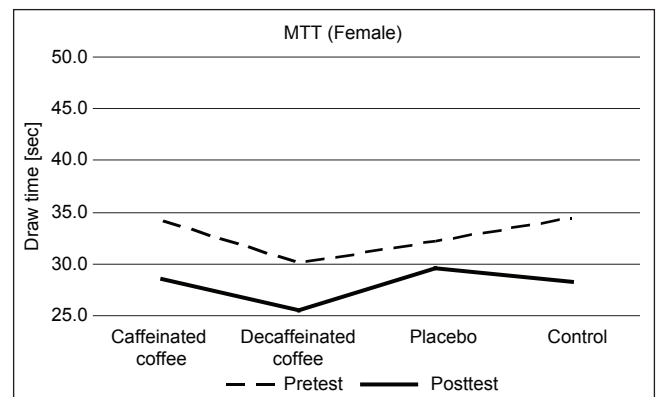
**Figure 5.** Error number of MTT in male



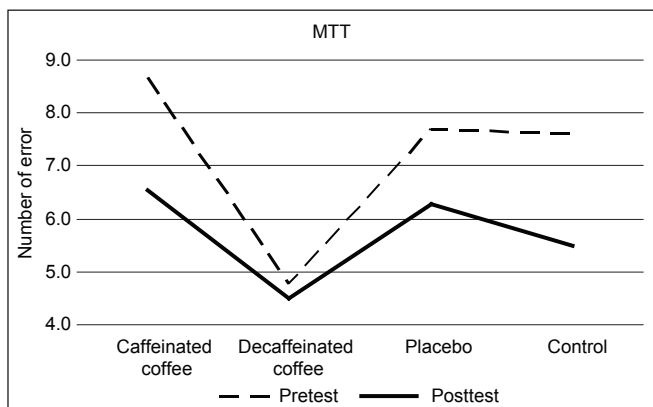
**Figure 8.** Total drawing time of MTT in male



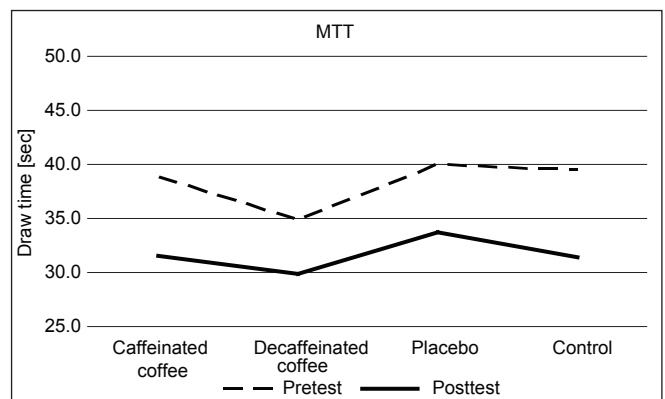
**Figure 6.** Error number of MTT in female



**Figure 9.** Total drawing time of MTT in female



**Figure 7.** Error number of MTT in total



**Figure 10.** Total drawing time of MTT in total

There was significant difference ( $F_{1, 23} = 58.292$ ,  $p = 0.02$ ), and large effect size ( $\eta^2 = 0.717$ ) for time. There was no significant difference for an interaction effect ( $F_{3, 69} = 0.386$ ,  $p = 0.763$ ,  $\eta^2 = 0.017$ ) at MTT draw time in male (Figure 8).

There was significant difference ( $F_{1, 40} = 61.282$ ,  $p = 0.03$ ), and large effect size ( $\eta^2 = 0.605$ ) for time. There was no significant difference for an interaction effect ( $F_{3, 120} = 1.665$ ,  $p = 0.178$ ,  $\eta^2 = 0.044$ ) at MTT draw time in female (Figure 9).

## Discussion

The purpose of this study was to investigate the acute effect of different MR intervention (caffeinated coffee, decaffeinated coffee, placebo, control) on attention and hand-eye coordination in recreationally active adults. The main findings were that 1) there was no main effect for time and no interaction effect on time of SCWT in total, female and male; 2) there was significant difference just for time at number of error of MTT in total and male. But there was no interaction effect in total, female and male. There was significant difference just for time at draw time of MTT in total, female and male. But there was no interaction effect in total, female and male. According these results we can conclude that for all time, four different mouth rinse interventions has shown similar effect.

To our knowledge, this research is the first to determine the effect of coffee alone MR on attention and hand-eye coordination. Previously, only a few studies examined the effects of caffeine MR on cognitive function. De Pauw et al. investigated the acute effect of caffeine and maltodextrin MR on cognitive performance and brain activity using EEG [11]. Ten healthy males received MR with caffeine (0.3 g/25 ml), maltodextrin (1.6 g/25 ml), or placebo for 20 s. Caffeine MR exerted a likely beneficial effect on reaction time due to the subsequent activation of both orbitofrontal and dorsolateral prefrontal cortexes. Pomportes et al. have similarly demonstrated improvements in cognitive function during 40-min submaximal exercise following a caffeinated MR [32]. In this study twenty-four participants were instructed to mouth rinse with caffeine (67 mg/25 mL), carbohydrate (1.6 g/25 mL), guarana complex (0.4 g/25 mL) or placebo for 20 s before and twice during exercise. In this study it was reported that caffeine, carbohydrate, or guarana MR may increase brain activation and arousal compared to placebo. These results conflict with our findings. Our data suggest that a caffeinated coffee (0.13%/25 ml) or decaffeinated coffee MR for 10 s provided immediately prior to SCWT or MTT did not improve attention and hand-eye coordination.

It is indicated that the only source of the bitter taste in coffee is not caffeine [6] and quinides in decaffeinated

coffee could be related to bitter taste [18], although it is not clear, decaffeinated coffee demonstrate the potential of expectancy and placebo by bitter [31]. Therefore, in our study, we thought that although not as much as caffeinated coffee, decaffeinated coffee would also have an ergogenic effect according to water and control condition. The reason for the lack of an effect may have been due to a relatively low dose of caffeine or rinsing for a shorter duration or low frequency. The concentrations of caffeine in commercially available coffee and the bitter taste of decaffeinated coffee may not be sufficient to produce an ergogenic response. Higher caffeine concentration or more frequent or longer duration MR may activate more receptors in oral cavity.

The ergogenic mechanisms related to caffeine MR are not exactly clear, but some mechanisms have also been speculated. Caffeine MR may inhibit adenosine through direct binding to adenosine receptors found in oral cavity and/or through activation of bitter taste receptors located in the oropharyngeal epithelia which can activate gustatory neural pathways and stimulate regions of the brain associated with information processing and reward [1, 20, 30]. Caffeine mouth rinsing is a new form of caffeine supplementation. Therefore, future research is required to better understand the mechanisms of caffeine MR on attention and hand-eye coordination.

The strengths of our study include our randomized, crossover design with both a control and placebo conditions. This study is limited by recreational training status of the participants. Well-trained subjects could experience a benefit from the coffee MR. We could not measure serum caffeine concentration, brain imaging technique for brain activity. Another population (well-trained subjects, habitual caffeine consumers or nonconsumer), higher caffeine concentrations or more frequent or longer duration MR might have led to alternative results.

## Conclusions

In conclusion, caffeinated coffee or decaffeinated coffee MR for 10 s provided immediately prior to SCWT or MTT did not improve attention and hand-eye coordination. Further studies involving absorption through the buccal mucosa, plasma caffeine concentrations, measures of brain activation, effects of fasted or fed state, effects of caffeine consumer and nonconsumer, caffeine solution concentration, timing of MR and MR frequent, are needed to examine the effects of caffeine MR on attention and hand-eye coordination. MR in comparison with ingestion of caffeine may be safer and alternative ergogenic aid in athletes who may suffer from side effects, or in some cases such as Ramadan or in sports requiring attention (e.g., such as archery, marksmanship) or where quick decision-making is important (e.g., soccer and basketball).

**Conflict of interest:** Authors state no conflict of interest.

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