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Caffeine mouth rinsing in the Fed state does not enhance 3-km cycling performance in the morning or evening

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Caffeine Mouth Rinsing in the Fed State Does Not Enhance 3-km Cycling Performance In the Morning or Evening

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Abstract

PURPOSE: To assess the effect of a caffeine mouth rinse on performance at different times of day. METHODS: 12 recreational cyclists completed a simulated 3-km time trial and 4 sets of 5 leg extension repetitions, twice in the morning and twice in the evening. 25 ml of 1.14% caffeine, or placebo solution was mouth rinsed before sets 3 and 4 of the leg extension and before the time trial. Treatments include: caffeine + morning, placebo + morning, caffeine + evening, and placebo + evening. RESULTS: The caffeine rinse had a negative impact on time trial performance in both the morning and the evening. Both the caffeine and placebo rinse had a positive impact on peak torque in leg extensions. CONCLUSIONS: The effect of the caffeine rinse had no difference between times of day, leading us to believe feeding state has an impact on the effects of the caffeine rinse on performance.

Chapter 1

Introduction

Caffeine is the most commonly used stimulant in the world. Accessible in many foods, drinks, and supplements, caffeine is used by people of all ages in an attempt to enhance energy levels, and cognitive and physical performance. Caffeine has well documented effects on the central nervous system (CNS); caffeine can increase neuron excitability in the cortex and spinal region and inhibit andesine receptors involved in pain sensitivity (Black et al, 2014). Caffeine ingestion has also been found to increase neurotransmitters such as dopamine, responsible for increasing motivation and motor drive (Kasper et al, 2015).

The effect of caffeine ingestion on physical performance across a wide range of activities has been detailed over the past 25 years. Specifically, caffeine can enhance 45 minute running (Kasper et al, 2015), 1500 meter swimming (Macintosh and Wright, 1994), 1k cycling (Wiles et al, 2007), peak muscle force production (Mora-Rodriguez et al, 2012 and 2014), 2 minute max cycling (Doherty et al, 2007), 1 hour cycling (Kovacs et al, 1998), and 8k running (Bridge and Jones, 2006). The mechanisms by which caffeine elicits positive outcomes is not completely clear but may relate to a reduction in RPE, reduction in muscle discomfort, or enhanced neural excitably, all secondary to the aforementioned neurological effects. From these data, it is clear that caffeine supplementation has the potential to enhance physical performance. However, not all individuals reliably benefit from caffeine and some individuals experience negative side effects, such as jitters, anxiety, cardiac palpitations, headaches and irritability (Nawrot et al, 2010).

Interestingly, caffeine mouth rinsing, without ingestion, may be a viable strategy to enhance performance in individuals that otherwise would not benefit from caffeine consumption

or among individuals susceptible to negative side effects. Studies have indicated that there may be a potential influence on performance from a caffeine mouth rinse. Caffeine mouth rinsing has been shown to enhance repeated cycling sprints (Beavens et al, 2013), 30 minutes of cycling (Bottoms et al, 2014), and 30 minutes of arm cranking (Sinclair and Bottoms, 2015). The positive influence on performance may be similar to the mechanism of a carbohydrate mouth rinse whereby receptors in the mouth are stimulated, changing brain stimulation effecting performance (Beavens et al, 2013), but there is currently no evidence to support this. However, some findings illustrate that there may not be as large of an effect from caffeine mouth rinsing as expected. There has been no significant influence on performance from a caffeine mouth rinse on a 1-hour cycling (Doering et al, 2014) or muscular strength training (Clark et al, 2015). Therefore, there is no current consensus regarding the efficacy of a caffeine mouth rinse for performance enhancement.

One factor that may partially mediate the efficacy of caffeine is time of day. Recently, our laboratory examined the impact of caffeine mouth rinse on performance of a 3k cycling time trial. While we found that caffeine mouth rinse did not have a significant effect overall on performance, the time of day that the mouth rinse trial was performed appeared to partially influence the performance response to the mouth rinse (Pataky et al. 2015). Specifically, subjects who completed the time trial before 10 am experienced a 2.0 ± 2.5% improvement in performance whereas subjects that performed their trials after 10 am had a 3.5 ± 4.3% decline in performance. However, the experimental design was not created to deliberately examine time of day and, as a result, subjects were not exposed to both ‘early’ and ‘late’ conditions. Further, the difference in the effect of the caffeine rinse on time of day was potentially influenced by the fact

that all but one of the early subjects completed the trial after an overnight fast while most of the late subjects had eaten the day of the trial (Pataky et al. 2015).

Therefore, this study is designed to directly examine the impact of a caffeine mouth rinse at different times of the day and under similar feeding conditions. We hypothesize that a caffeine mouth rinse will have a greater effect on performance in a 3k cycling time trial in the morning rather than the afternoon.

Chapter 2

Methods

Subjects

Approximately 15 recreationally trained cyclists will be recruited from James Madison University and the greater Harrisonburg/Rockingham County area. To be eligible for the study, participants must cycle at least 2 days per week in their typical weekly exercise routine. Testing will occur in the Human Performance Lab in Godwin Hall at James Madison University. Participants will be recruited by word of mouth, email, and social media. All procedures will be approved by the James Madison University Institutional Review Board prior to data collection.

Experimental Design

Subjects will be asked to report to the laboratory for exercise testing on seven separate occasions – one pre-testing session, two familiarization trials to minimize performance variability associated with learning, and four experimental trials consisting of a 3-km cycling time trial (4-7 minutes). The experimental trials will be separated by no fewer than three days and no more than seven days. A questionnaire will also be distributed to screen for health history, physical activity habits, and dietary habits. Following completion of all seven trials, subjects will be asked to complete a brief questionnaire about suspected treatment order.

Preliminary Test

Prior to testing, subjects will be given consent forms to read and sign that provide a comprehensive description of the study, the risks and benefits associated with the study, and the ways in which confidentiality will be maintained. Subjects will then have their body weight

measured to the nearest 0.2 kg, and height measured to the nearest 0.5 cm., after which they will perform a graded exercise test to determine their maximal oxygen uptake (VO_{2max}). Subjects will ride a cycle ergometer at a self-selected workload estimated as "a comfortable, but not easy pace for a 1-hour ride". Workload will be increased by 25 Watts every 2 minutes until subjects voluntarily request to stop due to fatigue or are unable to continue at a cadence >50 rpm. Oxygen uptake will be assessed at each stage during this test. VO_{2max} will be assessed directly from data obtained during the test and used as a descriptive characteristic.

Experimental Trials

For the four experimental trials, subjects will perform a 5-minute self-selected warm up followed by a 3-km computer simulated time trial on a stationary cycle ergometer (Velotron, Racermate, Inc., Seattle, WA). A rinse of either placebo or caffeine will be given immediately before the warm-up and immediately before the 3-k time trial using a nose clip to hide the difference in taste from placebo and caffeine rinse. Subjects will swirl the mouth rinse in their mouth for five seconds and then will expel the rinse. Standardization of no heavy physical activity 48 hours prior to testing; no caffeine, tobacco or alcohol 24 hours prior; and a standardized meal 2 hours prior will be required for the experimental trials. The familiarization trials will be identical to the experimental trials except the familiarization trials will be performed without the standardization procedures of physical activity and diet. Additionally, the mouth rinse will be practiced using water mouth rinse during the familiarization trials instead of the placebo or caffeine rinse. There will be no treatments provided prior to the familiarization trials.

Supplementation Protocol

A randomly counterbalanced, double blind, placebo controlled design will be implemented to compare the effects of the four treatment conditions. During the experimental trials subjects will be given two 25ml mouth rinse solutions at room temperature containing either: a) 300mg caffeine (NutraBio Labs, Inc., Middlesex, NJ), 1g saccharine (Sweet'N Low, Brooklyn, NY), and 25ml of water (rinse containing 1.14% caffeine) or b) a flavor-matched placebo containing 6g saccharine, and 25ml of water. Additionally, the subjects will complete the trials either: a) in the morning (before 10:00 AM) or b) in the afternoon (after 4:00 PM). The four treatments consist of a 2 x 2 factorial of time of day and mouth rinse treatments: 1. morning + placebo mouth rinse (*Early Placebo*); 2. morning + caffeine mouth rinse (*Early Rinse*); 3. afternoon + placebo mouth rinse (*Late Placebo*); 4. afternoon + caffeine mouth rinse (*Late Rinse*).

Dietary and Exercise Controls

Subjects will be asked to record food intake 24 hours prior to the first familiarization trial. Subjects will then be given a copy of the dietary log and asked to replicate food intake for 24 hours prior to each subsequent trial. Additionally, subjects will be asked to abstain from tobacco, alcohol and caffeine consumption for 24 hours prior to testing in all trials. Finally, subjects will be asked to arrive at the laboratory two hours after consuming a standardized meal consisting of ~500 kcals. Additionally, subjects will be asked to refrain from heavy exercise for 48 hours prior to testing, as well as record any physical activity during the 48 hours prior to testing. Subjects will be asked to maintain consistent physical activity habits before all trials.

Statistical Analysis

Mean power output (watts) from each 3-km time trial will be used as the performance measure. All data will be log transformed to diminish the effects of non-uniformity. Magnitude-based inferences about treatment effects will be derived using methods described by Hopkins and colleagues (Hopkins et al., 2009). A previously established "smallest worthwhile change" in performance will be used as the threshold value for a substantial treatment effect (separate treatment conditions vs. placebo) (Hopkins, 2004). The smallest worthwhile change in performance will be defined as 0.3 × the within-subject variability of select groups of elite cyclists across repeated time trials (CV = 1.3% for time and estimated 3.25% for power) (Paton & Hopkins, 2006).

Published spreadsheets (Hopkins, 2006a,b) will be used to determine the likelihood of the true treatment effect (of the population) reaching the substantial change threshold; these percent likelihoods will be classified as < 1% = almost certainly no chance, 1–5% = very unlikely, 5–25% = unlikely, 25–75% = possible, 75–95% = likely, 95–99% = very likely, and > 99% = almost certain. Clinical inference criteria will be used to classify the effects of treatment on performance. Specifically, if the percent chance of the effect reaching the substantial change threshold is < 25% and the effect is clear, it will be classified as "trivial." If the percent chance of the effect reaching the substantial change threshold for benefit exceeds 25% but the chance for harm is > 0.5% the effect will be classified as unclear.

Chapter 3

Manuscript

Caffeine Mouth Rinsing in the Fed State Does Not Enhance 3-km Cycling Performance In the Morning or Evening

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Introduction

Caffeine is the most commonly used stimulant in the world. Accessible in many foods, drinks, and supplements, caffeine is used in an attempt to enhance energy levels, as well as cognitive and physical performance. Caffeine has well documented effects on the central nervous system (CNS); caffeine can increase neuron excitability in the cortex and spinal region and inhibit adenosine receptors involved in pain sensitivity (Black et al, 2014). Caffeine ingestion has also been found to increase neurotransmitters such as dopamine, responsible for increasing motivation and motor drive (Kasper et al, 2015).

The effect of caffeine ingestion on physical performance across a wide range of activities has been detailed over the past 25 years. Specifically, caffeine can enhance performances from peak force production (Mora-Rodriguez et al, 2012 and 2014), to 60 minute cycling performance (Kovacs et al, 1998). The mechanisms by which caffeine elicits positive outcomes is not completely clear but may relate to a reduction in ratings of perceived exertion (RPE), reduction in muscle discomfort, or enhanced neural excitably, all secondary to the aforementioned neurological effects. From these data, it is clear that caffeine supplementation has the potential to enhance physical performance. However, not all individuals reliably benefit from caffeine and some individuals experience negative side effects, such as jitters, anxiety, cardiac palpitations, headaches and irritability (Nawrot et al, 2010).

Interestingly, caffeine mouth rinsing, without ingestion, may be a viable strategy to enhance performance in individuals that otherwise would not benefit from caffeine consumption or among individuals susceptible to negative side effects. Caffeine mouth rinsing has been shown to enhance repeated cycling sprints (Beavens et al, 2013), 30 minutes of cycling (Bottoms et al, 2014), and 30 minutes of arm cranking (Sinclair and Bottoms, 2015). The positive influence on

performance may be similar to the mechanism of a carbohydrate mouth rinse whereby receptors in the mouth are stimulated, changing brain stimulation effecting performance (Beavens et al, 2013). However, there is also evidence calling into question the benefits of caffeine mouth rinsing on performance; caffeine mouth rinse did not influence 1-hour cycling (Doering et al, 2014) or muscular strength and endurance (Clark et al, 2015). Therefore, the efficacy of caffeine mouth rinsing on performance has not reached a consensus.

One factor that may partially mediate the efficacy of caffeine is time of day. Recently, our laboratory examined the impact of caffeine mouth rinse on performance of a 3-km cycling time trial. While we found that caffeine mouth rinse did not have a significant effect on overall performance, the time of day that the mouth rinse trial was performed appeared to mediate the outcomes (Pataky et al. 2015). Specifically, subjects who completed the time trial before 10:00 am experienced a 2% improvement in power output whereas subjects that performed their trials after 10:00 am had a 4% decline in power output. However, the experimental design was not created to deliberately examine time of day and, as a result, subjects were not exposed to both 'early' and 'late' conditions. Further, the difference in the effect of the caffeine rinse on time of day was potentially influenced by the fact that all but one of the early subjects completed the trial after an overnight fast while most of the late subjects had eaten the day of the trial (Pataky et al. 2015).

Therefore, this study was designed to directly examine the impact of a caffeine mouth rinse at different times of the day and under similar feeding conditions. We hypothesized that caffeine mouth rinse would have a greater effect on performance in a 3k cycling time trial in the morning than the afternoon.

Methods

Subjects

12 recreationally trained male (n=6) and female (n=6) cyclists were recruited from James Madison University and the greater Harrisonburg/Rockingham County area for this study. Descriptive data are shown in Table 1. Potential subjects were required to cycle for a minimum of an hour of cycling in their typical week. The study was approved by the James Madison Review Board. Subjects completed a questionnaire regarding their caffeine habits (coffee, tea, soda, chocolate, etc.); daily caffeine intake was calculated by assigning typical caffeine values to each respective item.

Table 1. Descriptive data for all subjects and the male and female cyclists

	All Subjects (n=12)	**Male (n=6)**	**Female (n=6)**
Height (m)	1.71 ± 0.08	1.77 ± .054	1.66 ± .044
Body Mass (kg)	65.7 ± 9.0	69.1 ± 10.8	62.4 ± 6.0
Age (year)	21.2 ± 3.2	21.7 ± 4.7	20.7 ± 0.5
VO_{2max} ($mL*kg^{-1}*min^{-1}$)	50.5 ± 8.1	56.0 ± 7.8	44.6 ± 3.3
Cycle Training (hr/week)	3.9 ± 2.9	5.1 ± 3.3	2.7 ± 2.0

Data are reported as means ± SD

Preliminary Test

Following height and body weight measures, subjects performed a graded exercise test on a bicycle ergometer (Velotron, Racermate, Inc., Seattle, Washington, USA) to determine maximal oxygen consumption (VO_{2max}). Subjects selected their initial workload. They were instructed to choose "a comfortable, but not easy pace for a 1-hour ride". Workload was increased by 25 Watts every 2 minutes until subjects voluntarily requested to stop due to fatigue or were unable to continue at a cadence >50 rpm. Metabolic measurements were assessed via Moxus Modular Metabolic System (AEI Technologies, Pittsburgh, Pennsylvania, USA)

throughout the test and VO_{2max} was determined by the highest 30 s mean oxygen uptake value.

Experimental Design

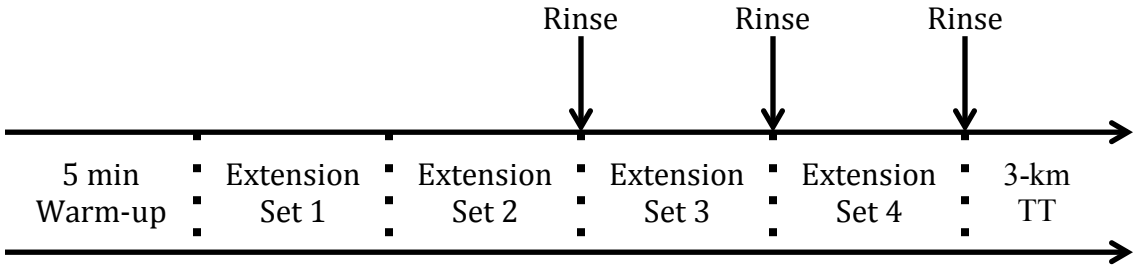
Following preliminary testing, subjects performed two familiarization trials followed by four experimental trials consisting of a 3-km cycling time trial (4-7 minutes). Experimental trials were separated by 3-7 days. A randomly counterbalanced, double blind, placebo controlled design was implemented to compare the effects of the four treatment conditions. During the experimental trials subjects were given three 25 ml mouth rinse solutions at room temperature containing either: A) 300 mg caffeine (NutraBio Labs, Inc., Middlesex, NJ), 1 g saccharine (Sweet'N Low, Brooklyn, NY), and 25 ml of water (rinse containing 1.14% caffeine) or B) a flavor-matched placebo containing 6 g saccharine, and 25 ml of water. Additionally, the subjects completed the trials either: A) in the morning (before 10:00 AM) or B) in the afternoon (after 4:00 PM). The four treatments consisted of each combination of time of day and mouth rinse treatments: 1. Morning + placebo mouth rinse (*Early Placebo*); 2. Morning + caffeine mouth rinse (*Early Rinse*); 3. Evening + placebo mouth rinse (*Late Placebo*); and 4. Evening + caffeine mouth rinse (*Late Rinse*).

Experimental Trials

Peak isokinetic knee extensor force was assessed using an isokinetic dynamometer (Biodex Multi-Joint System—PRO, Biodex Medical Systems, Inc., Shirley, NY, USA), after a standardized 5-min walking warm-up at 3.5 mph. Following the warm-up, subjects completed four sets of five leg extension repetitions on an isokinetic dynamometer (two warm up repetitions followed by three peak torque measurements) at 120 degrees/s with the left leg. The assigned mouth rinse (placebo or caffeine) was provided 15 seconds before the 3rd and 4th set. Subjects

swirled the rinse in their mouths for five seconds and then spat into a paper cup. Following muscle function testing, subjects completed a 3-minute warm-up and then a 3-km computer simulated time trial on an electronically braked cycle ergometer was performed where total time and average watts was recorded. Another rinse was provided immediately before the 3-km time trial. The timing of the rinses is illustrated in Figure 1.

Figure 1. Experimental trial design with mouth rinse timing



Dietary and Exercise Controls

Subjects recorded food intake for 24 hours prior to the first experimental trial. Copies of completed food logs were provided so that subjects could replicate food intake for 24 hours prior to each subsequent trial. Additionally, subjects were asked to abstain from tobacco, alcohol and caffeine consumption for 12 hours prior to testing in all trials. Subjects were provided with a standardized meal (~500 kcals consisting of cereal, yogurt, and juice) that was consumed two hours prior to the experimental trials. Additionally, subjects avoided heavy exercise for 24 hours prior to testing and were instructed to maintain consistent physical activity habits throughout the course of the investigation.

Statistical Analysis

Finishing time (seconds) from each 3-km time trial was used as the primary performance measure. Isokinetic peak torque was used as a second performance measure. All data was log-transformed to diminish the effects of non-uniformity. Magnitude-based inferences about treatment effects were derived using methods described by Hopkins and colleagues (Hopkins et al., 2009). A previously established "smallest worthwhile change" in performance was used as the threshold value for a substantial treatment effect (separate treatment conditions vs. placebo) (Hopkins, 2004). The smallest worthwhile change in performance was defined as 0.3 × the within-subject variability of select groups of elite cyclists across repeated time trials (CV = 1.3% for time and estimated 3.3% for power) (Paton & Hopkins, 2006).

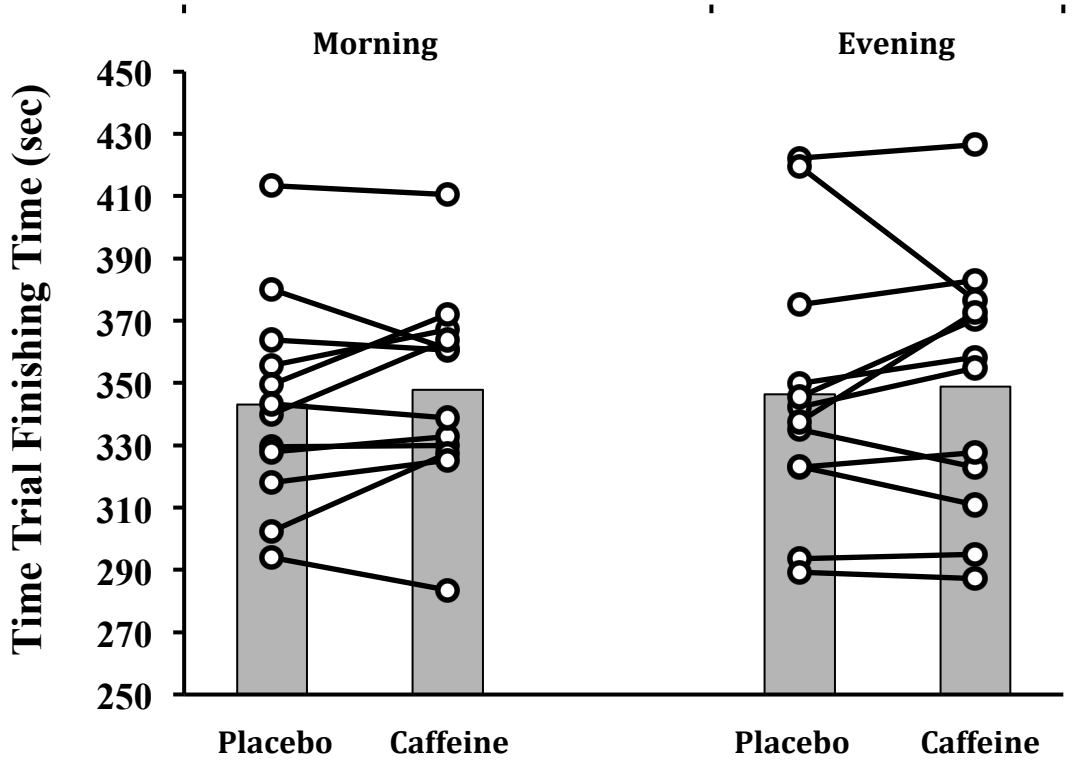
Published spreadsheets (Hopkins, 2006a,b) were used to determine the likelihood of the true treatment effect (of the population) reaching the substantial change threshold; these percent likelihoods were classified as < 1% = almost certainly no chance, 1–5% = very unlikely, 5–25% = unlikely, 25–75% = possible, 75–95% = likely, 95–99% = very likely, and > 99% = almost certain. Clinical inference criteria will be used to classify the effects of treatment on performance. Specifically, if the percent chance of the effect reaching the substantial change threshold is < 25% and the effect is clear, it will be classified as "trivial." If the percent chance of the effect reaching the substantial change threshold for benefit exceeds 25% but the chance for harm is > 0.5% the effect will be classified as unclear.

Results

3-km Time Trial Performance

The caffeine mouth rinse 'possibly' harmed performance in the morning compared to placebo (1.4 ± 2.2%; 62% likelihood). Similarly, the caffeine rinse 'possibly' harmed performance in the evening compared to placebo (0.7 ± 2.8%; 43% likelihood). The impact of the caffeine rinse (relative to placebo) on performance between morning and evening was 'unclear' (0.7 ± 3.4%). Average and individual finishing times are displayed in Figure 2.

Figure 2. 3-km Time Trial Performance



Peak Torque

In the morning, the difference between peak torque before and after the rinse was "possibly" beneficial for both the caffeine (3.6 ± 4.9%) and placebo (5.7 ± 3.9%). There was an

"unclear" difference (2.1 ± 6%) between the caffeine rinse and placebo in the morning. In the evening, the difference between peak torque had a "very likely trivial" effect from the caffeine rinse (-0.3 ± 3%) and a "likely trivial" effect from the placebo (2.9 ± 2.6%). In the evening, there was a "likely beneficial" difference in peak torque between the caffeine rinse and the placebo (3.2 ± 3.8%). While there was a likely difference with the caffeine rinse, torque was not altered in either condition. Average peak torque is displayed in Table 2.

Table 2. Average peak torque (Nm)

	Pre Rinse	**Post Rinse**
Caffeine		
Morning	139 ± 37	145 ± 42*
Evening	144 ± 46	147 ± 43+
Placebo		
Morning	134 ± 36	141 ± 35*
Evening	144 ± 42	144 ± 41#

Data are reported as means ± SD. * represents "possibly beneficial", + represents "very likely trivial", and # represents "likely trivial" difference

Discussion

The primary goal of this project was to determine if time of day impacted the effect of a caffeine mouth rinse on 3-km cycling time trial performance. In contrast to our hypothesis that caffeine mouth rinsing would enhance performance, especially in the morning, the caffeine mouth rinse possibly harmed performance both in the morning and evening, Consequently, there was not a clear difference between morning and evening responses to caffeine mouth rinsing. Further, there was no clear difference in peak torque in the morning and evening from the caffeine rinse. These data suggest that mouth rinsing with caffeine does not improve, and may actually impair, 3-km TT performance in the fed state.

The current findings are an important extension of recent data from our laboratory. Specifically, in our previous study, we found that a caffeine mouth rinse benefits performance in the morning and 'possibly' hinders performance in the evening (Pataky et al, 2015). However, in the previous study, most of the morning subjects performed their trials after an overnight fast whereas the subjects that performed the trials later in the day had eaten up until 2 hours prior to the trials (Pataky et al, 2015), thereby leading to large discrepancies in fasting duration prior to the early and late trials. Thus, we were unable to definitively attribute the beneficial effects of caffeine mouth rinsing on 3-km time trial performance to the time of day, as feeding status could have conceivably confounded the results. To provide further insight, in the current study, subjects performed their trials in the morning and evening under similar feeding conditions (i.e. standardized meal 2 hours prior to testing). When taken together, the data indicate that caffeine mouth rinsing is not influenced by time of day but rather is sensitive to feeding status, such that caffeine rinsing in a fasted state may enhance performance but rinsing in a fed state will not benefit and may actually impair performance.

Previous studies have reported equivocal findings on the ergogenic effects of caffeine rinsing, and the collective data support our conclusion above that feeding status may be a mediating factor. Specifically, 1-hour cycling performance was not enhanced by a caffeine rinse among subjects that performed 60 minutes after eating (Doering et al., 2014), whereas caffeine rinsing enhanced repeated cycling sprints (Beavens et al, 2013), 30-minute cycling (Bottoms et al, 2014), and 30 minutes of arm-cranking (Sinclair and Bottoms, 2015) among subjects that had pre-trial minimum fasting requirement of 2 hours, 4 hours and 4 hours respectively. The effect of a caffeine rinse may be similar to a carbohydrate rinse in a fasted verse fed state. While a carbohydrate rinse has a positive effect on performance in both a fed and fasted state, the effect is larger in a faster state (Lane et al, 2013). There has been evidence suggesting that the degree of activation from the brain after a carbohydrate rinse can be influenced by feelings of hunger (Lane et al, 2013). This can suggest that the magnitude of effect of the caffeine rinse may be due to the interaction of caffeine with the central nervous system (CNS). The magnitude of response from the CNS may be greater when a subject is fasted than fed. While we were not necessarily surprised by the lack of a positive performance effect, the possible detrimental influence of caffeine mouth rinsing on performance is difficult to explain. It is possible that caffeine mouth rinsing could lead to poor pacing strategies such that the rinse increased power output at the beginning of the trial but then led to premature fatigue. However, this was not the case, as power output was lower during each 0.5 km increment of the trial. Further studies should be designed to explore the effects of feeding status on the impact of the caffeine rinse.

In addition to measuring cycling performance, we also assessed peak torque before and following placebo/caffeine mouth rinsing. When comparing the caffeine rinse to the placebo, there was an “unclear” difference in the morning, but a “likely beneficial” difference in the

evening. While there was a likely difference from the caffeine rinse in the evening, there was no alteration in torque between the caffeine and placebo. This supports the idea that time of day does not effect performance but feeding status may. Clark et al found that there was no improvement in muscle strength from a caffeine rinse, however there was no mention on feeding status of the subjects (2015). When observing how the individual rinses affected the peak torque, we found that there was a "possibly beneficial" effect from both the caffeine and placebo rinse in the morning, and a "very likely trivial" and a "likely trivial" effect from the caffeine and placebo rinses in the evening respectively. Both the caffeine mouth rinse and the placebo left a similar, bitter taste in the mouth. After both rinses, peak torque increased slightly. A bitter taste can increase autonomic nervous system responses, increase skin temperature, increase vasoconstriction, and increase heart rate (Rousmans et al, 2000). An increase in autonomic system response can lead to greater force of muscle activation, increasing peak torque after the rinse. The caffeine rinse itself had no effect on peak torque when compared to the placebo, however the bitter taste may have played a role on why torque increased after both rinses.

Our results provide evidence that the caffeine mouth rinse has a negative effect on a 3-km cycling performance in a fed state. The time of day in which the rinse is given did not affect the consequences from the rinse. The bitter taste of the rinse may have a minimal effect on muscle torque, but not enough to impact short distances in cycling. It remains unclear why caffeine rinsing improves performance in some studies; however further research should be completed to examine if the feeding state of participants affects the outcomes of caffeine rinsing.

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Appendices

James Madison University
Department of Kinesiology
Consent for Investigative Procedure

I, _____, hereby agree on _____ (date) to participate in the research project conducted by Nicholas D. Luden, Ph.D., and Kayla Sweeney from James Madison University titled *The Effect of a Caffeine Mouth Rinse on Performance at Different Times of Day.*

The purpose of this study is to determine the impact of a caffeine mouth rinse at different times of day on cycling performance.

Subject Responsibility

I understand that I will undergo the following testing in the study:

You will be asked to perform seven separate exercise tests performed on a stationary bike (cardiovascular fitness test, two familiarization tests, and four 3km time trial tests) as well as peak leg muscle strength testing. All testing will occur in Godwin Hall, room 209, on the campus of James Madison University. You will also be asked about lifestyle behaviors such as smoking, caffeine use, and physical activity. The total time commitment is estimated to be no more than 4-5 hours.

Pre-testing 1 (60 min):

After completing this consent form and the health history screening, if you meet the inclusion criteria for the study, researchers will measure your height and body weight.

You will then be asked to perform a maximal cardiovascular fitness test to determine your peak oxygen consumption (VO_{2max}). You will be asked to ride a stationary bike at an initial workload that is 'fairly easy'. The workload will then be increased every two minutes until: 1) you request to stop due to fatigue, or 2) cannot maintain a cadence of ≥50 revolutions per minute. You will be verbally encouraged to continue to obtain an accurate measurement of VO_{2max}. To access oxygen consumption, you will need to breathe through a mouthpiece/breathing apparatus which collects expired air throughout the test (10-15 minutes).

Familiarization Trials (n =2; 30 minutes each):

During two separate visits, you will be asked to perform a peak leg muscle strength test and a 3-km cycling practice trial on a stationary bike. You will warm-up with a 5-minute treadmill test at 3.5 mph and then be asked to push with maximum effort against a shin pad connected to an electronic device that controls your speed of movement. Your muscular strength will be assessed at three different speeds. Immediately following the muscle strength test, you will be asked to

perform a 5-minute bicycle warm-up at your own pace and then a 3-km computer-simulated time trial. You will be encouraged to treat the time trial like a competition. During these visits, you will practice the mouth rinse before and after the warm-up using water. You will be prompted to swirl 25 milliliters of water around in your mouth for 5 seconds.

Experimental Trials (n=4; 30 minutes each):

You will be asked to perform the same exercises as the familiarization trials (see above). However, instead of receiving a water mouth rinse, you will be asked to rinse with either a placebo or caffeine mouth rinse.

You will be randomly assigned to a treatment order that that will dictate the mouth rinse type and time of day that you report to the laboratory. The experimental trials are as follows: 1. Placebo rinse trial before 10:00 AM, 2. Caffeine rinse trial before 10:00 AM, 3. Placebo rinse trial after 4:00 PM, and 4. Caffeine rinse trial after 4:00 PM.

Standardization Procedures

You will be asked to record food intake during the day of the first experimental trial. You will then be given a copy of the dietary log and asked to replicate food intake on the days of all subsequent experimental trials. Additionally, you will be asked to abstain from alcohol and caffeine consumption for 24 hours prior to testing in all trials. Finally, you will be asked to arrive at the laboratory two hours after consuming a standardized meal consisting of ~500 kcals (the investigators will provide meals consisting of – breakfast cereal, orange juice, yogurt). Additionally, you will be asked to refrain from heavy exercise for 48 hours prior to testing, as well as record any physical activity during the 48 hours prior to testing. You will also be asked to maintain consistent physical activity habits before all trials.

Risks/Benefits

Cardiovascular Exercise (3-km Time Trial and VO_{2max} test):

According to the American College of Sports Medicine's Guidelines for Exercise Testing and Prescription, the risk associated with heavy exercise for individuals categorized as "low risk" is very minimal, and physician supervision is not necessary. The conditions that the exercise sessions are to take place are likely safer than the typical exercise environments of the subjects. If you do not meet ACSM criteria for "low risk", you will not be allowed to participate in the study. In the unlikely event of cardiac or other complications during exercise, an emergency plan is in place. This includes immediate access to a phone to call emergency personnel. In addition, at least one of the listed investigators will be present during the exercise sessions, and all are CPR certified.

Caffeine Mouth Rinse:

There are no known side effects of risks associated with a caffeine mouth rinse.

Muscle Function Test:

The risks of the muscle function testing include soreness from exertion 24-48 hours post and potential lightheadedness or loss of consciousness if correct form is not utilized. You will be instructed in correct form and breathing techniques prior to testing.

Benefits:

You will receive a free assessment of your cardiovascular fitness (VO_{2max}). The top performing males and females will also be entered into a drawing for a monetary reward. The top 5 male performers (fastest finishing placebo time) will be entered into a drawing to win $150. Male participants with finish times that place them 6-10 will be entered into a drawing to win $75. The same incentive plan will be implemented for females. Finally, participation in this research will help the investigators formulate caffeine recommendations for athletes.

Confidentiality

The results of this research will be presented at a symposium and published in the JMU honors thesis database and possibly in a peer-reviewed journal article. The results of this project will be coded in such a way that your identity will not be attached to the final form of this study. The researcher retains the right to use and publish non-identifiable data. However, you can ask that your data be removed from the study at any point prior to presentation and publication. While individual responses are confidential, aggregate data will be presented representing averages or generalizations about the responses as a whole. All data will be stored in a secure location accessible only to the researcher. Final aggregate results and your individual results will be provided at your request.

Participation & Withdrawal

Your participation is entirely voluntary. You are free to choose not to participate. Should you choose to participate, you can withdraw at any time without consequences of any kind.

Questions

You may have questions or concerns during the time of your participation in this study, or after its completion. If you have any questions about the study, contact Kayla Sweeney at sweeneka@dukes.jmu.edu or by phone at (703)-740-6613.

Giving of Consent

I have read this consent form and I understand what is being requested of me as a participant in this study. I freely consent to participate. I have been given satisfactory answers to my questions. The investigator provided me with a copy of this form. I certify that I am at least 18 years of age.

Name of Participant (Printed)	Name of Researcher(s) (Printed)
Name of Participant (Signed)	Name of Researcher(s) (Signed)
Date	Date

For questions about your rights as a research subject, you may contact the chair of JMU's Institutional Review Board (IRB). Dr. David Cockley, (540) 568-2834, cocklede@jmu.edu.

AHA/ACSM Health/Fitness Facility Pre-participation Screening Questionnaire

Assess your health status by marking all *true* statements

History

You have had:

_____ a heart attack
_____ heart surgery
_____ cardiac catheterization
_____ coronary angioplasty (PTCA)
_____ pacemaker/implantable cardiac
_____ defibrillator/rhythm disturbance
_____ heart valve disease
_____ heart failure
_____ heart transplantation
_____ congenital heart disease

Symptoms

_____ You experience chest discomfort with exertion
_____ You experience unreasonable breathlessness
_____ You experience dizziness, fainting, or blackouts
_____ You take heart medications

Other Health Issues

_____ You have diabetes
_____ You have asthma or other lung disease
_____ You have burning or cramping sensation in your lower legs when walking short distances
_____ You have musculoskeletal problems that limit your physical activity
_____ You have concerns about the safety of exercise
_____ You take prescription medication(s)

If you marked any of these statements in this section, consult your physician or other appropriate health care provider before engaging in exercise. You may need to use a facility with a ***medically qualified staff***.

Cardiovascular risk factors

_____ You are a man older than 45 years
_____ You smoke, or quit smoking within the previous 6 months
_____ Your blood pressure is > 140/90 mmHg
_____ You do not know your blood pressure
_____ You take blood pressure medication
_____ Your blood cholesterol level is > 200 mg/dl
_____ You do not know your cholesterol level
_____ You have a close blood relative who had a heart attack or heart surgery before age 55 (father or brother) or age 65 (mother or sister)
_____ You are physically inactive (i.e. you get < 30 minutes of physical activity on at least 3 days of the week)
_____ You are > 20 pounds overweight

If you marked two or more of the statements in this section, you should consult your physician or other appropriate health care provider before engaging in exercise. You might benefit from using a facility with a ***professionally qualified exercise staff*** to guide your exercise program.

_____ None of the above

You should be able to exercise safely without consulting your physician or other appropriate health care provider in a self-guided program or almost any facility that meets your exercise program needs.

Subject Prescreening Information and Caffeine Habits

Age: _____years

Height: _____ Weight: _____

Typical Exercise Habits over the past 3 months

Average number of days cycling per week: _____

Average number of hours cycling per week: _____

Do you have a muscle or join injury that precludes you from completing the cycling protocol?

Caffeine Habits

Please list your approximate weekly intake of the following:

Cups of coffee:

Cups of tea:

Cans (12oz) caffeinated soda:

Servings of chocolate:

Doses of caffeinated pills:

Other caffeinated beverages or supplements not listed:

SPIT Study – VO_{2max} Test

Date: **Sex:**
Subject: **Body Weight (kg):**
Age: **Height (cm):**

Seat Height: Handlebar Height:

Seat Fore/aft: Handlebar fore/aft:

Nosepiece

Subject will determine starting workload; Increase workload 25 W every 1 min

Time (min)	Work-Watts	HR (bpm)	VO_2 (L/min)	VO_2 (ml/kg/min)	RPE
5 min warm-up					
Exercise min 1					
Min 2					
Min 3					
Min 4					
Min 5					
Min 6					
Min 7					
Min 8					
Min 9					
Min 10					
Min 11					
Min 12					
Min 13					
Min 14					
Min 15					
Min 16					
Min 17					
Min 18					
Min 19					
Min 20					

Max Heart Rate:

RER at end of test:

VO_{2max} (mL/kg/min):

VO_{2max} (L/min):

SPIT Study
Familiarization Trial

Subject #: _____ Date:_____

Trial: FAM1 FAM2

Explain the protocol in detail to ensure the subject knows what is expected.

5 minute warm up on treadmill at 3.5 mph

BIODEX

Chair position:_____

Seatback position:_____

Machine position:_____

Seat height:_____

Arm attachment position:_____

Speed	**Extension PeakT**
120	_____
120	_____
Rinse	
120	_____
120	_____

Velotron

Seat height:_____

Time Trial Time:_____ Average watts:_____

SPIT Study
Experimental Trial

Subject #: _____ Date: _____ Time: _____ AM PM

Treatment given: Purple Gold

Trial: 1 2 3 4

Explain the protocol in detail to ensure that the subject knows what is expected.

Verify that the subject is fasted (eaten standardized meal 2 hours prior, 12hr caffeine and alcohol)

5 minute warm up on treadmill (3.5mph)

BIODEX – *Remind subjects to push and pull as hard as they possibly can*

Speed	**Extension PeakT**
120	_____
120	_____
Rinse	
120	_____
120	_____

Velotron – *Remind subjects to treat this like a championship caliber race*

Time trial time: _____ Average Watts: _____

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