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The Influence of Time-of-Day Consumption and Training Status
on the Ergogenic Properties of Caffeine

James Boyett

A thesis submitted to the Graduate Faculty of

JAMES MADISON UNIVERSITY

In

Partial Fulfillment of the Requirements

for the degree of

Master of Science

Department of Kinesiology

May 2016

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Acknowledgements

Firstly, I would like to thank Dr. Nicholas Luden for serving as both my thesis and educational advisor. You have provided support and insight numerous times throughout my career and thesis which has helped me to not only get to where I am right now, but grow as a person and a researcher.

Secondly, I would like to thank Dr. Christopher Womack and Dr. Michael Saunders for serving as committee members on my thesis. Both of you have helped me when I had questions about research and writing process, as well as other questions related to academia.

I would also like to thank my co-investigator, Gabrielle Giersch. Working alongside you made the whole data collection process more enjoyable as well manageable.

Thank you to David Pumphrey, Andrew Marquez, Jordan Parker, and Hannah Kotarski for your help with data collection as well as subject recruitment and data entry.

To the rest of the members of the Human Performance Lab, thank you for helping when I had any questions about equipment, needed help with edits, and generally making the lab an enjoyable place.

Finally, I would like to thank my mother, father, and maternal grandmother for their support, both financially and emotionally. This has been a long road, but they have always been there for me.

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Abstract

PURPOSE: The objectives were to determine the effects of time-of-day consumption and training status on the benefits of caffeine supplementation for cycling performance and peak muscle strength. **METHODS:** Twenty untrained and trained subjects completed four trials consisting of isokinetic peak torque testing and 3-km time trials (TT). Subjects ingested either 6 mg/kg of caffeine or a placebo one hour prior to each trial. Treatments were: morning + placebo, morning + caffeine, evening + placebo, evening + caffeine. Magnitude based inferences were used to evaluate treatment differences. **RESULTS:** Caffeine ('very likely' and 'likely') improved 3-km TT performance in the morning and evening. 3-km TT performance was 'likely' improved more in the morning than evening for total subject pool and trained subjects. Untrained subjects 'likely' benefited more during the 3-km TT from supplementation than trained in both the morning and evening. Caffeine supplementation was 'likely' trivial and 'unclear' for the majority of peak muscle strength conditions. **CONCLUSIONS:** Caffeine supplementation improved 3-km TT performance in the morning for trained and untrained, with lesser benefits in the evening, while untrained benefited more than trained. Peak muscle strength was largely unaffected by caffeine supplementation, regardless of time-of-day consumption or training status.

Chapter One

Introduction

The ease and accessibility of caffeine is undeniable, and its use within sport is pervasive; among a sample of 20,686 urine samples, 33% of athletes testing positive for urinary caffeine levels above 5 µg/mL, approximate to the amount of caffeine in 3 cups of coffee (7). Caffeine is believed to exert its ergogenic effect through three possible mechanisms: 1) central nervous system (CNS) adenosine antagonism, 2) increased calcium release from the sarcoplasmic reticulum (within skeletal muscle), and 3) alteration of sodium-potassium ion pump activity; and these mechanisms may not be mutually exclusive. Caffeine and its metabolites are potent adenosine receptor antagonists (43, 56) due to similarity in molecular structure to adenosine (20, 59). This antagonism may enhance motor unit recruitment, as caffeine has been observed to increase maximum voluntary contraction (MVC) and recruitment above placebo levels (29, 54, 63). *In vitro* observation of caffeine on peripheral tissues showed increased calcium release from the sarcoplasmic reticulum, thought to have been produced by adenosine antagonism at the level of ryanodine receptors (RyR) (59). This antagonism allows for increased calcium release from the sarcoplasmic reticulum, allowing for greater levels of excitation contraction coupling to occur (36, 67). Finally, there is evidence of caffeine altering sodium-potassium pump dynamics, resulting in decreased plasma potassium concentrations (35). Better maintenance of electrochemical gradients extends the ability to deliver continuous action potentials. However, physiological doses do not appear to alter plasma potassium concentration (21). Inconsistent mechanistic findings may be due to discrepancies in dosages for research, with physiological dosing between 2-9mg/kg body

weight with a common dose of 6mg/kg body weight (3, 6, 8, 10, 12, 13, 15, 19, 63, 64), and supra-physiological dosages above toxic levels in humans for mechanistic actions (20, 35, 59, 67). It is entirely possible all of these factors contribute to the ergogenic effect of caffeine and adenosine antagonism mediates all of these mechanisms (35, 43, 59). On balance, the current literature points to adenosine antagonisms working both centrally and peripherally.

Regardless of the mechanism of action, the ergogenic properties of caffeine have been extensively studied during aerobic endurance performance, with enhancement observed during both long-duration (≥ 60 minutes) (12, 19, 25, 33, 57, 68), and short-duration aerobic performance (2-60 minutes) (8, 19, 27, 45, 64). In 1979, Ivy et al. reported that 250mg of caffeine increased work output and oxygen consumption during two hours of isokinetic cycle ergometry (25). Since then, caffeine has been associated with improved work output (33), time to fatigue (19), and cycling time trial (TT) performance (12, 33, 68). In a brief review of the literature, Graham found overwhelming support for the ergogenic properties of caffeine in long-duration performance, only noting three instances where no effect occurred (20). Research investigating caffeine supplementation prior to short-duration aerobic performance is less established than long-duration aerobic performance, although studies report faster race performance with caffeine (8, 45, 64). Our laboratory recently observed that caffeine enhanced 3km cycling TT performance by 1.1% (45). Additionally, improvements in time to fatigue at a given work rate have been observed with up to a 10 minute increase in exercise time at 85% $\text{VO}_{2\text{max}}$ (19, 27).

Relative to caffeine and endurance performance, the effect on speed and power during anaerobic performance (≤ 2 minutes) is less clear (4). Caffeine can improve

swimming velocities and times during 100m swimming sprints in trained but not untrained individuals (10). Similar improvements have been noted in mean power (13, 65), peak power (65), and TT performance (65). Importantly, the benefits of caffeine for anaerobic performance have not been universal. One study reported no change in peak power, total work, or the rate of power decay (66). Additionally, while Woolf et al. observed increased peak power in 78% of the subjects during administration of the Wingate test (69), other studies and meta-analyses found no effect (6, 15, 20, 21), with one even observing a detrimental effect (21) on Wingate performance.

The effect of caffeine on muscular strength, muscular power, MVC, and muscular endurance is equivocal, with more studies showing an effect (6, 15, 18, 41, 60, 69) than no effect (2, 3, 69). A recent meta-analysis performance on 27 muscular strength and 23 muscular endurance studies yielded effect sizes of 4% and 14% gains in performance, respectively (63). However, there are inconsistencies in who benefits from caffeine supplementation, with some individuals responding more than others. This may be due to factors which have yet to be investigated in-depth, such as training status (2, 4, 20, 28, 33, 60) and genetics (3, 4, 60).

Many environmental factors – such as diet, smoking, obesity, exercise, and menstrual related factors – have been linked to altered caffeine pharmacokinetics. Environmental factors inhibiting the activity of the enzyme responsible for caffeine metabolism, cytochrome P450 1A2 (CYP1A2), include: diet (16, 48, 49), menstrual related factors (oral contraceptives, luteal phase, post-menopausal estrogen replacement) (1, 17, 50) and obesity (1, 31); whereas factors inducing CYP1A2 activity include: diet (1, 34, 49, 55) and smoking (1, 9, 16, 17, 42, 52, 53). Regarding exercise, chronic (61), but

not acute (31, 38) exercise appears to increase CYP1A2 activity and the pharmacokinetics of caffeine. When compared to resting metabolism, acute endurance exercise of 90 minutes failed to alter caffeine pharmacokinetics in lean or obese individuals (31, 38). Conversely, CYP1A2 activity increased after 30 days of endurance training (61). Separately from CYP1A2 activity, chronic endurance training causes increased adenosine receptor density (39). Training-induced physiological changes associated with caffeine metabolism and physiological interactions prove promising for differences in who benefits from caffeine.

As mentioned above, training status may also mediate the magnitude of the ergogenic effect of caffeine. A meta-analysis indicated that untrained subjects may benefit more than trained subjects (63). However, virtually all of the insight on this topic has been derived from comparing separate studies conducted with trained and untrained subjects. To our knowledge, only three studies have included both trained and untrained in parallel design to distinguish the effect of training status. Collomp et al. evaluated the influence of training status on caffeine consumption during 100 m swim performance and observed trained, but not untrained swimmers benefited from consumption (10). Conversely, two studies observing cycling time-to-fatigue and peak strength noted no significant differences between trained and untrained (7, 51). However, it should be noted neither study observed that caffeine elicited a significant effect on performance. The influence of the ergogenic properties of caffeine as they pertain to training status on muscular strength and power or short-duration aerobic performance is still unclear.

Time-of-day variations have been identified in both CYP1A2 activity and physical performance. Increases in CYP1A2 activity in the morning when compared to the afternoon occur in both South Asian and Caucasian individuals (47). Similarly, CYP1A2

activity appears to be elevated during sleeping hours and directly after waking, when compared to the rest of day (30). Few studies have evaluated the effect of time-of-day caffeine consumption on performance. To date, one study from this lab on aerobic performance with coincidental design (45), and two deliberately designed time-of-day studies observing muscular strength and power (40, 41), witnessed time-of-day influencing the ergogenic effect of caffeine, with caffeine raising performance levels in the morning to match those observed in the afternoon or evening. However, no deliberately designed study has observed time-of-day influence ergogenic effect in aerobic performance.

The ease and accessibility of caffeine is undeniable, and its use within sport is pervasive. However, a number of major candidate factors involved in caffeine and performance have yet to be resolved. Caffeine metabolism is regulated by many factors; some controllable such as time-of-day (30, 47) and diet (16, 34, 48, 49, 55); while others are not controllable, such as aspects related to menstruation (1, 17, 38, 50, 53). Additionally, training status appears to impact whether or not an individual benefits from supplementation (8, 15, 19, 27, 40, 41, 64). Therefore, it is important to study each aspect and its role in the ergogenic properties of caffeine for physical performance.

Purpose

The purpose of the current study is to detail the extent that time-of-day consumption and training status influence caffeine's effect on 3km time trial performance and peak isokinetic leg strength.

Aims and Hypotheses

Aim 1: To determine if time-of-day consumption mediates the effects of caffeine supplementation on peak isokinetic leg torque.

Hypothesis 1: Caffeine will elicit larger improvements in peak torque among morning than evening consumption.

Aim 2: To determine if time-of-day consumption mediates the effects of caffeine supplementation on a 3km cycling time trial.

Hypothesis 2: Caffeine will elicit larger improvements in 3km cycling time trial among morning than evening consumption.

Aim 3: To determine if training status mediates the effects of caffeine supplementation on peak isokinetic leg torque.

Hypothesis 3: Caffeine will elicit larger improvements in peak torque among untrained than trained individuals.

Aim 4: To determine if training status mediates the effects of caffeine supplementation on a 3km cycling time trial.

Hypothesis 4: Caffeine will elicit larger improvements in 3km cycling time among untrained than trained individuals.

Significance

Major candidate factors affecting who benefits from caffeine supplementation have yet to be resolved, warranting investigation into the factors influencing caffeine's ergogenic properties. Time-of-day consumption appears to affect the value of caffeine, but the literature investigating this interaction is too young to make any definitive verdict on the interaction. Further, untrained individuals appear to experience greater benefits from caffeine supplementation, yet only a few study have observed trained versus untrained in the same design. Relatively few studies have randomized, double-blind, crossover, placebo controlled designs investigating the effect of time-of-day consumption and training status on the ergogenic properties of caffeine, and none have investigated both of these parameters and the cross-treatment interaction on caffeine supplementation in both short-duration aerobic and muscular strength performance that may occur in a single study. The findings from the current study have the potential to determine which of the major candidate factors (time-of-day consumption and training status) affect whom benefits from caffeine supplementation regarding short-duration aerobic and muscular performance.

Chapter Two

Methodology

Subjects

Sixty healthy male subjects – thirty trained cyclists and thirty novice cyclists – from James Madison University will participate in this study. All subjects are required to have performed, at minimum, either “occasional” cycling (one day per month) in their weekly exercise routine for the novice cyclists, or “consistent” cycling (four days per week) in their weekly exercise routine over the previous three months for the trained cyclists. Females are excluded from the current study because of fluctuating caffeine pharmacokinetics. Specifically caffeine clearance is reduced during the luteal phase of menstrual cycle as well as a result of certain types of oral contraception usage (1, 17, 50). Subjects will be informed of the experimental procedures and risks prior to giving written consent. The study was approved by the James Madison University Institutional Review Board.

Fitness Testing

Following height and body weight measurements, subjects perform an incremental exercise test to exhaustion on a bicycle ergometer (Velotron, Racermate, Inc., Seattle, WA, USA) to determine maximum oxygen consumption (VO_{2max}). The workload starts at 100 W for untrained and 150 W for trained, and increases every minute in 25 W increments until volitional fatigue, or inability of the subject to maintain a cadence of 50 RPM or higher for more than 10 seconds. Metabolic measurements is assessed via Moxus Modular

Metabolic System (AEI Technologies, Pittsburgh, PA, USA) throughout the test and VO_{2max} will be determined by the highest 30-s mean oxygen uptake value.

Supplementation

A randomly counterbalanced, double blind, placebo controlled design will be implemented to compare the effects of the four different treatment conditions. During the experimental trials subjects are given 6mg/kg body weight in capsule form containing either anhydrous caffeine or all-purpose flour (placebo), which will be ingested 1 hour prior to each treatment trial. Additionally, subjects will perform four trials: two morning (6:00am to 10:00am), and two evening (4:00pm to 8:00pm). Morning and evening trials will be repeated at identical times, with an eight-hour minimum separation between designated morning and evening times. The four treatment conditions are: 1. Morning + placebo capsule (AM_{PLA}) 2. Morning + caffeine capsule (AM_{CAF}) 3. Evening + placebo capsule (PM_{PLA}) 4. Evening + caffeine capsule (PM_{CAF}).

Performance Trials

Each subject performs six exercise trials (two familiarization trials followed by four experimental trials) on both an isokinetic dynamometer (Biodex Multi-Joint System - PRO, Biodex Medical Systems, Inc., Shirley, NY, USA), and the aforementioned cycle ergometer, with 3-14 days between each trial. The subjects perform a 5-minute treadmill warm-up at 3.5 mph. Following warm-up, subjects complete six sets composed of two warm-up repetitions on the isokinetic dynamometer, followed by two maximal exertion isokinetic peak torque measurements at 30, 120, and 240°/sec. One repetition consists of

knee extension immediately followed by knee flexion. Once all exertions are completed in a given set, 60-seconds of rest is given before proceeding to the next set. Once two sets are completed at a given velocity, the subject moves to the next velocity for two sets, moving from slowest to fastest velocity in set progression. After the isokinetic peak torque measurements, subjects perform a 3km time trial. The familiarization trials are identical to the experimental trials, with the exception of treatment. Subjects are instructed to treat each trial as a competition. Subjects will not receive verbal feedback or encouragement from the investigators and no visual feedback from the time trial will be provided, with the exception of elapsed distance for the 3km time trial.

A mean of the peak torques from both maximal attempts during the isokinetic testing during both knee flexion and knee extension will be analyzed to determine if differences are present between treatments. Similarly, time trial time and mean power output will be analyzed to determine if any differences are present between treatments.

After the completion of every trial, subjects are given a questionnaire asking which supplementation protocol (caffeine or placebo) subjects believed was administered during each trial, as well as asked to give a confidence rating for each trial's prediction.

Dietary and Exercise Control

Subjects are provided with instructions for recording food intake so subsequent dietary intake could be replicated. All subjects record food intake for 24 hours prior to all experimental trials and are instructed to replicate food intake for each experimental trial. Subjects are instructed to abstain from any alcohol (24 hrs), caffeine (12 hrs), and food intake (4 hrs; post-absorptive state) prior to each experimental trial. All subjects record

daily physical activity for 48 hours prior to experimental trials. Subjects will be instructed to maintain consistent exercise habits between trials and to abstain from any heavy and/or unaccustomed exercise 48 hours prior to each experimental trial. Dietary and exercise records will be obtained prior to each experimental trial.

Statistical Analysis

All data will be log transformed to diminish the effects of nonuniformity. Magnitude-based inferences about the data will be derived using methods described by Hopkins and colleagues (22). A previously established ‘smallest worthwhile change’ in performance is used as the threshold value for a substantial treatment effect (separate treatment conditions vs. placebo) (24). The smallest worthwhile change in performance is defined as $0.3 \times$ 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), which translates to a difference in mean power output of 1% or 2.4 watts and 0.4% or 1.2 -seconds in the current project (46). For the isokinetic data, $0.2 \times SD$ of the AM_{PLA} trial will be used to determine smallest worthwhile change (24).

A published spreadsheet (23) will then be used to determine the likelihood of the true treatment effect (of the population) reaching the substantial change threshold ($0.3 \times CV$); these 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. If the percent chance of the effect reaching the substantial change threshold is $<25\%$ and the effect will be clear, it will be classified as a ‘trivial’ effect. If 90% confidence intervals include values exceeding the substantial change threshold for both a

positive and negative effect, effects will be classified as unclear (>5% chance of reaching the substantial threshold for both a positive and negative effect).

Chapter Three
Manuscript

**The Influence of Time-of-Day Consumption and Training Status
on the Ergogenic Properties of Caffeine**

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ABSTRACT

PURPOSE: The objectives were to determine the effects of time-of-day consumption and training status on the benefits of caffeine supplementation for cycling performance and peak muscle strength. **METHODS:** Twenty untrained and trained subjects completed four trials consisting of isokinetic peak torque testing and 3-km time trials (TT). Subjects ingested either 6 mg/kg of caffeine or a placebo one hour prior to each trial. Treatments were: morning + placebo, morning + caffeine, evening + placebo, evening + caffeine. Magnitude based inferences were used to evaluate treatment differences. **RESULTS:** Caffeine ('very likely' and 'likely') improved 3-km TT performance in the morning and evening. 3-km TT performance was 'likely' improved more in the morning than evening for total subject pool and trained subjects. Untrained subjects 'likely' benefited more during the 3-km TT from supplementation than trained in both the morning and evening. Caffeine supplementation was 'likely' trivial and 'unclear' for the majority of peak muscle strength conditions. **CONCLUSIONS:** Caffeine supplementation improved 3-km TT performance in the morning for trained and untrained, with lesser benefits in the evening, while untrained benefited more than trained. Peak muscle strength was largely unaffected by caffeine supplementation, regardless of time-of-day consumption or training status.

INTRODUCTION

The ease and accessibility of caffeine is undeniable, and its use within sport is pervasive; among a sample of 20,686 urine samples, 33% of athletes testing positive for urinary caffeine levels above 5 $\mu\text{g/mL}$, approximate to the amount of caffeine in 3 cups of coffee (7). The benefits of caffeine have consistently been shown to enhance performance longer in events lasting longer than 2 minutes (17, 21, 25, 32, 34), whereas data on shorter duration events and peak muscle strength are less conclusive. Specifically, caffeine intake can enhance anaerobic power and speed (6, 8, 33), yet repeated 30-second Wingate Anaerobic Test performance does not appear to be impacted by caffeine supplementation (4, 10, 13). Likewise, the influence of caffeine on muscular strength, power, endurance, and maximal voluntary contraction (MVC) is equivocal, with some (4, 10, 11, 23) but not all studies (1, 2) supporting an ergogenic effect of caffeine. Altogether, it is clear that caffeine has the capacity to improve physical performance. Importantly, there are a number of unresolved factors that may impact the magnitude of the effect of caffeine, including time of day and training status.

Time-of-day has recently been shown to influence the performance impact of caffeine supplementation. To date, only two studies have investigated the potential time-of-day \times caffeine interaction and both indicated that caffeine benefits may be heightened in the morning compared to the evening. Mora-Rodríguez et al. investigated caffeine's effect on bench press, squat, and MVC, noting placebo trials in the morning were below the performance of all other trials for bench press. No differences were observed between the morning caffeine trials and either of the evening trials (caffeine or placebo) (23), indicating caffeine attenuated morning deficits in somatic performance. Additionally, our lab recently

studied caffeine supplementation on 3-km time trial performance, and added post-hoc grouping into 'before 10am' vs. 'after 10am' performance for parallel comparison (25). Caffeine ingestion improved 'before 10am' performance while the improvement of performance 'after 10am' with caffeine was unclear. Because only one crossover designed study assessed time-of-day caffeine consumption and muscular performance and our recent post-hoc analysis revealed caffeine may elicit greater improvements in the morning, the primary purpose of this study was to observe the effect of time-of-day on caffeine ergogenics.

As mentioned above, training status may also mediate the magnitude of the ergogenic effect of caffeine. A meta-analysis indicated that untrained subjects may benefit more than trained subjects (31). However, virtually all of the insight on this topic has been derived from comparing separate studies conducted with trained and untrained subjects. To our knowledge, only three studies have included both trained and untrained in parallel design to distinguish the effect of training status. Collomp et al. evaluated the influence of training status on caffeine consumption during 100 m swim performance and observed trained, but not untrained swimmers benefited from consumption (6). Conversely, two studies observing cycling time-to-fatigue and peak strength noted no significant differences between trained and untrained (5, 27). However, it should be noted neither study observed that caffeine elicited a significant effect on performance. The influence of the ergogenic properties of caffeine as they pertain to training status on muscular strength and power or short-duration aerobic performance is still unclear. Therefore, the second purpose of this study was to observe the effect of training status on caffeine ergogenics.

METHODOLOGY

Subjects

Twenty-two healthy male subjects (twelve trained and ten novice cyclists) from James Madison University and the surrounding area volunteered for the study. However, one trained and one novice cyclist withdrew for reason unrelated to the study, resulting in a sample of eleven trained and nine novice cyclists who completed the study. Descriptive data are shown in *Table 1*. Subjects were required to have performed, at minimum, either “occasional” cycling (one day per month) for the novice cyclists, or “consistent” cycling (four days per week) in their weekly exercise routine over the past three months for the trained cyclists. Cycling frequency and duration were self-reported. Trained and untrained cyclists were determined by the number of hours cycling per week for the 3km data, with comparison based off the top (trained) vs. bottom (untrained) tertiles. Subjects were asked about their resistance training habits and this information was used to permit post-hoc separation into binomial groups of resistance trained (10.1 ± 7.8 hrs/wk, range: 3.5-22.5 hrs/wk) and untrained (0.6 ± 0.7 hrs/wk, range: 0-2 hrs/wk) for use as a covariate. Subjects were informed of the experimental procedures and risks prior to giving written consent. The study was approved by the James Madison University Institutional Review Board.

Cardiovascular Fitness Testing

Following height and body weight measurements, subjects performed an incremental exercise test to exhaustion on a bicycle ergometer (Velotron, Racermate, Inc., Seattle, WA, USA) to determine maximum oxygen consumption (VO_{2max}). The workload started at either 100 or 150 W for untrained and trained, respectively, and was increased

by 25 W every minute until volitional fatigue. Metabolic measurements were assessed using a Moxus Modular Metabolic System (AEI Technologies, Pittsburgh, PA, USA) throughout the test and VO_{2max} was determined by the highest 30-second mean oxygen uptake.

Experimental Design

A randomly counterbalanced, double blind, placebo controlled design was implemented to compare the effects of the four different treatment conditions. During the experimental trials subjects ingested a capsule one hour prior to exercise containing either 6 mg/kg body weight anhydrous caffeine or all-purpose flour (placebo). Subjects performed four trials: two morning (6:00am to 10:00am), and two evening (4:00pm to 8:00pm). Morning and evening trials were repeated at their respective identical time, with an eight-hour minimum separation between designated morning and evening times. The four treatment conditions were: 1. Morning + placebo capsule (AM_{PLA}) 2. Morning + caffeine capsule (AM_{CAF}) 3. Evening + placebo capsule (PM_{PLA}) 4. Evening + caffeine capsule (PM_{CAF}).

Performance Trials

Each subject performed six exercise trials (two familiarization trials followed by four experimental trials) on both an isokinetic dynamometer (Biodex Multi-Joint System - PRO, Biodex Medical Systems, Inc., Shirley, NY, USA), and cycle ergometer, with 3-14 days between each trial. The subjects performed a 5-minute treadmill warm-up at 3.5 mph. Following warm-up, subjects completed six sets composed of two warm-up repetitions on

the isokinetic dynamometer, followed by two maximal exertion isokinetic peak torque measurements at 30, 120, and 240 degrees/sec on their right leg. Each repetition consisted of knee extension immediately followed by knee flexion. Once all repetitions were completed in a given set, 60-seconds of rest was given before proceeding to the next set. After completing two sets at a given velocity, subjects performed the next velocity for two sets, progressing from slowest to fastest in velocity. A mean of the peak torques from both maximal attempts during the isokinetic testing during both knee flexion and knee extension were analyzed to determine if differences were present between treatments. After the isokinetic peak torque measurements, subjects performed a 3-km time trial on the cycle ergometer. The familiarization trials were identical to the experimental trials, with the exception of treatment. Subjects were instructed to treat each trial as a competition. 3-km time trial time and mean power output were analyzed to determine if any differences were present between treatments. Subjects did not receive verbal feedback or encouragement from the investigators during testing and no visual feedback from the time trial were provided, with the exception of elapsed.

After the completion of the all trials, subjects were given a questionnaire asking which supplementation protocol (caffeine or placebo) subjects believed was administered during each trial, as well as asked to give a confidence interval for each trial's prediction.

Dietary and Exercise Control

Subjects were provided with instructions for recording food intake so dietary intake could be replicated across trials. All subjects recorded food intake for 24 hours prior to all experimental trials and were instructed to replicate food intake for each experimental trial.

Subjects were also instructed to abstain from any alcohol (24 hrs), caffeine (12 hrs), and food intake (4 hrs; post-absorptive state) prior to each experimental trial. Daily physical activity was also recorded for 48 hours prior to experimental trials. Subjects were instructed to maintain consistent exercise habits between trials and to abstain from any heavy and/or unaccustomed exercise 48 hours prior to each experimental trial. Dietary and exercise records were collected prior to each experimental trial.

Statistical Analysis

All data were log transformed to diminish the effects of nonuniformity. Magnitude-based inferences about the data were derived using methods described by Hopkins and colleagues (14). A previously established ‘smallest worthwhile change’ in performance was used as the threshold value for a substantial treatment effect (separate treatment conditions vs. placebo) (16). The smallest worthwhile change in performance was defined as $0.3 \times$ 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), which translated to a difference in mean power output of 1% or 2.4 watts and 0.4% or 1.2-seconds in the current project (26). For the isokinetic data, $0.2 \times$ SD of the AM_{PLA} trial was used to determine smallest worthwhile change (16).

A published spreadsheet (15) was then used to determine the likelihood of the true treatment effect (of the population) reaching the substantial change threshold ($0.3 \times$ CV); these 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. If the percent chance of the effect reaching the substantial change threshold was

<25% and the effect was clear, it was classified as a 'trivial' effect. If 90% confidence intervals included values exceeding the substantial change threshold for both a positive and negative effect, effects were classified as unclear (>5% chance of reaching the substantial threshold for both a positive and negative effect).

RESULTS

3-km Time Trial Performance

All Subjects

In all subjects, AM_{CAF} 3-km time and power output (3-km TT) was ‘very likely’ better than AM_{PLA}, while PM_{CAF} ‘likely’ improved performance vs. PM_{PLA} (*Figures. 1 and 2*). AM_{CAF} ‘likely’ improved 3-km TT performance to a greater extent than PM_{CAF} when compared to the respective placebo condition (PLA) (*Table 2*).

Trained Subjects

For trained subjects, AM_{CAF} performance was ‘likely’ improved vs. AM_{PLA}, whereas caffeine’s effect was ‘unclear’ between PM_{PLA} and PM_{CAF} for 3-km TT. AM vs. PM comparison revealed that AM_{CAF} ‘likely’ improved performance more than PM_{CAF} when compared to PLA.

Untrained Subjects

AM_{CAF} and PM_{CAF} ‘likely’ improved time trial performance vs. AM_{PLA} and PM_{PLA}, respectively, in untrained subjects. Time-of-day AM vs. PM comparison was ‘unclear’.

Training Status

It was ‘unclear’ whether trained or untrained benefited more from caffeine in the AM condition, but untrained subjects ‘likely’ benefited more from caffeine supplementation than trained in the PM condition.

Peak Muscle Force

All Subjects

For all peak muscle force data, see *Table 2 and 3*. Caffeine ‘possibly’ decreased torque 30 deg/sec flexion (30FLX) in the AM, 120 deg/sec flexion (120FLX), and 240 deg/sec flexion (240FLX) conditions, whereas caffeine’s influence was ‘likely’ trivial in the AM for all other conditions. 30EXT performance was ‘possibly’ improved by caffeine in the PM_{CAF} trial when compared to PM_{PLA}, but all other conditions were ‘likely’ trivial regarding PM performance and caffeine. Caffeine ‘possibly’ increased PM_{CAF} torque more than AM_{CAF} torque in the 30EXT condition when compared to PLA. All other conditions from AM vs. PM comparison revealed trivial or ‘unclear’ findings.

Trained Subjects

AM_{CAF} performance was ‘unclear’ or ‘likely’ trivial for all conditions when compared to AM_{PLA} for trained subjects. For PM_{CAF} compared to PM_{PLA}, trained subjects ‘possibly’ improved with caffeine for 30EXT, ‘possibly’ were harmed by caffeine for 240FLX, and the remaining conditions were ‘unclear’ or ‘likely’ trivial. All conditions were ‘unclear’ regarding AM_{CAF} vs. PM_{CAF} when compared to PLA.

Untrained Subjects

Caffeine supplementation ‘possibly’ benefited in the AM_{CAF} trial over the AM_{PLA} trial for the 30EXT condition for untrained subjects. All other conditions for AM_{PLA} vs. AM_{CAF}, PM_{PLA} vs. PM_{CAF}, and AM_{CAF} vs. PM_{CAF} when compared to PLA were ‘unclear’ for caffeine ingestion in untrained subjects.

Training Status

All conditions were 'unclear' regarding caffeine and training status in both the AM and PM trials.

DISCUSSION

The purpose of the current study was to investigate how time-of-day and training status consumption influences the ergogenic effects of caffeine for both 3-km TT performance and peak muscle strength. We observed several key findings related to both time-of-day and training status. Specifically, caffeine increased 3-km TT performance in the AM more than in the PM (all subjects and trained subjects). Caffeine also improved cycling performance for the untrained in both the AM and PM, but benefit for trained was ‘likely’ in the AM and ‘unclear’ in the PM. Caffeine intake also benefited 3-km performance more among untrained subjects, compared to their trained counterparts. Peak muscle force data was less conclusive, as almost all non-trivial and ‘unclear’ findings were with the total subject pool. Additionally, all data regarding peak strength – except for the total subjects during the 30EXT condition – showed no interaction between time-of-day and caffeine performance.

Consistent with our general hypothesis, caffeine enhanced 3-km TT performance among trained subjects in the morning but not the evening. This may be related to the slower time trial performances in the morning compared to the evening, in the absence of caffeine. Research has documented morning deficits in somatic control and performance in both trained and untrained subjects (3, 20, 24, 28). In support of our data, both Mora-Rodríguez et al. and Pataky et al. observed caffeine supplementation in strength trained and recreationally trained cyclist, respectively. AM caffeine supplementation returned performance to the level of, but not beyond both PM trials with supplementation, indicating an attenuation these deficits (23, 25). Unlike the trained subjects, untrained subjects ‘likely’ rode faster with caffeine in both AM and PM, compared to PLA. While the time-of-day

interaction for the untrained group was ‘unclear’, subjects appeared to experience more of a caffeine benefit in the morning ($5.5\% \pm 4.3$) than in the evening ($2.9\% \pm 2.6\%$). Slower AM_{PLA} performances when compared to PM_{PLA} may be culpable, but both AM_{CAF} and PM_{CAF} achieved the same finishing times. While attenuation of morning deficits may explain some of these findings, this cannot completely explain the increase in PM_{CAF} from PM_{PLA} . Though speculative, improved pacing patterns and time-to-fatigue in the untrained subjects may have resulted in improvements in PM_{CAF} performance as caffeine has previously been found to increase time-to-fatigue (12). Untrained subject pacing patterns may not be as optimized as trained, leading to premature fatigue. Increased time-to-fatigue would allow for less influence of suboptimal pacing, leading to larger increases in AM_{CAF} performance, as well as improvements in PM_{CAF} performance.

Our data indicate that untrained subjects respond more favorably to caffeine supplementation than trained subjects. Porterfield et al. did not observe any benefit from caffeine in either trained nor untrained. However, these investigators did report trends suggesting greater increases in cycling time-to-fatigue in untrained (10.2%) vs. trained (3.1%) (27), which partially supports our findings. Further, while Collomp et al. reported that trained but not untrained individuals benefited from caffeine supplementation (6), our data suggest that untrained subjects ‘likely’ benefited more from caffeine than trained for 3-km TT performance with both morning and evening supplementation. However, our data does support previous meta-analysis data with untrained benefiting more from caffeine than trained (31). Furthermore, we did not observe any obvious effect of caffeine on peak muscle force nor an influence from training status as previously seen by Brooks et al. (5). Differences in the current findings and those of Collomp et al. may be due to exercise

mode. Collomp et al. examined swimmers, which has a large upper-body component to it, whereas the current study and the majority of previous literature used lower-body dominant exercise modes. We question the ability of untrained swimmers to translate increases in power output to faster swimming times, as swimming is a less trained, more technical biomechanical movement than running or cycling.

Overall, peak muscle strength is largely unaffected by caffeine except 'possibly' at slow speeds of contraction for knee extension. These data agree with some findings in the literature showing no benefit from caffeine in muscular performance (1, 2, 35), but are in opposition to the majority of studies which suggest an ergogenic benefit from caffeine supplementation (4, 10, 11, 23, 29, 35). Timmins et al. found caffeine elicited a smaller magnitude of effects in muscle groups with smaller mass (29), which may explain the trivial and 'unclear' results found as hamstring muscle mass is smaller than quadriceps mass (9). Moreover, a recent meta-analysis revealed that caffeine effects are more noticeable in knee extensor data ($ES = 0.37$) when compared to other muscle groups ($ES = 0.06$) (31). Further, as angular velocity increases, so do the number of 'unclear' and trivial analyses, indicating there may be a velocity interaction. As velocity increases, the recruitment of faster motor units (fast-twitch muscle) increases (30), indicating a greater percentage of the working muscle to be fast-twitch and less to be slow-twitch contribution (18). Jacobson et al. supports this idea as isokinetic data on elite athletes showed significant findings for caffeine consumption during knee extensor, and not knee flexor data, with increased performance at slower angular velocities (19). Lynge and Hellsten found greater adenosine receptor density in slow-twitch muscle, which would suggest caffeine to benefit slow-twitch fibers as caffeine's mechanism of action is adenosine antagonism (22).

Therefore, as velocity increases, it is possible that the fiber type that benefits from caffeine supplementation would represent a smaller percentage of recruited motor units, resulting in less of an effect. This would explain why greater results were noted in the 3-km data while peak strength data, with the exception of 30EXT, was largely unaffected.

One of the limitations of the current study is the relatively small sample size for parallel comparisons. Large numbers of ‘unclear’ semantic inferences may be caused by sensitivity of the data to individual differences and outliers (no subject data surpassed 3 SD above the mean) due to the small sample size. Further studies should aim to increase sample size to allow for more definitive results regarding any training status differences, as there is a great deal still unknown. Despite these limitations, our data still supports caffeine attenuating AM deficits in trained, and more benefit in untrained when compared to trained.

The findings of this study support the idea that time-of-day and training status influences caffeine ergogenics. Our data supported previous data regarding both benefit from caffeine in AM performance for time-of-day, as well as a larger ergogenic effect in untrained compared to trained individuals. The research on both factors is still sparse, and more information is needed before personalized prescription for optimal performance outcomes can be made. Due to the equivocal results of the current study and literature on training status and caffeine, subsequent studies should observe larger sample sizes for training status. Additionally, research should inspect the possibility of a time-of-day \times genetic interaction which has been proposed by previous studies and the current, as well as try to pinpoint the conditions in which caffeine is ergogenic for muscular performance.

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Table 1. Descriptive Data

	Cycling		
	TOT (n = 20)	T (n = 7)	U (n = 7)
Height (cm)	175.4 ± 7.4	175.4 ± 6.9	175.9 ± 8.3
Weight (kg)	73.6 ± 10.9	70.2 ± 10.7	76.0 ± 10.6
Age (yrs)	24.6 ± 7.7	24.7 ± 8.4	24.3 ± 8.8
VO _{2max} (ml/kg/min)	57.2 ± 9.3	64.8 ± 7.9	49.2 ± 5.6
Cycling (hrs/week)	4.9 ± 2.8	7.5 ± 1.8	2.4 ± 0.8
Resistance (hrs/week)	3.9 ± 6.5	4.9 ± 8.2	3.1 ± 3.3
Caffeine (mg/week)	648 ± 869	1101 ± 1075	339 ± 529

Descriptive data reported as Mean±SD. TOT = Total Subjects, T = Trained, U = Untrained

Table 2. 3-km, 30EXT, and 30FLX Data

Variable	Treatment	All Subjects				Trained				Untrained				Trained vs. Untrained CAF vs. PLA
		PLA	CAF	CAF vs. PLA	AM vs. PM	PLA	CAF	CAF vs. PLA	AM vs. PM	PLA	CAF	CAF vs. PLA	AM vs. PM	
3-km Power (W)	AM	246.35 ±57.88	258.05 ±51.97	7.16±4.38 (5.64±4.42) 99/1/0 Very Likely	4.23±5.27 (1.71±5.34) 85/10/5 Likely	272.00 ±61.06	278.71 ±62.46	4.48±4.55 (2.59±3.99) 91/6/3 Likely	7.54±8.04 (3.54±6.31) 91/4/4 Likely	203.43 ±38.69	230.43 ±39.93	13.75±22.49 (13.86±12.08) 86/3/11 Likely	4.99±24.08 (5.94±13.32) 62/5/33 Unclear	-9.27±22.95 (-11.27±12.40) 21/5/75 Unclear
	PM	248.25 ±49.80	258.45 ±53.70	2.93±3.14 (3.93±3.20) 85/13/2 Likely		277.71 ±55.01	277.00 ±66.31	-3.06±7.31 (-0.95±5.46) 16/13/70 Unclear		215.71 ±26.02	234.71 ±41.94	8.77±11.28 (7.92±7.62) 90/4/6 Likely		-11.82±12.54 (-8.86±8.74) 5/3/93 Likely
30 EXT	AM	192.65 ±39.05	194.10 ±47.51	0.91±4.37 (-0.25±4.26) 12/85/3 Likely (trivial)	-4.34±5.52 (-6.19±5.35) 1/46/53 Possibly	214.68 ±28.88	210.94 ±36.56	0.29±9.65 (-2.18±6.61) 24/56/21 Unclear	-6.44±13.65 (-10.35±8.80) 10/28/62 Unclear	190.55 ±25.07	199.80 ±32.28	4.38±7.41 (4.44±4.00) 53/43/3 Possibly	-0.09±15.49 (0.09±8.35) 32/36/32 Unclear	-4.09±11.24 (-6.62±7.29) 11/39/50 Unclear
	PM	190.74 ±38.67	202.29 ±41.84	5.24±3.57 (5.94±3.45) 72/28/0 Possibly		202.92 ±30.89	219.07 ±25.70	6.73±11.18 (8.36±6.85) 68/27/5 Possibly		197.51 ±22.71	207.56 ±36.44	4.47±14.39 (4.36±7.75) 52/34/14 Unclear		2.26±16.84 (4.00±9.56) 43/32/26 Unclear
30 FLX	AM	146.35 ±31.46	142.36 ±34.98	-2.69±6.11 (-3.24±5.67) 4/62/34 Possibly	-0.50±6.81 (-1.72±6.38) 12/70/18 Unclear	162.39 ±21.56	159.50 ±23.08	-1.20±8.54 (-1.94±4.85) 13/61/26 Unclear	-0.74±11.13 (-1.26±6.24) 22/49/29 Unclear	143.12 ±22.39	140.37 ±24.87	-2.54±21.07 (-2.20±11.47) 28/28/44 Unclear	-1.86±23.05 (-0.61±13.19) 32/26/43 Unclear	1.34±22.17 (0.26±11.92) 40/27/32 Unclear
	PM	145.69 ±31.84	143.65 ±31.58	-2.19±3.26 (-1.52±3.16) 0/84/16 Likely (trivial)		156.55 ±26.69	155.04 ±21.93	-0.46±8.50 (-0.68±4.70) 16/63/21 Unclear		147.08 ±18.66	146.45 ±29.03	-0.67±12.36 (-1.59±8.27) 23/47/30 Unclear		0.21±13.99 (0.90±8.98) 30/41/29 Unclear

Values for Placebo (PLA) and Caffeine (CAF) reported as Mean±SD. Comparison values reported as adjusted (actual in parenthesis) Mean±90% CI for differences between change scores (i.e. AM vs. PM), % likelihoods of positive effect/trivial effect/negative effect and semantic inferences.

Table 3. 120EXT, 120FLX, 240EXT, and 240FLX Data

Variable	Treatment	All Subjects				Trained				Untrained				Trained vs. Untrained CAF vs. PLA
		PLA	CAF	CAF vs. PLA	AM vs. PM	PLA	CAF	CAF vs. PLA	AM vs. PM	PLA	CAF	CAF vs. PLA	AM vs. PM	
120 EXT	AM	171.34 ±31.67	171.27 ±32.99	-0.25 ±3.54 (-0.13±3.29) 4/91/6 Likely (trivial)	-1.57±4.56 (-2.00±4.29) 3/75/22 Likely (trivial)	185.27 ±31.06	186.52 ±30.33	1.07±6.20 (0.78±3.46) 22/69/9 Unclear	0.67±7.12 (-0.38±4.13) 23/62/15 Unclear	171.11 ±22.71	173.60 ±27.64	1.44±10.77 (1.11±6.05) 35/46/19 Unclear	-1.38±14.28 (-1.69±7.85) 27/34/39 Unclear	-0.36±11.73 (-0.33±6.57) 27/42/31 Unclear
	PM	171.70 ±29.52	174.73 ±29.18	1.32 ±3.05 (1.86±2.92) 10/90/1 Likely (trivial)		183.49 ±25.88	185.03 ±20.30	0.41±4.57 (1.16±2.84) 11/83/7 Unclear		173.22 ±23.26	178.78 ±29.81	2.81±11.08 (2.80±5.96) 44/42/14 Unclear		-2.40±11.69 (-1.65±6.32) 18/40/42 Unclear
120 FLX	AM	147.00 ±31.40	141.90 ±32.35	-4.58±5.24 (-3.74±5.00) 1/43/56 Possibly	-3.28±5.94 (-2.69±5.65) 2/59/39 Possibly	157.31 ±22.08	156.72 ±18.80	-1.45±4.77 (-0.18±4.57) 3/81/15 Likely (trivial)	-0.40±5.82 (0.98±5.29) 9/78/13 Unclear	147.43 ±30.92	137.59 ±25.61	-6.09±23.11 (-6.37±12.42) 21/22/57 Unclear	-3.50±24.98 (-3.84±13.43) 29/23/48 Unclear	4.64±23.60 (6.20±12.92) 51/24/25 Unclear
	PM	147.61 ±32.51	146.04 ±32.43	-1.30±3.04 (-1.04±2.84) 0/94/6 Likely (trivial)		156.16 ±23.62	154.26 ±22.28	-1.04±4.11 (-1.16±3.44) 3/88/9 Likely (trivial)		153.99 ±28.46	150.89 ±32.96	-2.59±12.92 (-2.54±6.95) 17/42/41 Unclear		1.54±13.22 (1.38±7.42) 35/43/22 Unclear
240 EXT	AM	154.59 ±28.61	158.43 ±33.59	2.02±3.14 (1.95±2.91) 18/81/0 Likely (trivial)	1.27±4.62 (0.15±4.50) 19/77/4 Likely (trivial)	161.08 ±22.62	165.26 ±28.98	3.81±9.81 (2.21±5.97) 51/40/9 Unclear	4.45±11.41 (1.82±6.92) 55/34/11 Unclear	156.57 ±33.40	158.44 ±35.18	1.26±9.76 (0.88±5.63) 32/50/18 Unclear	-4.07±15.12 (-3.65±9.32) 19/30/52 Unclear	2.54±12.79 (1.32±7.58) 44/36/20 Unclear
	PM	157.85 ±29.86	159.99 ±26.08	0.75±3.55 (1.80±3.58) 9/89/2 Likely (trivial)		166.08 ±30.33	166.23 ±27.07	-0.64±7.49 (0.39±4.53) 15/62/23 Unclear		158.73 ±30.29	164.80 ±23.26	5.33±13.12 (4.54±8.25) 59/30/11 Unclear		-5.97±14.25 (-4.15±8.88) 12/27/61 Unclear
240 FLX	AM	143.41 ±33.58	140.54 ±36.89	-2.77±6.69 (-2.69±6.20) 4/63/33 Possibly	-2.14±7.57 (-2.94±7.11) 7/65/29 Unclear	152.35 ±12.96	151.95 ±16.11	-1.29±5.99 (-0.41±3.65) 5/78/16 Unclear	1.45±8.12 (1.31±5.00) 24/65/10 Unclear	145.04 ±37.96	141.52 ±36.68	-2.60±26.82 (-2.48±14.35) 31/25/45 Unclear	-3.07±28.02 (-2.49±15.19) 31/23/46 Unclear	1.31±27.48 (2.07±14.81) 41/25/34 Unclear
	PM	143.95 ±33.31	144.51 ±34.20	-0.64±3.85 (0.25±3.77) 2/94/5 Likely (trivial)		154.69 ±19.86	152.11 ±20.63	-2.74±6.43 (-1.71±3.99) 4/66/30 Possibly		148.92 ±33.65	149.97 ±37.10	0.47±11.75 (0.01±6.78) 26/53/21 Unclear		-3.21±12.63 (-1.72±7.42) 14/44/42 Unclear

Values for Placebo (PLA) and Caffeine (CAF) reported as Mean±SD. Comparison values reported as adjusted (actual in parenthesis) Mean±90% CI for differences between change scores (i.e. AM vs. PM), % likelihoods of positive effect/trivial effect/negative effect and semantic inferences.

Figure 1. 3-km Time Trial Performance

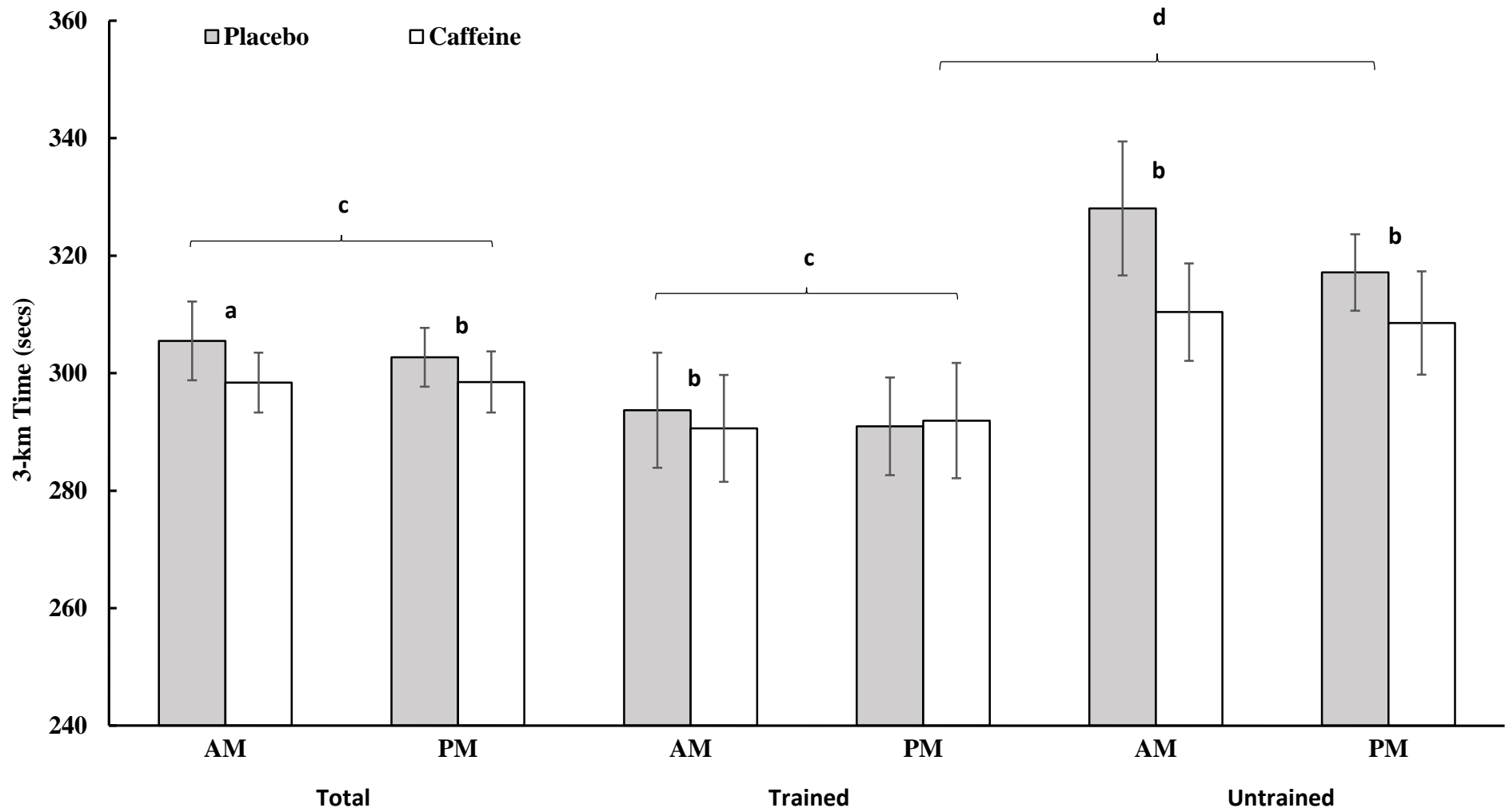


Figure 2. Percent Change 3-km Time

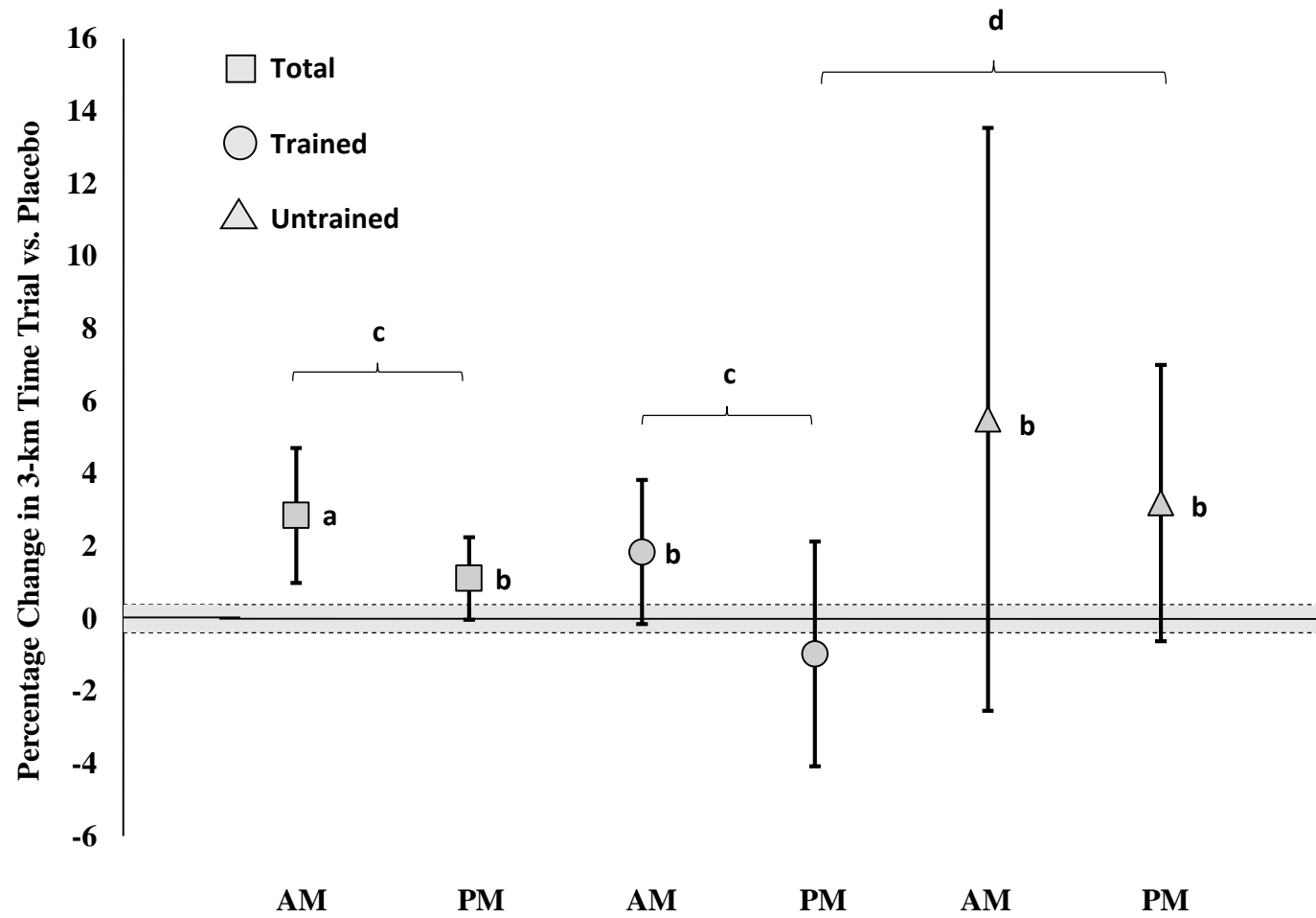


FIGURE LEGENDS

Figure 1.

Effects of Caffeine during 3-km Time Trial performance in seconds: Bars depict mean time in seconds (\pm SE). (a) Signifies a ‘very likely’ improvement between placebo and caffeine. (b) Signifies a ‘likely’ improvement between placebo and caffeine. (c) Signifies a ‘likely’ increased benefit in the AM over PM. (d) Signifies a ‘likely’ increased benefit in the untrained over trained.

Figure 2.

Effects of Caffeine during 3-km Time Trial performance in seconds: Markers indicate mean percent change from placebo and bars depict \pm 90% confidence interval. Dashed lines signify threshold value of a meaningful effect ($0.3 \times CV$). (a) Signifies a ‘very likely’ improvement between placebo and caffeine. (b) Signifies a ‘likely’ improvement between placebo and caffeine. (c) Signifies a ‘likely’ increased benefit in the AM over PM. (d) Signifies a ‘likely’ increased benefit in the untrained over trained.

James Madison University
Department of Kinesiology
Consent for Investigative Procedure

I, _____, hereby agree on _____ (date) to participate in the research project conducted by Christopher J. Womack, Ph.D., Nicholas D. Luden, Ph.D., James Boyett, and Gabe Giersch from James Madison University titled *The Effect of Genetics, Training Status, and Time-of-day Consumption on the Ergogenic Properties of Caffeine*.

The purpose of this study is to determine whether or not genetics influences the effects of caffeine supplementation on performance. Additionally, this study aims to determine whether trained individuals benefit more from caffeine supplementation than untrained during cycling and muscular strength performance. The final purpose of this study is to determine whether the time of day affects whether caffeine is beneficial for cycling performance.

Subject Responsibility

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

This study consists of seven separate exercise tests performed on both on a muscle strength device and a stationary bike (cardiovascular fitness test, two familiarization tests, and four 3km time trial tests). 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 and physical activity and complete dietary and physical activity records. The total time commitment is estimated to be less than 10 hours over the course of 4-6 weeks.

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 exhaustion is reached, determined by either: 1) your request to stop due to fatigue, or 2) inability to 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):

On two occasions, you will be asked to perform peak skeletal muscle function testing 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, followed by a strength test. This will consist of two warm-up repetitions followed by two maximal exertion isokinetic peak torque measurements at 30, 120, and 240 degrees/sec. One set consists of two sub-maximal repetitions immediately followed by two maximal repetitions, with sixty seconds of rest between two sets at the same velocity. Once all exertions are completed at a given velocity, 60 seconds of rest will be given before proceeding to the next velocity. After strength testing is complete, you will move to a stationary bicycle to complete the 3-km time trial. You will be encouraged to treat the time trial like a competition.

Experimental Trials (n=4; 120 minutes each):

You will report to the laboratory 60 minutes prior to exercise testing. You will rest in a seated position for 5 minutes, after which a blood sample will be obtained for measurement of caffeine/caffeine metabolite levels (one of the samples will also be used to extract DNA for genotyping). Immediately following the blood draw, you will ingest either placebo or caffeine capsules, after which you will wait for ~60 minutes in the laboratory until exercise testing. Immediately prior to exercise testing, a second blood sample will be obtained for the measurement of caffeine/caffeine metabolite levels. Following the blood draw, you will complete the peak muscle function test and 3-km time trial described above.

Supplementation Protocol:

No supplementation will be given during the familiarization trials. You will be randomly assigned a treatment order. Treatments will be: 1. Placebo capsule containing flour administered 1hr prior to exercise at 8:00am 2. Caffeine capsule containing 6 mg/kg bodyweight caffeine administered 1hr prior to exercise at 8:00am 3. Placebo capsule containing flour administered 1hr prior to exercise at 6:00pm 4. Caffeine capsule containing 6 mg/kg bodyweight caffeine administered 1hr prior to exercise at 6:00pm.

Dietary and Exercise Controls:

You will be asked to record food intake 24 hours prior to the first familiarization trial. You will then be given a copy of the dietary log and asked to replicate food intake for 24 hours prior for each subsequent trial. Additionally, you will be asked to abstain from alcohol and caffeine consumption for 24 hours prior to testing in all trials. Additionally, you will be asked to arrive at the laboratory in a fasted state (no food intake within the past 4 hours). Finally, 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 be asked to maintain consistent physical activity habits before all trials.

DNA Sampling:

We will extract a sample of your DNA from one of your blood samples. DNA and blood samples will be stored in our laboratory freezer for at least 3 years to allow us to conduct follow-up studies in the event that new discoveries are made related to DNA and caffeine metabolism. Your sample will be coded so that nobody except the primary investigators can identify which sample is yours. The DNA testing will involve determining sequences of DNA for specific genes that are related to caffeine metabolism. We will not use this DNA for any other purpose. The results of this genetic testing will only be available to the primary investigator and you. These results will not be made public and will be stored in a locked file cabinet.

Risks/Benefits:

Skeletal Muscle Function

The risks of 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.

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.

Blood Sampling

The risks of blood sampling using venipuncture include possible mild bruising, and the risk of transfer of blood-borne pathogens, as well as possible risks of infection or skin irritation. These risks are considered to be minimal, and all safety precautions for handling blood samples will be followed according to OSHA protocols, including: investigators will wear latex gloves at all times during blood sampling and testing. A sharps container lined with a biohazard bag will be used for all sharp objects involved in the blood sampling; all other materials (i.e. gloves, gauze pads, etc.) used during the sampling will be put in a separate waste disposal unit lined with a biohazard bag. All investigators who will be involved in blood draws (and handling of blood) have been trained in these phlebotomy techniques, and completed JMU blood-borne pathogen training. The total amount of blood obtained

during this study is approximately 24 ml. For reference, this amount is ~ 6% of a can of soda, or 5% of the amount given when donating blood in a single session (approximately 1 pint, or 473 ml).

Caffeine Ingestion

The risks and side effects associated with caffeine supplementation include: rapid heart rate, elevated blood pressure, headache, nausea, vomiting, restlessness, agitation, and anxiety.

Performance incentive:

The top 5 trained performers (fastest finishing placebo time, use of caffeine would necessitate an unfair advantage to possible genetic responders) will be entered into a drawing to win \$150. Trained individuals in the top 6-10 placebo time will be entered into a drawing to win \$75. An identical incentive method will be used for the untrained subjects (1 \$150 and 1 \$75).

Confidentiality

The results of this research will be presented at conferences and published in exercise science journals. 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 will be made available to you upon 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. Your right to withdraw includes the right to request that your DNA and blood samples be discarded at any time. You should be aware that the DNA sample is subject to court subpoena. To dispose of your samples, your samples will be rinsed down a chemical drain in our laboratory or will be disposed of in a biohazard container. Again, your sample will not be identifiable without the coding document that will be locked away in a filing cabinet.

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 Nicholas D. Luden, Ph.D. at ludennd@jmu.edu or by phone at 540-568-4068

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

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**.

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)
-

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 & Caffeine Habits

Age: _____ years

Height _____ Weight _____

Typical Exercise Habits over the Past 3-6 Months:

Average number of days of cycling per week _____

Average number of hours of cycling per week _____

Briefly describe your cycling habits over the past 3-6 months:

Average number of days of resistance exercise/weight lifting per week _____

Average number of hours of resistance exercise/weight lifting per week _____

Briefly describe your resistance training habits over the past 3-6 months:

Do you have a muscle or joint injury/condition that precludes the completion of the cycling or muscle function protocol? If yes, please explain.

Are you allergic to wheat?

Do you have gluten intolerance?

Are you allergic to latex?

Caffeine Habits:

Please list your approximate WEEKLY intake of the following:

Cups of coffee:

Cups of tea:

Cans (12 oz) of caffeinated soda:

Servings of chocolate:

Doses of caffeinated pills (No-Doz, Vivarin, etc.):

Other caffeinated beverages not listed (please list specific drink and weekly intake):

24-HOUR DIET RECORD

Subject number _____

Date _____ Day of Week _____

Time	Food and/or Drink	Method of Preparation	Quantity Consumed	Brand Name

Adapted From: Lee RD, Nieman DC. *Nutritional Assessment*. 2nd ed. United States of America: Mosby; 1996

Physical Activity Records

Subject # _____

Trial # _____

Date: _____

Date	Type of Exercise Performed	Duration of Exercise (minutes)	Intensity of Exercise (use scale below)

Intensity Scale

- 6
- 7 Very, very light
- 8
- 9 Very light
- 10
- 11 Fairly light
- 12
- 13 Somewhat hard
- 14
- 15 Hard
- 16
- 17 Very hard
- 18
- 19 Very, very hard
- 20

TRAIT Study*Subject Information*

Subject #: _____

Demographic Information

Height: _____

Weight: _____

BIODEX

Chair position: _____

Seatback position: _____

Machine position: _____

Seat height: _____

Arm attachment position: _____

VELOTRON

Seat height: _____

Seat fore/aft: _____

Handlebar height: _____

Handlebar fore/aft: _____

TRAIT Study

VO₂ Peak Test

Velotron Settings: Information

- Seat height: _____
- Seat fore/aft: _____
- Handlebar height: _____
- Handlebar fore/aft: _____

Demographic

Height: _____
 Weight: _____
 Age: _____
 RHR: _____

VO₂ Peak Test:

- Starting workload: _____ (watts)
- Increase workload 25 watts every minute. Proceed with 1-minute stages until subject requests to stop due to fatigue or when subject is no longer able to maintain >50rpm for 10 seconds

Stage	Time	Watts	HR	RPE
1	_____	_____	_____	_____
2	_____	_____	_____	_____
3	_____	_____	_____	_____
4	_____	_____	_____	_____
5	_____	_____	_____	_____
6	_____	_____	_____	_____
7	_____	_____	_____	_____
8	_____	_____	_____	_____
9	_____	_____	_____	_____
10	_____	_____	_____	_____
11	_____	_____	_____	_____
12	_____	_____	_____	_____
13	_____	_____	_____	_____
14	_____	_____	_____	_____
15	_____	_____	_____	_____

TRAIT Study

Experimental Trial

Subject #: _____ Date: _____ Time: _____ AM/PM
 Purple/Gold

Trial: FAM1 FAM2 1 2 3 4

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

Verify that the subject is fasted (4hr food/drink, 12hr caffeine)

- Subject sits in standard position for 5 minutes
- Pre-treatment blood draw (time: _____ am/pm) * **Let clot for 30 minutes**
- Treatment given (time: _____ am/pm)
- Subject remains in laboratory for 60 minutes
- Post-treatment blood draw (time: _____ am/pm)

5 minute warm up on treadmill (3.5mph)

Biodex

Speed	Flexion PeakT	Extension PeakT
30	_____	_____
30	_____	_____
120	_____	_____
120	_____	_____
240	_____	_____
240	_____	_____

Velotron

Time trial time: _____ Avg Watts: _____

Subject: _____

Date: _____

	Trial 1		Trial 2		Trial 3		Trial 4	
Capsule	PLA	CAF	PLA	CAF	PLA	CAF	PLA	CAF
Confidence	_____		_____		_____		_____	

**Confidence rated on a scale from 1-10. 10 being 100% confidence and 1 being not confident at all

Subject #: _____

Preferred Contact: _____

Do you want an End-Of-Study Packet? Y / N ?

Appendix IX

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