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The effect of menstrual phase and oral contraceptive steroids on caffeine during cycling performance

Annette M. Lemanski
James Madison University

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The Effect of Menstrual Phase and Oral Contraceptive Steroids on Caffeine during
Cycling Performance

Annette Lemanski

A thesis submitted to the Graduate Faculty of

JAMES MADISON UNIVERSITY

In

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FACULTY COMMITTEE:
Committee Chair: Nicholas D. Luden, Ph.D.
Committee Members/ Readers:
Michael J. Saunders, Ph.D.
Christopher J. Womack, Ph.D.
David L. Wenos, Ph.D.

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Table of Contents

Acknowledgements.....	ii
Table of Contents.....	iii
List of Tables.....	iv
List of Figures.....	v
Abstarct.....	vi
I. Introduction.....	1
Background	
Significance	
Aims and Hypotheses	
II. Methodology.....	7
Subjects	
Experimental Design	
Preliminary Testing	
Supplementation	
Familiarization Trials	
Performance Trials	
Blood Analysis	
Dietary and Exercise Control	
Statistical Analysis	
III. Manuscript.....	11
Introduction	
Methodology	
Results	
Discussion	
IV. Appendices.....	34
References.....	41

List of Tables

Table 1.0 Descriptive Data.....	31
Table 2.0 3-km Performance Time and Power Output Data.....	32

List of Figures

Figure 1 Average 3-km Time Trial Performance.....	33
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ABSTRACT

PURPOSE: There is evidence that female sex hormones impact caffeine metabolism, with decreased CYP1A2 activity and diminished caffeine clearance in women with higher estrogen levels. Therefore, the objectives of this project were to determine the effects of oral contraceptives and menstrual cycle on the benefits of caffeine supplementation for cycling performance. **METHODS:** Sixteen recreationally trained female cyclists, oral contraceptive steroid (OCS) users ($n=8$, age = 21.4 ± 1.4 years, height = 168.4 ± 3.6 cm, weight = 63.6 ± 7.2 kg, $VO_{2max} = 48.0 \pm 4.0$ ml•kg⁻¹•min⁻¹) and non-users ($n=8$, age = 20.9 ± 2.1 years, height = 161.0 ± 7.7 cm, weight = 59.5 ± 9.2 kg, $VO_{2max} = 50.9 \pm 7.8$ ml•kg⁻¹•min⁻¹), completed four separate computer-simulated 3-km cycling time trials (TT). Subjects ingested either 6mg/kg of caffeine or a placebo capsule one hour prior to each trial. Magnitude based inferences were used to evaluate treatment differences. **RESULTS:** Caffeine enhanced 3-km TT performance (compared to placebo) in early and late phases of the menstrual cycle for OCS users 'possibly' and 'very likely' by $1.6 \pm 2.1\%$, $2.7 \pm 1.4\%$ and non-users 'likely' by $2.6 \pm 2.3\%$, $2.1 \pm 2.2\%$ respectively. All other comparisons for OCS users and non-users were 'unclear'. **CONCLUSIONS:** Caffeine supplementation improved 3-km TT finishing time and average power output in follicular and luteal phases of the menstrual cycle, with unclear differences between the phases for both OCS users and non-users. This indicates that menstrual status is not a significant source of variability on the ergogenic effect of caffeine supplementation on cycling performance.

Chapter One

Introduction

Caffeine (1,3,7-trimethylxanthine) is often consumed for its stimulating effects and ability to improve physical performance (6). There is good evidence that caffeine supplementation can enhance physical performance ranging from peak strength to endurance performance (4–6, 11, 16, 21, 23, 55, 57, 61, 62). Early research suggested that the ergogenic effect of caffeine on aerobic exercise stem from adrenaline-augmented free-fatty acid oxidation and glycogen sparing (13). Recently, the physiological responses to exercise altered by caffeine are suggested to be through stimulation of the central nervous system mediated by antagonizing adenosine receptor sites (51), which increases motor-unit recruitment in large muscle groups (5, 60) and reduces the rate of perceived exertion during endurance exercise (5, 6, 13, 17). It is also accepted that caffeine binds to adenosine receptors limiting the relaxation of smooth muscles and vasodilation in the visceral organs in order to increase blood to the muscles (19).

The optimal caffeine dose for performance benefits is between 3 and 6 mg of caffeine per kilogram of body mass (14, 21, 23, 57). Higher doses of ≥ 9 mg/kg do not result in further performance enhancement (21), and may even impair performance due to various side effects (i.e. tachycardia, anxiety, dizziness, nausea) (15, 23, 51). The pharmacokinetics of caffeine is predominantly regulated by hepatic cytochrome P450 (CYP1A2), the enzyme responsible for metabolizing the majority of caffeine (18, 39, 46, 47, 58, 65). Therefore, different concentration/activity levels of CYP1A2 represents a major source of variability in the pharmacokinetics of

caffeine (64), and consequently the dose-response. The enzyme-saturating dose of caffeine has been shown to be 6 mg/kg (39), suggesting that this dosage reaches the upper limit of hepatic caffeine metabolism, which is in line with the aforementioned dose-performance response relationship.

CYP1A2 is involved in the biotransformation of drugs such as caffeine, along with being involved in the bioactivation of pro-carcinogens such as estradiol (33).

Demethylation is the first step to caffeine metabolism (31). The three dimethylxanthine metabolites can be measured in plasma as done in time-concentration studies (1, 3, 31, 34, 39, 53). Of the three dimethylxanthine metabolites, paraxanthine (1-7 dimethylxanthine) is more abundant than theobromine (3,7-dimethylxanthine) and theophylline (1-3-dimethylxanthine)(31, 37); approximately 70-80% of caffeine is converted to paraxanthine (47). Less is known about paraxanthine kinetics and its potential role in facilitating beneficial effects of caffeine on exercise performance (11), however, caffeine metabolites have an even higher affinity for the adenosine receptors (12, 19), thereby plausibly having an even greater effect on performance than caffeine itself. This indicates that the rate of caffeine metabolism and consequential metabolite levels could be an important factor in the ergogenic properties of caffeine. Previous evidence observing the influence of genotype on caffeine metabolism suggests caffeine affect those who have faster caffeine metabolism to a greater degree within cycling performance, which was thought to be due to higher levels caffeine metabolites (63). This was thought to be due to caffeine metabolites. Indeed, several studies have reported that specific variations of the CYP1A2 enzyme that impact enzyme

levels are linked to caffeine-related performance and health outcomes (22, 25, 27, 43, 63).

Directly relevant to the rationale for the current project is that the vast majority of what is known about the impact on caffeine on performance and physiology has been derived from males (6, 11, 14, 20, 23, 35, 40, 54, 61, 63). Moreover, the little that is known about females in this context suggests that there may be sex specific responses to caffeine intake (10, 20, 36, 38). The rate of caffeine metabolism is not only sensitive to genetic variations of the enzyme, but also biological sex (6, 31); specifically, female sex hormones, estrogen and estradiol (1, 3, 18, 24, 48). There is an evident trend for higher plasma caffeine and lower plasma paraxanthine concentrations in women compared to men, suggesting that women metabolize caffeine slower than men (7). Women with higher estrogen levels, [such as oral contraceptive steroid (OCS) users, females in the luteal phase, or pregnant], have been found to have decreased CYP1A2 activity and decreased caffeine clearance (3, 34, 48). Despite the varying clearance rates across the menstrual cycle, currently nothing is known about whether or not this impacts the performance response to caffeine intake. Therefore, one of the primary aims of this study was to test the hypothesis that there will be a magnified effect of caffeine on performance in the follicular phase compared to the luteal phase.

The use of hormonal contraceptives is another condition when estrogen levels are elevated, (56), possibly effecting the ergogenic effect of caffeine through dampening the rate of caffeine metabolism. OCS's can diminish elimination kinetics of several drugs, including caffeine (53). OCS's containing ethinyl-estradiol/estradiol/estrogen

are commonly used by young females as birth control and as treatment for female athlete triad to prevent bone loss (52), and to treat menstrual disturbances (32, 52). Chronic OCS use has been previously associated with impaired metabolism of caffeine (1, 3, 46, 49, 53, 59). There is evidence of delayed caffeine clearance in as little as the first two weeks of starting OCS use (53). Caffeine half-life was significantly prolonged (94% longer) in women on OCS as compare to nonusers (46). A 3-5.5 hour half-life of caffeine is common, however women on OCS's may experience increased half-life to approximately 10 hours (7, 34). It is commonly reported that OCS's inhibit CYP1A2 activity (1, 3, 26, 49, 50, 59). Whether the mechanism of CYP1A2 inhibition by estradiol is due to competition or down-regulation of enzyme synthesis is unclear (8, 48). OCS's appear to modify the metabolism of caffeine by reducing the formation of paraxanthine, which is the primary pathway of caffeine metabolism, possibly limiting its physiological effects (50).

The rate at which caffeine is absorbed by the gastrointestinal tract and reaches peak concentrations in the blood (\approx 60 minutes) is similar for OCS users and non-users (1, 39, 46). This finding indicates that absorption of caffeine and availability of plasma caffeine is not altered by OCS use, solely the breakdown of caffeine into its metabolites is affected. Regardless of the implicit compounding effects of OCS use on caffeine metabolism, there is no current evidence of the interactive effect OCS use has on the ergogenic effect of caffeine. Therefore, the second aim of this project is to test the influence of OCS use on the performance response to caffeine supplementation compared to non-OCS users.

Significance:

Despite good evidence that caffeine enhances performance, data among female participants is sparse. Menstrual status is acknowledged as a contaminate of caffeine metabolism, but is avoided within performance studies as females are compared within the same phase, often the early follicular phase (10, 56). Moreover, the effect that oral contraceptives have on caffeine metabolism has led to the exclusion of oral contraceptive users in studies examining the effects that caffeine and caffeine metabolism have on performance. To our knowledge, nothing is known about how oral contraceptive use may influence the ergogenic effect of caffeine. The purpose of this study is to determine the effect of oral contraceptive use on caffeine metabolism and performance following caffeine consumption. Additionally, this study will yield further insight to the factors that can effect performance improvement in regards to caffeine ingestion including oral contraceptives and caffeine metabolism.

Aims and Hypotheses:

Aim 1: To determine if the phase of the menstrual cycle mediates the effects of caffeine supplementation on 3k cycling performance time.

Hypothesis 1: Caffeine will elicit a greater improvement in cycling performance time during the follicular phase compare to the luteal phase.

Aim 2: To determine if oral contraceptive steroid use mediates the effects of caffeine consumption on 3k cycling performance times.

Hypothesis 2: Caffeine will elicit a greater improvement in cycling performance time in non-oral contraceptive users compared to the oral contraceptive users.

Chapter Two

Methodology

Subjects

Thirty recreationally trained female cyclists from James Madison University and the greater Harrisonburg/ Rockingham County area will be included in this study. Inclusion criteria include: female, 18-35 years of age, non-smokers, cycle ≥ 30 minutes at least two days a week, and $VO_{2max} \geq 40$ ml/kg/min. Further, subjects must be eumenorrhoeic with menstrual cycle length between 21-35 days over the past 6 months. Subjects who are not on any form of contraception will be assigned to the control group (n=15), and the subjects on monophasic or tri-phasic oral contraceptives will be assigned to the OCS user group (n=15). In the OCS user group, subjects will be limited to monophasic oral contraceptives or tri-phasic oral contraceptives containing ethinyl estradiol and levonorgestrel for at least 6 months (9). Subjects will be fully informed about the experimental procedures and possible risks before given written informed consent. Informed consent includes information of the experimental procedures and risks prior to participation. James Madison University Institutional Review Board approved this study.

Experimental Design

Subjects will report to the laboratory for two familiarization trials and four experimental trials. Two experimental trials (1 placebo and 1 caffeine) will be conducted during the luteal phase and two experimental trials (1 placebo and 1 caffeine) will be performed in the follicular phase of the menstrual cycle. The follicular phase trials will be performed from days 7 to 13 of the menstrual cycle,

and the luteal phase trials will be performed from days 18 to 24. Each treatment trial will be performed in the morning between 6:00am and 10:00am. Trials will be separated by at least 48 hours.

Preliminary Testing

Following height and body mass measurements, VO_{2max} will be assessed during an incremental test on a bicycle ergometer (Velotron™ cycle ergometer, Racermate, Inc. Seattle, WA, USA). Workload will begin at 50 W and will be increased in 25 W increments every minute until volitional fatigue, or pedaling rate drops below 50 rpm for >10 seconds. Breath samples will be collected and oxygen consumption will be analyzed with a Moxus® Modular Metabolic System (AEI Technologies, Pittsburgh, PA, USA). VO_{2max} will be determined as the highest 30 second mean oxygen uptake value.

Supplementation

In a crossover, double-blind design, subjects will be provided caffeine treatment or placebo. Anhydrous caffeine at 6mg/kg body weight will be measured into opaque gelatin capsules. For placebo pills, gluten-free rice flour will be put into opaque gelatin capsules. Supplementation will be ingested with water, 60 minutes prior to the performance trial.

Familiarization Trials

Two familiarization trials will be completed on a Velotron™ cycle ergometer before each subjects begins experimental trials. A 5-min warm-up will be performed before all 3-km time-trials. Subjects will be instructed to approach the time trial as a competition, emphasizing the importance to perform each trial as a race and put

forth 100% effort. All trials will be completed without verbal feedback and only elapsed distance will be displayed. The familiarization trial protocol will be identical to the performance trials with the exception of caffeine/placebo supplementation.

Performance Trials

Subjects warmed up on a treadmill for 5 minutes at 3.5 mph, followed by the 3k-time trial on a Velotron cycle ergometer. Performance will be recorded in time to complete and average watts for the 3-km time trial.

Dietary and Exercise Control

Subjects will complete 24-hour food logs and be instructed to replicate their diet for 24 hours prior to each subsequent trial. Subjects will be asked to refrain from any food or drink (besides water) for four hours before and from caffeine and alcohol for 12 hours before each trial. The OCS user group will be required to authenticate intake of their respective oral contraceptive within the past 24 hours. In addition, 48-hour physical activity logs will be completed prior to each trial, and will be instructed to maintain consistent exercise habits between trials. Subjects will be asked to refrain from any vigorous physical activity 12 hours prior to each trial.

Statistical Analysis

Magnitude-based inferences about the data will be derived using methods described by Hopkins and colleagues (29). 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 1.7 watts and 0.4% or 1.4 seconds in the current project (45).

A published spreadsheet by Hopkins (28) will then be used to determine the likelihoods of the true treatment effect of the population reaching the substantial change threshold ($0.2 \times CV$); these 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 were used to classify the effects of treatment on performance. Specifically, if the percent chance of the effect reaching the substantial change threshold was $<25\%$ and the effect was clear, it was classified as “trivial.” If the percent chance of the effect reaching the substantial change threshold for benefit exceeded 25% but the chance for harm was $>0.5\%$ the effect was classified as unclear. An exception to the 0.5% chance of harm criterion was made if the benefit/harm odds ratio was >66 , in which case the effect was interpreted as clear and an inference was assigned.

Chapter Three

Manuscript

**The Effect of Menstrual Phase and Oral Contraceptive Steroids on Caffeine
during Cycling Performance**

Authors: Annette M. Lemanski, Nicholas D. Luden, Michael J. Saunders,
Christopher J. Womack, David L. Wenos

Institution: James Madison University, Harrisonburg VA, 22807

Contacts: Annette Lemanski, lemansam@dukes.jmu.edu
Michael Saunders, saundemj@jmu.edu
Christopher Womack, womackcx@jmu.edu
David Wenos, wenosdl@jmu.edu

Address of Correspondence:

Nicholas D. Luden, Ph.D.
Department of Kinesiology
James Madison University
Harrisonburg VA, 22807
Phone: (540) 568-4069
Fax: (540) 568-3338
Email: ludennd@jmu.edu

ABSTRACT

PURPOSE: There is evidence that female sex hormones impact caffeine metabolism, with decreased CYP1A2 activity and diminished caffeine clearance in women with higher estrogen levels. Therefore, the objectives of this project were to determine the effects of oral contraceptives and menstrual cycle on the benefits of caffeine supplementation for cycling performance. **METHODS:** Sixteen recreationally trained female cyclists, oral contraceptive steroid (OCS) users (n=8, age = 21.4 ± 1.4 years, height = 168.4 ± 3.6 cm, weight = 63.6 ± 7.2 kg, $VO_{2max} = 48.0 \pm 4.0 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) and non-users (n=8, age = 20.9 ± 2.1 years, height = 161.0 ± 7.7 cm, weight = 59.5 ± 9.2 kg, $VO_{2max} = 50.9 \pm 7.8 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), completed four separate computer-simulated 3-km cycling time trials (TT). Subjects ingested either 6mg/kg of caffeine or a placebo capsule one hour prior to each trial. Magnitude based inferences were used to evaluate treatment effects. **RESULTS:** Caffeine enhanced 3-km TT performance (compared to placebo) in early ('possibly') and late ('very likely') phases of the menstrual cycle for OCS users by 1.6 ± 2.1% and 2.7 ± 1.4% respectively. Likewise, caffeine enhanced 3-km TT performance in early and late ('likely') phases of the menstrual cycle for non-users by 2.6 ± 2.3% and 2.1 ± 2.2%. All other comparisons for OCS users and non-users were 'unclear'. **CONCLUSIONS:** Caffeine supplementation improved 3-km TT finishing time and average power output in follicular and luteal phases of the menstrual cycle, with unclear effects between the phases for both OCS users and non-users. This indicates that menstrual status is not a significant source of variability on the ergogenic effect of caffeine supplementation on cycling performance.

INTRODUCTION

Caffeine is commonly consumed for its stimulating effects on the central nervous system and ability to improve physical performance (6). There is good evidence that caffeine supplementation can enhance physical performance ranging from peak strength to prolonged endurance performance (~150 minutes)(4–6, 11, 16, 21, 23, 55, 57, 61, 62), with optimal dosing falling somewhere between 3 and 6 mg of caffeine per kilogram of body mass (14, 21, 23, 57).

Caffeine metabolism is predominantly regulated by hepatic cytochrome P450 (CYP1A2), the enzyme responsible for metabolizing the majority of caffeine (18, 39, 46, 47, 58, 65). Therefore, different concentrations/activity levels of CYP1A2 represents a major source of variability in the pharmacokinetics of caffeine (64). Demethylation is the first step to caffeine metabolism, which is mostly metabolized to form paraxanthine (31). Less is known about paraxanthine kinetics and its potential role in facilitating beneficial effects of caffeine on exercise performance (11). However, caffeine metabolites have an even higher affinity for the adenosine receptors, which stimulate the central nervous system (12, 19), thereby plausibly having an even greater effect on performance than caffeine itself.

The rate of caffeine metabolism is not only sensitive to genetic variations of the enzyme, but also biological sex (6, 31). Specifically, female sex hormones, estrogen and estradiol influence caffeine levels following supplementation (1, 3, 18, 24, 48). There is an evident trend for higher plasma caffeine and lower plasma paraxanthine concentrations in women compared to men, suggesting that women metabolize caffeine slower than men (7). Moreover, women with higher estrogen levels, [such

as oral contraceptive steroid (OCS) users, females in the luteal phase, or during pregnancy], have been found to have lower CYP1A2 activity and decreased caffeine clearance (3, 34, 48). Whether the mechanism of CYP1A2 inhibition by estrogen is due to competition or down-regulation of enzyme synthesis is unclear (8, 48). Despite the varying clearance rates across the menstrual cycle, currently nothing is known about whether or not this impacts the performance response to caffeine intake. Therefore, one of the primary aims of this study was to test the hypothesis that there will be a magnified effect of caffeine on performance in the follicular phase compared to the luteal phase.

In addition to menstrual phase, estrogen levels are also elevated with certain types of oral contraceptives, (56), conceivably altering the ergogenic effect of caffeine via an attenuated rate of caffeine metabolism. Chronic OCS use has been previously associated with impaired metabolism of caffeine (1, 3, 46, 49, 53, 59), and it is commonly reported that OCS's inhibit CYP1A2 activity (1, 3, 26, 49, 50, 59). OCS's appear to modify the metabolism of caffeine by reducing the formation of paraxanthine, which is the primary pathway of caffeine metabolism, possibly limiting its physiological effects (50). There is no current evidence of the interactive effect OCS use has on the ergogenic effect of caffeine. Therefore, the second aim of this project is to test the influence of OCS use on the performance response to caffeine supplementation compared to non-OCS users.

METHODOLOGY

Subjects

Twenty-eight healthy, recreationally trained female cyclists (15 OCS users, 13 non-users) from James Madison University and the greater Harrisonburg/Rockingham County area volunteered for the study. However, seven OCS users and five non-users withdrew from the study due to injuries, Lyme disease diagnosis, switching birth control prescription, seasonal flu, vasovagal syncope, or scheduling difficulties.

Inclusion criteria included: female, 18-35 years of age, non-smokers, cycle \geq 30 minutes at least two days a week, and $VO_{2max} \geq 40.0 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. Further, subjects were required to be eumenorrhoeic with menstrual cycle length between 21-35 days over the past 6 months. Descriptive data are shown in *Table 1*. Subjects who were not on any form of contraception were assigned to the control group (n=8), and the subjects on monophasic or tri-phasic oral contraceptives were assigned to the OCS user group (n=8). In the OCS user group, subjects were limited to monophasic oral contraceptives or tri-phasic oral contraceptives containing levonorgesterel and ethinyl-estradiol for at least 6 months to ensure consistent levels of estradiol (9).

Subjects were fully informed about the experimental procedures and possible risks before given written informed consent. Informed consent included information of the experimental procedures and risks prior to participation. James Madison University Institutional Review Board approved this study.

Experimental Design

Subjects reported to the laboratory for two familiarization trials and four experimental trials. Two experimental trials (1 placebo and 1 caffeine) were conducted during the follicular phase and two experimental trials (1 placebo and 1 caffeine) were performed in the luteal phase of the menstrual cycle. Experimental trials during the follicular phase were performed from days 7 to 13 of the menstrual cycle, and the luteal phase trials were performed from days 18 to 24. Menstrual status was not verified by secondary measures (i.e. estrogen levels, body temperature, etc.). As such, the presumed follicular and luteal phases will be referred to as 'early' and 'late' phases, respectively.

Preliminary Testing

Following height and body mass measurements, maximal oxygen consumption (VO_{2max}) was assessed during an incremental test on a bicycle ergometer (Velotron™ cycle ergometer, Racermate, Inc. Seattle, WA, USA). Workload started at 50 W and was increased in 25 W increments every minute until volitional fatigue, or pedaling rate dropped below 50 rpm for >10 seconds. Breath samples were collected and oxygen consumption (VO_2) was analyzed with a Moxus® Modular Metabolic System (AEI Technologies, Pittsburgh, PA, USA). VO_{2max} was determined as the highest 30 second mean oxygen uptake value.

Supplementation

In a crossover, double-blind design, subjects were provided caffeine treatment or placebo. Anhydrous caffeine (6 mg/kg body mass) was measured into opaque gelatin capsules. For placebo pills, gluten-free rice flour was put into opaque

gelatin capsules. Supplementation was ingested with water 60 minutes prior to the performance trial.

Familiarization Trials

Prior to the experimental trials, two separate familiarization trials were completed on a Velotron™ cycle ergometer. A 5-min self-selected warm-up was performed before all 3-km time-trials. Subjects were instructed to approach the time trial as a competition, emphasizing the importance to perform each trial as a race. All trials were completed without verbal feedback and only elapsed distance was displayed. The familiarization trial protocol was identical to the performance trials with the exception of caffeine/placebo supplementation.

Performance Trials

The subjects performed a 5-min warm-up, followed by a computer-simulated 3-km time trial on the Velotron cycle ergometer. Average power output during the time trial and finishing times were recorded and used as the performance measures.

Dietary and Exercise Control

Subjects completed 24-hour food logs and were instructed to replicate their diet for 24 hours prior to each subsequent trial. Subjects were asked to refrain from any food or drink (besides water) for four hours before and from caffeine and alcohol for 12 hours before each trial. The OCS user group was required to authenticate intake of their respective oral contraceptive within the past 24 hours. In addition, 48-hour physical activity logs were completed prior to each trial, and subjects were instructed to maintain consistent exercise habits between trials. In addition, subjects were asked to refrain from any vigorous physical activity 12

hours prior to each trial. Each treatment trial was performed in the morning between 6:00am and 10:00am. Trials were separated by at least 48 hours.

Statistical Analysis

Magnitude-based inferences were derived using methods described by Hopkins and colleagues (29). 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 1.7 watts and 0.4% or 1.4 seconds in the current project (45).

A published spreadsheet by Hopkins (28) was used to determine the likelihoods of the true treatment effect of the population reaching the substantial change threshold; 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 'trivial' effect. If 90% confidence intervals included values that exceeded 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

In general, caffeine enhanced 3-km TT performance (compared to placebo) in OCS users and non-users in both menstrual phases. 3-km TT performance data (finishing time) are reported in Figure 1 and Table 2. In addition, the 3-km TT power output data (average wattage) are reported in Table 2.

For the non-user group, caffeine 'likely' improved performance time by $2.6 \pm 2.3\%$ during the early phase and by $2.1 \pm 2.2\%$ in the late phase. Likewise, caffeine 'likely' increased power output by $6.7 \pm 6.1\%$ in the early phase and 'very likely' increased power output by $6.7 \pm 4.8\%$ in the late phase. It was 'unclear' whether there were differences in the magnitude of benefit from caffeine between the early and late phases for finishing time and average wattage.

Similarly for the OCS users, caffeine 'possibly' improved performance by $1.6 \pm 2.1\%$ during the early phase and 'very likely' improved performance by $2.7 \pm 1.4\%$ during the late phase. While, power output was 'likely' increased by $4.7 \pm 5.6\%$ in the early phase, and was 'very likely' increased by $7.2 \pm 3.7\%$ in the late phase.

When comparing finishing time for non-users and OCS users by average of both phases, the outcome is 'unclear' to whether non-users or OCS users benefitted more altogether.

Discussion

The purpose of the current study was to investigate how two distinct phases of the menstrual cycle and the use of oral contraceptives influenced the ergogenic effects of caffeine on 3-km cycling performance. The major finding was that caffeine

improved 3-km time trial performance for female cyclists during both the early and late phases of the menstrual cycle, with no clear difference between the early or late phase of the menstrual cycle. Further, oral contraceptive status (users vs. non-users) did not appear to systematically influence the ergogenic effect of caffeine supplementation. The collective data suggest that menstrual status is not a significant source of variation in the performance response to caffeine supplementation.

While not measured in the current study, the concentrations of endogenous female sex hormones vary considerably during the menstrual cycle, with estradiol concentration in serum rising from 10-30 pg/ml in the follicular phase to 80-200 pg/ml during the luteal phase (65). Given the prior evidence of slowed caffeine metabolism with higher levels of estrogen (3, 34, 48), we suspected that high estrogen levels associated with the late menstrual phase (luteal) would slow caffeine metabolism. As a result, we hypothesized that non-OCS users would experience higher caffeine: metabolite levels following supplementation in the late, versus early phase, thus leading to a greater caffeine benefit. To our knowledge, this is the first study to investigate the menstrual phase as a possible source of the variation in the ergogenic effect of caffeine supplementation. Therefore it is not possible to compare to previous findings. However, the absence of a systematic difference in the performance benefit of caffeine between menstrual phases observed here is indirectly supported by prior studies indicating the males and females benefit similarly to caffeine ingestion (2, 10, 20). It may be that alterations in hepatic P-450 enzyme activity between phases of the menstrual cycle are not

physiologically relevant, as shown in drug therapy (65), Also noted by a meta-analysis of the effect of the menstrual cycle on metabolism during exercise, a limitation to this study is the participation following an overnight fast, as a positive nutritional state may lessen the impact of ovarian hormones on metabolism during exercise (41). Thus, the effect of estrogen on CYP1A2 enzyme activity may not be enough to influence the ergogenic effect of caffeine despite the fact that we tested subjects under dietary conditions that should have magnified the impact of estrogen. Estrogen may have less of an effect following usual dietary intake.

Using the same logic outlined above, because we suspected that OCS users would have had higher estrogen levels than non-users, we therefore hypothesized OCS users would have slowed caffeine metabolism, thus lower caffeine: metabolite ratio leading to less benefit of caffeine compared to non-users. Again, in contrast to our hypothesis, there was no clear difference in the performance response to caffeine between OCS users and non-users. Therefore, the difference of estrogen levels in modern OCS may not compromise caffeine metabolism to a degree influences the ergogenic effect of caffeine. It is important to note that the outcome was 'unclear', which does not indicate consistent/similar responses between groups, but rather marked variability in the responses to caffeine. This suggests that more data needs to be gathered before drawing firm conclusions about potential differences.

It is well known that there is significant individual variability in the performance response to caffeine. Our laboratory, and others, have suggested that this variability may be related to differences in the rate of caffeine metabolism and

absorption between individuals (42, 44, 63). There is evidence that peak blood caffeine levels occur one hour after ingestion (4), and that these levels are similar for both OCS users and non-users (46). Thus we know absorption of caffeine is similar in OCS users and non-users, but rates of metabolism are highly variable. Therefore, we speculate that the difference in ergogenic effect of caffeine between these two groups would be greater beyond one hour post-ingestion, as the half-life of caffeine is prolonged in OCS users (46). Future research should consider evaluating performance more than one-hour post caffeine ingestion to establish the effect of caffeine metabolism rate, or caffeine and metabolite concentrations, on ergogenic effect of caffeine.

One major limitation of the current study is the presumption of different estrogen levels between the early and late phases. Although there is strong evidence of diminished caffeine metabolism with high levels of estradiol (53), we have no measure to determine caffeine elimination kinetics within our sample. In order to evaluate the influence of the menstrual cycle, it was essential to determine menstrual phases to keep hormone levels consistent within an investigation, which was done by examining women outside of the days of menses and the days of ovulation (where spikes in other female hormones, such as progesterone and follicular-stimulating hormone occur) (30, 56).

The primary strength of this project was the concomitant investigation of oral contraceptive status and menstrual phase, two conditions that presumably impact estrogen levels, on the benefit that caffeine has on simulated performance. Unfortunately the outcomes from this investigation are considerably weakened by

the absence of blood estrogen measurements. Therefore, we cannot definitely conclude that estrogen levels, per se, do not alter the performance response to caffeine. Altogether, the results from this investigation indicate that females derive performance benefits from caffeine irrespective of menstrual phase and despite use of OCS, however, the magnitude to which benefits occur under these conditions are unclear.

Manuscript References

1. Abernethy DR, Todd EL. Impairment of Caffeine Clearance By Chronic Use of Low-Dose Estrogen-Containing Oral-Contraceptives. *Eur J Clin Pharmacol* 1985;28(4):425–8.
2. Amendola CA, Gabrieli JD, Lieberman HR. Caffeine's Effects on Performance and Mood are Independent of Age and Gender. *Nutr Neurosci* 1998;1(4):269–80.
3. Balogh A, Henschel L, Klinger G, Vollanath R, Borner A, Kuhn W. Influence of ethinylestradiol-containing combination oral contraceptives with gestodene or levonorgestrel on caffeine elimination [Internet]. *Eur J Clin Pharmacol* 1995;48(2) Available from: <http://link.springer.com/10.1007/BF00192743>
4. Bell DG, McLellan TM. Exercise endurance 1, 3, and 6 h after caffeine ingestion in caffeine users and nonusers. *J Appl Physiol* 2002;93(4):1227–34.
5. Black CD, Waddell DE, Gonglach AR. Caffeine's ergogenic effects on cycling: Neuromuscular and perceptual factors. *Med Sci Sports Exerc* 2015;47(6):1145–58.
6. Burke LM. Caffeine and sports performance. *Indian J Nat Prod Resour* 2009;8(3):216.
7. Callahan MM, Robertson RS, Branfman AR, McComish MF, Yesair DW. Comparison of caffeine metabolism in three nonsmoking populations after oral administration of radiolabeled caffeine. [Internet]. *Drug Metab Dispos* 1983;11(3):211–7. Available from: <http://online.liebertpub.com/doi/10.1089/jcr.2015.0029>
8. Carrillo J a, Benitez J. Clinically significant pharmacokinetic interactions between dietary caffeine and medications. *Clin Pharmacokinet* 2000;39(2):127–53.
9. Casazza GA, Suh S, Miller BF, Navazio FM, Brooks GA. Effects of oral contraceptives on peak exercise capacity. [Internet]. *J Appl Physiol* 2002;93(5):1698–702. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/12381756>
10. Chen H-Y, Wang H-S, Tung K, Chao H-H. Effects of Gender Difference and Caffeine Supplementation on Anaerobic Muscle Performance [Internet]. *Int J Sports Med* 2015;36(12):974–8. Available from: <http://www.thieme-connect.de/DOI/DOI?10.1055/s-0035-1550048>
11. Conway KJ, Orr R, Stannard SR. Effect of a divided caffeine dose on endurance cycling performance, postexercise urinary caffeine concentration, and plasma paraxanthine. *J Appl Physiol* 2003;94(4):1557–62.
12. Daly JW. Review Caffeine analogs : biomedical impact. 2007;64:2153–69.
13. Davis JK, Green JM. Ergogenic Value and Mechanisms of Action. *Sport Med*

- 2009;39(10):813–32.
14. Desbrow B, Biddulph C, Devlin B, Grant GD, Leveritt MD. The effects of different doses of caffeine on endurance cycling time trial performance. 2011;(December):37–41.
 15. Desbrow B, Leveritt M. Well-Trained Endurance Athletes' Knowledge, Insight, and Experience of Caffeine Use. 2007;(4):328–39.
 16. Doherty M, Smith P, Hughes M, Davison R. Caffeine lowers perceptual response and increases power output during high-intensity cycling. *J Sports Sci* 2004;22(7):637–43.
 17. Doherty M, Smith PM. Effects of caffeine ingestion on rating of perceived exertion during and after exercise: A meta-analysis. *Scand J Med Sci Sport* 2005;
 18. Eugster HP, Probst M, Würigler FE, Sengstag C. Caffeine, estradiol, and progesterone interact with human CYP1A1 and CYP1A2. Evidence from cDNA-directed expression in *Saccharomyces cerevisiae*. [Internet]. *Drug Metab Dispos* 2008;21(1):43–9. Available from: <http://dmd.aspetjournals.org/cgi/doi/10.1124/dmd.108.022301>
 19. Fredholm BB. Adenosine, Adenosine Receptors and the Actions of Caffeine *. 1995;93–101.
 20. Glenn JM, Gray M, Gualano B, Roschel H, Paulo S. The Ergogenic Effects of Supplemental Nutritional Aids on Anaerobic Performance in Female Athletes. *Strength Cond J* 2016;38(2):105–20.
 21. Goldstein ER, Ziegenfuss T, Kalman D, et al. International society of sports nutrition position stand: caffeine and performance. [Internet]. *J Int Soc Sports Nutr* 2010;7(1):5. Available from: <http://dx.doi.org/10.1186/s12970-015-0090-y>
 22. Goodman MT, Tung K-H, McDuffie K, Wilkens LR, Donlon TA. Association of Caffeine Intake and CYP1A2 Genotype With Ovarian Cancer [Internet]. *Nutr Cancer* 2003;46(1):23–9. Available from: http://www.tandfonline.com/doi/abs/10.1207/S15327914NC4601_03
 23. Graham TE, Spriet LL. Metabolic, catecholamine, and exercise performance responses to various doses of caffeine. *J Appl Physiol* 1995;78(3):867–74.
 24. Granfors MT, Backman JT, Laitila J, Neuvonen PJ. Oral contraceptives containing ethinyl estradiol and gestodene markedly increase plasma concentrations and effects of tizanidine by inhibiting cytochrome P450 1A2. *Clin Pharmacol Ther* 2005;78(4):400–11.
 25. Grosso LM, Bracken MB. Caffeine metabolism, genetics, and perinatal outcomes: A review of exposure assessment considerations during pregnancy. *Ann Epidemiol* 2005;15(6):460–6.
 26. Gunes A, Ozbey G, Vural EH, et al. Variation in CYP1A2 activity and its clinical

- implications: influence of environmental factors and genetic polymorphisms. [Internet]. *Pharmacogenomics* 2008;9(5):769–78. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/18466106><http://www.ncbi.nlm.nih.gov/pubmed/19450128>
27. Hallström H, Melhus H, Glynn A, Lind L, Syvänen A-C, Michaëlsson K. Coffee consumption and CYP1A2 genotype in relation to bone mineral density of the proximal femur in elderly men and women: a cohort study [Internet]. *Nutr Metab (Lond)* 2010;7(12):1–9. Available from: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2842270/pdf/1743-7075-7-12.pdf>
 28. Hopkins W. A Spreadsheet for Deriving a Confidence Interval, Mechanistic Inference and Clinical Inference from a P Value. 2007;16–20.
 29. Hopkins WG, Marshall SW, Batterham AM, Hanin J. Progressive statistics for studies in sports medicine and exercise science. *Med Sci Sports Exerc* 2009;41(1):3–12.
 30. de Jonge XAKJ. Effects of the Menstrual Cycle on Exercise Performance. [Internet]. *Sport Med* 2003;33(11):833–51. Available from: <http://search.ebscohost.com/login.aspx?direct=true&db=s3h&AN=10929510&site=ehost-live>
 31. Kalow W, Tang B-K. Use of caffeine metabolite ratios to explore CYP1A2 and xanthine oxidase activities [Internet]. *Clin Pharmacol Ther* 1991;50(5/1):508–19. Available from: <http://doi.wiley.com/10.1038/clpt.1991.176>
 32. Kanarkowski R, Tornatore KM, D'Ambrosio R, Gardner MJ, Jusko WJ. Pharmacokinetics of single and multiple doses of ethinyl estradiol and levonorgestrel in relation to smoking. [Internet]. *Clin Pharmacol Ther* 1988;43(1):23–31. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/3121231>
 33. Kochańska-Dziurawicz AA, Janikowska G, Bijak A, Stanjek-Cichoracka A, Mazurek U. The effect of maximal physical exercise on relationships between the growth hormone (GH) and insulin-like growth factor 1 (IGF-1) and transcriptional activity of CYP1A2 in young ice hockey players. [Internet]. *J Sports Med Phys Fitness* 2015;55(3):158–63. Available from: <http://dx.plos.org/10.1371/journal.pone.0122119>
 34. Lane J, Steege J, Rupp S, Kuhn C. Menstrual cycle effects on caffeine elimination in the human female [Internet]. *Eur J Clin ...* 1992;543–6. Available from: <http://link.springer.com/article/10.1007/BF02285099>
 35. Laurent D, Schneider KE, Prusaczyk WK, et al. Effects of caffeine on muscle glycogen utilization and the neuroendocrine axis during exercise. *J Clin Endocrinol Metab* 2000;85(6):2170–5.
 36. Lebrun CM. Effect of the Different Phases of the Menstrual Cycle and Oral Contraceptives on Athletic Performance [Internet]. *Sport Med*

- 1993;16(6):400–30. Available from:
<http://link.springer.com/10.2165/00007256-199316060-00005>
37. Lelo A, Birkett D, Robson R, Miners J. Comparative pharmacokinetics of caffeine and its primary demethylated metabolites paraxanthine, theobromine and theophylline in man. *Br J Clin Pharmacol* 1986;22(2):177–82.
 38. Mahdavi R, Daneghian S, Jafari A, Homayouni A. Effect of Acute Caffeine Supplementation on Anaerobic Power and Blood Lactate Levels in Female Athletes [Internet]. *J Caffeine Res* 2015;5(2):83–7. Available from:
<http://online.liebertpub.com/doi/10.1089/jcr.2014.0034>
 39. McLean C, Graham TE. Effects of exercise and thermal stress on caffeine pharmacokinetics in men and eumenorrheic women. *J Appl Physiol* 2002;93(4):1471–8.
 40. Michaud TJ, Bachmann KA, Andres FF, Flynn MG, Sherman GP, Rodriguez-Zayas J. Exercise training does not alter cytochrome P-450 content and microsomal metabolism. [Internet]. *Med Sci Sports Exerc* 1994;26(8):978–82. Available from: <http://doi.wiley.com/10.1002/art.23811>
 41. Oosthuysen T, Bosch AN. The Effect of the Menstrual Cycle on Exercise Metabolism. *Sport Med* 2010;40(3):207–27.
 42. Pasma WJ, van Baak MA, Jeurden AE, de Haan A. The Effect of Different Dosages of Caffeine on Endurance Performance Time. 1994;225–30.
 43. Pataky MW. The Influence of the CYP1A2 Polymorphism on the Effect of Caffeine Ingestion and Mouth Rinsing on 3KM Cycling Performance. 2015;
 44. Paton C, Costa V, Guglielmo L. Effects of caffeine chewing gum on race performance and physiology in male and female cyclists [Internet]. *J Sports Sci* 2014;414(January 2015):1–8. Available from:
<http://www.tandfonline.com/doi/abs/10.1080/02640414.2014.984752%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/25517202>
 45. Paton CD, Hopkins WG. Variation in performance of elite cyclists from race to race. *Eur J Sport Sci* 2006;6(1):25–31.
 46. Patwardhan R, Desmond P, Johnson R, Schenker S. Impaired elimination of caffeine by oral contraceptive steroids. *J Lab Clin Med* 1980;95(4):603–8.
 47. Perera V, Gross AS, McLachlan AJ. Measurement of CYP1A2 activity: a focus on caffeine as a probe. [Internet]. *Curr Drug Metab* 2012;13(5):667–78. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22554278>
 48. Pollock BG, Wylie M, Stack J a., et al. Inhibition of Caffeine Metabolism by Estrogen Replacement Therapy in Postmenopausal Women [Internet]. *J Clin Pharmacol* 1999;39(9):936–40. Available from:
<http://doi.wiley.com/10.1177/00912709922008560>
 49. Rasmussen BB, Brix TH, Kyvik KO, Brøsen K. The interindividual differences

- in the 3-demthylation of caffeine alias CYP1A2 is determined by both genetic and environmental factors. *Pharmacogenetics* 2002;12(6):473–8.
50. Ribeiro-Alves M a, Trugo LC, Donangelo CM. Use of oral contraceptives blunts the calciuric effect of caffeine in young adult women. *J Nutr* 2003;133(2):393–8.
 51. Ribeiro JA, Sebastio AM. Caffeine and adenosine. *J Alzheimer's Dis* 2010;20(SUPPL.1)
 52. Rickenlund A, Carlstro K, Schoultz BOVON, et al. Effects of Oral Contraceptives on Body Composition and. 2016;89(September):4364–70.
 53. Rietveld EC, Broekman MMM, Houben JJG, Eskes TKAB, van Rossum JM. Rapid onset of an increase in caffeine residence time in young women due to oral contraceptive steroids. *Eur J Clin Pharmacol* 1984;26(3):371–3.
 54. de Santos RA, Da Kiss MAPM, Da Silva-Cavalcante M, et al. Caffeine Alters Anaerobic Distribution and Pacing during a 4000-m Cycling Time Trial. *PLoS One* 2013;8(9)
 55. Sokmen B, Armstrong LE, Kraemer WJ, et al. Caffeine Use in Sports: Considerations for the Athlete. *J Strength Cond Res* 2008;22(3):978–86.
 56. Stachenfeld NS, Taylor HS. Challenges and methodology for testing young healthy women in physiological studies. [Internet]. *Am J Physiol Endocrinol Metab* 2014;306(8):E849-53. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/24569589>
 57. Talanian JL, Spriet LL. Low and Moderate Doses of Caffeine Late in Exercise Improve Performance in Trained Cyclists [Internet]. *Appl Physiol Nutr Metab* 2016;apnm-2016-0053. Available from: <http://www.nrcresearchpress.com/doi/10.1139/apnm-2016-0053>
 58. Tsuchiya Y, Nakajima M, Yokoi T. Cytochrome P450-mediated metabolism of estrogens and its regulation in human. *Cancer Lett* 2005;227(2):115–24.
 59. Vistisen K, Poulsen HE, Loft S. Foreign compound metabolism capacity in man measured from metabolites of dietary caffeine. [Internet]. *Carcinogenesis* 1992;13(9):1561–8. Available from: <http://doi.wiley.com/10.1046/j.1365-2125.1999.00918.x>
 60. Warren GL, Park ND, Maresca RD, McKibans KI, Millard-Stafford ML. Effect of caffeine ingestion on muscular strength and endurance: A meta-analysis. *Med Sci Sports Exerc* 2010;42(7):1375–87.
 61. Wiles JD, Bird SR, Hopkins J, Riley M. Effect of caffeinated coffee on running speed, respiratory factors, blood lactate and perceived exertion during 1500-m treadmill running. *Br J Sports Med* 1992;26(2):116–20.
 62. Wiles JD, Coleman D, Tegerdine M, Swaine IL. The effects of caffeine ingestion on performance time, speed and power during a laboratory-based 1 km cycling time-trial. [Internet]. *J Sports Sci* 2006;24(11):1165–71. Available

from: <http://www.ncbi.nlm.nih.gov/pubmed/17035165>

63. Womack CJ, Saunders MJ, Bechtel MK, et al. The influence of a CYP1A2 polymorphism on the ergogenic effects of caffeine. [Internet]. *J Int Soc Sports Nutr* 2012;9(1):7. Available from: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3334681&tool=pmcentrez&rendertype=abstract>
64. Yang A, Palmer AA, De Wit H. Genetics of caffeine consumption and responses to caffeine. *Psychopharmacology (Berl)* 2010;211(3):245–57.
65. Zaigler M, Rietbrock S, Szymanski J, Dericks-Tan JS, Staib AH, Fuhr U. Variation of CYP1A2-dependent caffeine metabolism during menstrual cycle in healthy women. [Internet]. *Int J Clin Pharmacol Ther* 2000;38(5):235–44. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/10839467>

Table 1.0 Descriptive Data

	Total (n=16)	Non-Users (n=8)	OCS Users (n=8)
Height (cm)	164.7 ± 6.9	161.0 ± 7.7	168.4 ± 3.6
Weight (kg)	61.2 ± 8.6	58.8 ± 9.7	63.6 ± 7.2
Age (yrs)	21.1 ± 1.8	20.6 ± 2.1	21.4 ± 1.4
VO ₂ max (ml/kg/min)	49.7 ± 6.5	51.3 ± 8.2	48.0 ± 4.0
Cycling (hrs/week)	3.1 ± 1.9	3.8 ± 2.0	2.4 ± 1.7
Caffeine (mg/week)	601.1 ± 494.9	623.4 ± 551.1	578.9 ± 469.0

Descriptive data reported as mean, SD

Table 2. 3-km Performance Time and Power Output Data

		Mean \pm SD		Within-Condition Differences; Δ PLA vs. CAF Mean, \pm 90%CI (raw) % Likelihoods (+/triv/-), Inference	Between-Condition Differences Δ Early vs. Late Mean, \pm 90%CI (raw) % Likelihoods (+/triv/-), Inference	
Variable	Condition	PLA	CAF	PLA vs. CAF	1 vs. 3 (Non-Users: Early vs. Late)	1&3, vs. 2&4 (Users vs. Non Users)
3-km TT Time in seconds (Power Output in Watts)	(1) Non-Users Early	358.9 \pm 43.1 (167.1 \pm 51.6)	349.9 \pm 44.2 (178.4 \pm 52.5)	2.6 \pm 2.3, % likelihoods (2, 4, 94) likely beneficial	0.5 \pm 3.0, % likelihoods (30.1,17.6, 52.3), unclear	0.1 \pm 1.9, % likelihoods (32.1,29,39) Unclear
	(2) Users Early	356.8 \pm 28.3 (162.3 \pm 28.5)	351.0 \pm 28.4 (170.1 \pm 31.8)	1.6 \pm 2.1, % likelihoods (3, 26, 71) possibly beneficial		
	(3) Non-Users Late	356.3 \pm 38.2 (167.6 \pm 47.9)	348.2 \pm 31.5 (177.0 \pm 41.4)	2.1 \pm 2.2, % likelihoods (3, 6, 91) likely beneficial		
	(4) Users Late	354.7 \pm 22.9 (164.1 \pm 24.5)	345.0 \pm 21.3 (176.0 \pm 27.0)	2.7 \pm 1.4, % likelihoods (0, 2, 98) very likely beneficial		

Values for Placebo (PLA) and Caffeine (CAF) reported as Mean \pm SD. Comparison values reported Mean \pm 90% CI for differences between change scores (i.e. Early vs. Late), % likelihoods of positive effect/trivial effect/negative effect and semantic inferences.

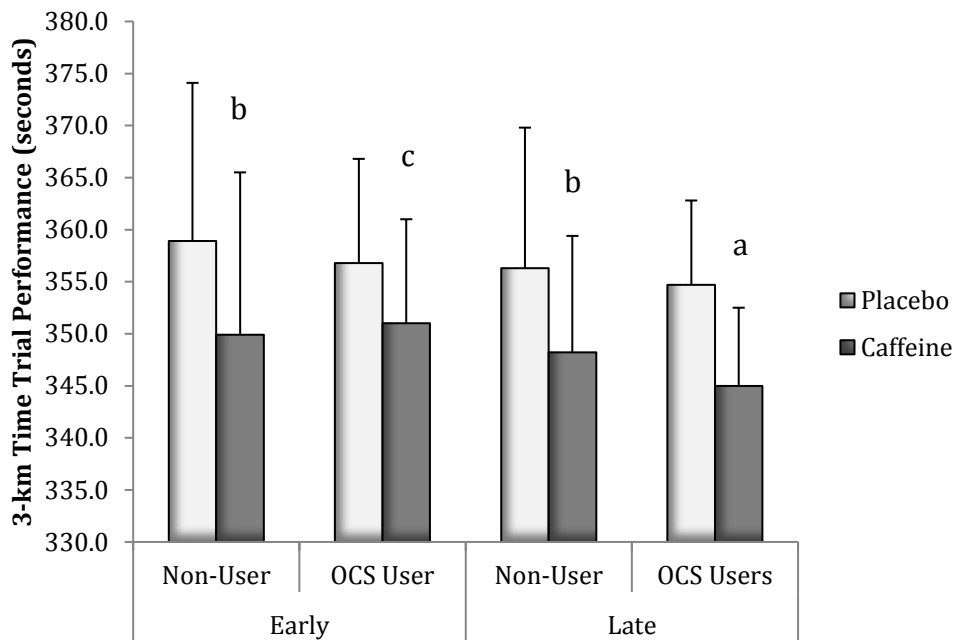


Figure 1 Average (mean \pm SE) 3-km Time Trial performance for the caffeine and placebo treatments for non-oral contraceptive users and oral contraceptive users during the early and late phases.

Effects of Caffeine during 3-km Time Trial performance in seconds: Bars depict mean time in seconds (\pm SE). (a) 'Very likely' improvement vs. placebo. (b) 'Likely' improvement vs. placebo. (c) 'Possible' improvement vs. placebo.

Appendix A

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 Annette Lemanski from James Madison University titled *The Effect of Oral Contraceptives on Caffeine Metabolism and Performance*.

The purpose of this study is to determine the impact of oral contraceptives on caffeine metabolism and 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 a stationary bike (cardiovascular fitness test, one familiarization test, 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 oral contraceptive use and duration of use. The total time commitment is estimated to be less than 6 hours over the course of 8 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 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):

On one occasion, you will be asked to perform 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 the stationary bicycle 3-km time trial. You will be encouraged to treat the time trial like a competition.

Experimental Trials (n=4; 90 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 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 levels. Following the blood draw, you will complete the 3-km time trial described above.

Supplementation Protocol:

No supplementation will be given during the familiarization trial. You will be randomly assigned a treatment order. Treatments will be: 1. Placebo capsule containing flour administered 1hr prior to exercise between 6-10am 2. Caffeine capsule containing 6 mg/kg bodyweight caffeine administered 1hr prior to exercise between 6-10am.

DNA Sampling:

We will extract a sample of your DNA from one of your blood samples to determine the type of enzyme that you have to breakdown caffeine. DNA and blood samples will be stored in our laboratory freezer indefinitely 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 destroyed immediately upon request. 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

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 25 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 10 performers will be entered into a drawing to win a \$150 and \$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 Annette Lemanski, lemansam@dukes.jmu.edu. Or by phone (715) 340- 4423

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.

Appendix C

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 joint injury that precludes you from completing the cycling protocol?

Allergies:

Are you allergic to rice flour?

Are you allergic to latex?

Contraceptive use:

Are you currently on any form of contraceptives (including the ring, IUD, etc)?

Are you currently using oral contraceptives?

If yes, what is the brand name of your current medication?

How long have you been using oral contraceptives?

Approximately how long is your menstrual cycle (in days)? Menstrual cycle is day one of menses to the next day one of menses.

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

References

1. Abernethy DR, Todd EL. Impairment of Caffeine Clearance By Chronic Use of Low-Dose Estrogen-Containing Oral-Contraceptives. *Eur J Clin Pharmacol* 1985;28(4):425–8.
2. Amendola CA, Gabrieli JD, Lieberman HR. Caffeine's Effects on Performance and Mood are Independent of Age and Gender. *Nutr Neurosci* 1998;1(4):269–80.
3. Balogh A, Henschel L, Klinger G, Vollanath R, Borner A, Kuhn W. Influence of ethinylestradiol-containing combination oral contraceptives with gestodene or levonorgestrel on caffeine elimination [Internet]. *Eur J Clin Pharmacol* 1995;48(2) Available from: <http://link.springer.com/10.1007/BF00192743>
4. Bell DG, McLellan TM. Exercise endurance 1, 3, and 6 h after caffeine ingestion in caffeine users and nonusers. *J Appl Physiol* 2002;93(4):1227–34.
5. Black CD, Waddell DE, Gonglach AR. Caffeine's ergogenic effects on cycling: Neuromuscular and perceptual factors. *Med Sci Sports Exerc* 2015;47(6):1145–58.
6. Burke LM. Caffeine and sports performance. *Indian J Nat Prod Resour* 2009;8(3):216.
7. Callahan MM, Robertson RS, Branfman AR, McComish MF, Yesair DW. Comparison of caffeine metabolism in three nonsmoking populations after oral administration of radiolabeled caffeine. [Internet]. *Drug Metab Dispos* 1983;11(3):211–7. Available from: <http://online.liebertpub.com/doi/10.1089/jcr.2015.0029>
8. Carrillo J a, Benitez J. Clinically significant pharmacokinetic interactions between dietary caffeine and medications. *Clin Pharmacokinet* 2000;39(2):127–53.
9. Casazza GA, Suh S, Miller BF, Navazio FM, Brooks GA. Effects of oral contraceptives on peak exercise capacity. [Internet]. *J Appl Physiol* 2002;93(5):1698–702. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/12381756>
10. Chen H-Y, Wang H-S, Tung K, Chao H-H. Effects of Gender Difference and Caffeine Supplementation on Anaerobic Muscle Performance [Internet]. *Int J Sports Med* 2015;36(12):974–8. Available from: <http://www.thieme-connect.de/DOI/DOI?10.1055/s-0035-1550048>
11. Conway KJ, Orr R, Stannard SR. Effect of a divided caffeine dose on endurance cycling performance, postexercise urinary caffeine concentration, and plasma paraxanthine. *J Appl Physiol* 2003;94(4):1557–62.
12. Daly JW. Review Caffeine analogs : biomedical impact. 2007;64:2153–69.
13. Davis JK, Green JM. Ergogenic Value and Mechanisms of Action. *Sport Med* 2009;39(10):813–32.
14. Desbrow B, Biddulph C, Devlin B, Grant GD, Leveritt MD. The effects of different doses of caffeine on endurance cycling time trial performance. 2011;(December):37–41.
15. Desbrow B, Leveritt M. Well-Trained Endurance Athletes' Knowledge, Insight, and Experience of Caffeine Use. 2007;(4):328–39.
16. Doherty M, Smith P, Hughes M, Davison R. Caffeine lowers perceptual response and increases power output during high-intensity cycling. *J Sports Sci* 2004;22(7):637–43.
17. Doherty M, Smith PM. Effects of caffeine ingestion on rating of perceived exertion during and after exercise: A meta-analysis. *Scand J Med Sci Sport* 2005;
18. Eugster HP, Probst M, Würzler FE, Sengstag C. Caffeine, estradiol, and progesterone interact with human CYP1A1 and CYP1A2. Evidence from cDNA-directed expression in *Saccharomyces cerevisiae*. [Internet]. *Drug Metab Dispos* 2008;21(1):43–9. Available from: <http://dmd.aspetjournals.org/cgi/doi/10.1124/dmd.108.022301>

19. Fredholm BB. Adenosine , Adenosine Receptors and the Actions of Caffeine *. 1995;93–101.
20. Glenn JM, Gray M, Gualano B, Roschel H, Paulo S. The Ergogenic Effects of Supplemental Nutritional Aids on Anaerobic Performance in Female Athletes. *Strength Cond J* 2016;38(2):105–20.
21. Goldstein ER, Ziegenfuss T, Kalman D, et al. International society of sports nutrition position stand: caffeine and performance. [Internet]. *J Int Soc Sports Nutr* 2010;7(1):5. Available from: <http://dx.doi.org/10.1186/s12970-015-0090-y>
22. Goodman MT, Tung K-H, McDuffie K, Wilkens LR, Donlon TA. Association of Caffeine Intake and CYP1A2 Genotype With Ovarian Cancer [Internet]. *Nutr Cancer* 2003;46(1):23–9. Available from: http://www.tandfonline.com/doi/abs/10.1207/S15327914NC4601_03
23. Graham TE, Spriet LL. Metabolic, catecholamine, and exercise performance responses to various doses of caffeine. *J Appl Physiol* 1995;78(3):867–74.
24. Granfors MT, Backman JT, Laitila J, Neuvonen PJ. Oral contraceptives containing ethinyl estradiol and gestodene markedly increase plasma concentrations and effects of tizanidine by inhibiting cytochrome P450 1A2. *Clin Pharmacol Ther* 2005;78(4):400–11.
25. Grosso LM, Bracken MB. Caffeine metabolism, genetics, and perinatal outcomes: A review of exposure assessment considerations during pregnancy. *Ann Epidemiol* 2005;15(6):460–6.
26. Gunes A, Ozbey G, Vural EH, et al. Variation in CYP1A2 activity and its clinical implications: influence of environmental factors and genetic polymorphisms. [Internet]. *Pharmacogenomics* 2008;9(5):769–78. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/18466106><http://www.ncbi.nlm.nih.gov/pubmed/19450128>
27. Hallström H, Melhus H, Glynn A, Lind L, Syvänen A-C, Michaëlsson K. Coffee consumption and CYP1A2 genotype in relation to bone mineral density of the proximal femur in elderly men and women: a cohort study [Internet]. *Nutr Metab (Lond)* 2010;7(12):1–9. Available from: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2842270/pdf/1743-7075-7-12.pdf>
28. Hopkins W. A Spreadsheet for Deriving a Confidence Interval, Mechanistic Inference and Clinical Inference from a P Value. 2007;16–20.
29. Hopkins WG, Marshall SW, Batterham AM, Hanin J. Progressive statistics for studies in sports medicine and exercise science. *Med Sci Sports Exerc* 2009;41(1):3–12.
30. de Jonge XAKJ. Effects of the Menstrual Cycle on Exercise Performance. [Internet]. *Sport Med* 2003;33(11):833–51. Available from: <http://search.ebscohost.com/login.aspx?direct=true&db=s3h&AN=10929510&site=ehost-live>
31. Kalow W, Tang B-K. Use of caffeine metabolite ratios to explore CYP1A2 and xanthine oxidase activities [Internet]. *Clin Pharmacol Ther* 1991;50(5/1):508–19. Available from: <http://doi.wiley.com/10.1038/clpt.1991.176>
32. Kanarkowski R, Tornatore KM, D’Ambrosio R, Gardner MJ, Jusko WJ. Pharmacokinetics of single and multiple doses of ethinyl estradiol and levonorgestrel in relation to smoking. [Internet]. *Clin Pharmacol Ther* 1988;43(1):23–31. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/3121231>
33. Kočańska-Dziurawicz AA, Janikowska G, Bijak A, Stanjek-Cichoracka A, Mazurek U. The effect of maximal physical exercise on relationships between the growth hormone (GH) and insulin-like growth factor 1 (IGF-1) and transcriptional activity of CYP1A2 in young ice hockey players. [Internet]. *J Sports Med Phys Fitness* 2015;55(3):158–63. Available from:

- <http://dx.plos.org/10.1371/journal.pone.0122119>
34. Lane J, Steege J, Rupp S, Kuhn C. Menstrual cycle effects on caffeine elimination in the human female [Internet]. *Eur J Clin ...* 1992;543–6. Available from: <http://link.springer.com/article/10.1007/BF02285099>
 35. Laurent D, Schneider KE, Prusaczyk WK, et al. Effects of caffeine on muscle glycogen utilization and the neuroendocrine axis during exercise. *J Clin Endocrinol Metab* 2000;85(6):2170–5.
 36. Lebrun CM. Effect of the Different Phases of the Menstrual Cycle and Oral Contraceptives on Athletic Performance [Internet]. *Sport Med* 1993;16(6):400–30. Available from: <http://link.springer.com/10.2165/00007256-199316060-00005>
 37. Lelo A, Birkett D, Robson R, Miners J. Comparative pharmacokinetics of caffeine and its primary demethylated metabolites paraxanthine, theobromine and theophylline in man. *Br J Clin Pharmacol* 1986;22(2):177–82.
 38. Mahdavi R, Daneghian S, Jafari A, Homayouni A. Effect of Acute Caffeine Supplementation on Anaerobic Power and Blood Lactate Levels in Female Athletes [Internet]. *J Caffeine Res* 2015;5(2):83–7. Available from: <http://online.liebertpub.com/doi/10.1089/jcr.2014.0034>
 39. McLean C, Graham TE. Effects of exercise and thermal stress on caffeine pharmacokinetics in men and eumenorrhic women. *J Appl Physiol* 2002;93(4):1471–8.
 40. Michaud TJ, Bachmann KA, Andres FF, Flynn MG, Sherman GP, Rodriguez-Zayas J. Exercise training does not alter cytochrome P-450 content and microsomal metabolism. [Internet]. *Med Sci Sports Exerc* 1994;26(8):978–82. Available from: <http://doi.wiley.com/10.1002/art.23811>
 41. Oosthuysen T, Bosch AN. The Effect of the Menstrual Cycle on Exercise Metabolism. *Sport Med* 2010;40(3):207–27.
 42. Pasma WJ, van Baak MA, Jeurden AE, de Haan A. The Effect of Different Dosages of Caffeine on Endurance Performance Time. 1994;225–30.
 43. Pataky MW. The Influence of the CYP1A2 Polymorphism on the Effect of Caffeine Ingestion and Mouth Rinsing on 3KM Cycling Performance. 2015;
 44. Paton C, Costa V, Guglielmo L. Effects of caffeine chewing gum on race performance and physiology in male and female cyclists [Internet]. *J Sports Sci* 2014;414(January 2015):1–8. Available from: <http://www.tandfonline.com/doi/abs/10.1080/02640414.2014.984752%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/25517202>
 45. Paton CD, Hopkins WG. Variation in performance of elite cyclists from race to race. *Eur J Sport Sci* 2006;6(1):25–31.
 46. Patwardhan R, Desmond P, Johnson R, Schenker S. Impaired elimination of caffeine by oral contraceptive steroids. *J Lab Clin Med* 1980;95(4):603–8.
 47. Perera V, Gross AS, McLachlan AJ. Measurement of CYP1A2 activity: a focus on caffeine as a probe. [Internet]. *Curr Drug Metab* 2012;13(5):667–78. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22554278>
 48. Pollock BG, Wylie M, Stack J a., et al. Inhibition of Caffeine Metabolism by Estrogen Replacement Therapy in Postmenopausal Women [Internet]. *J Clin Pharmacol* 1999;39(9):936–40. Available from: <http://doi.wiley.com/10.1177/00912709922008560>
 49. Rasmussen BB, Brix TH, Kyvik KO, Brøsen K. The interindividual differences in the 3-demethylation of caffeine alias CYP1A2 is determined by both genetic and environmental factors. *Pharmacogenetics* 2002;12(6):473–8.

50. Ribeiro-Alves M a, Trugo LC, Donangelo CM. Use of oral contraceptives blunts the calciuric effect of caffeine in young adult women. *J Nutr* 2003;133(2):393–8.
51. Ribeiro JA, Sebastiao AM. Caffeine and adenosine. *J Alzheimer's Dis* 2010;20(SUPPL.1)
52. Rickenlund A, Carlstro K, Schoultz BOVON, et al. Effects of Oral Contraceptives on Body Composition and. 2016;89(September):4364–70.
53. Rietveld EC, Broekman MMM, Houben JJG, Eskes TKAB, van Rossum JM. Rapid onset of an increase in caffeine residence time in young women due to oral contraceptive steroids. *Eur J Clin Pharmacol* 1984;26(3):371–3.
54. de Santos RA, Da Kiss MAPM, Da Silva-Cavalcante M, et al. Caffeine Alters Anaerobic Distribution and Pacing during a 4000-m Cycling Time Trial. *PLoS One* 2013;8(9)
55. Sokmen B, Armstrong LE, Kraemer WJ, et al. Caffeine Use in Sports: Considerations for the Athlete. *J Strength Cond Res* 2008;22(3):978–86.
56. Stachenfeld NS, Taylor HS. Challenges and methodology for testing young healthy women in physiological studies. [Internet]. *Am J Physiol Endocrinol Metab* 2014;306(8):E849-53. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/24569589>
57. Talanian JL, Spriet LL. Low and Moderate Doses of Caffeine Late in Exercise Improve Performance in Trained Cyclists [Internet]. *Appl Physiol Nutr Metab* 2016;apnm-2016-0053. Available from: <http://www.nrcresearchpress.com/doi/10.1139/apnm-2016-0053>
58. Tsuchiya Y, Nakajima M, Yokoi T. Cytochrome P450-mediated metabolism of estrogens and its regulation in human. *Cancer Lett* 2005;227(2):115–24.
59. Vistisen K, Poulsen HE, Loft S. Foreign compound metabolism capacity in man measured from metabolites of dietary caffeine. [Internet]. *Carcinogenesis* 1992;13(9):1561–8. Available from: <http://doi.wiley.com/10.1046/j.1365-2125.1999.00918.x>
60. Warren GL, Park ND, Maresca RD, McKibans KI, Millard-Stafford ML. Effect of caffeine ingestion on muscular strength and endurance: A meta-analysis. *Med Sci Sports Exerc* 2010;42(7):1375–87.
61. Wiles JD, Bird SR, Hopkins J, Riley M. Effect of caffeinated coffee on running speed, respiratory factors, blood lactate and perceived exertion during 1500-m treadmill running. *Br J Sports Med* 1992;26(2):116–20.
62. Wiles JD, Coleman D, Tegerdine M, Swaine IL. The effects of caffeine ingestion on performance time, speed and power during a laboratory-based 1 km cycling time-trial. [Internet]. *J Sports Sci* 2006;24(11):1165–71. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/17035165>
63. Womack CJ, Saunders MJ, Bechtel MK, et al. The influence of a CYP1A2 polymorphism on the ergogenic effects of caffeine. [Internet]. *J Int Soc Sports Nutr* 2012;9(1):7. Available from: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3334681&tool=pmcentrez&rendertype=abstract>
64. Yang A, Palmer AA, De Wit H. Genetics of caffeine consumption and responses to caffeine. *Psychopharmacology (Berl)* 2010;211(3):245–57.
65. Zaigler M, Rietbrock S, Szymanski J, Dericks-Tan JS, Staib AH, Fuhr U. Variation of CYP1A2-dependent caffeine metabolism during menstrual cycle in healthy women. [Internet]. *Int J Clin Pharmacol Ther* 2000;38(5):235–44. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/10839467>