The effects of creatine monohydrate loading on recovery in healthy women throughout the menstrual cycle

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A thesis submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Master of Arts in the Department of Exercise and Sport Science (Exercise Physiology).

Chapel Hill
2022

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ABSTRACT

Amanda Nicole Gordon: The Effects of Creatine Monohydrate Loading on Exercise Recovery in Healthy Women Throughout the Menstrual Cycle (Under the direction of: Abbie Smith-Ryan)

This study evaluated the effect of CrM supplementation on exercise recovery, measured from HRV and repeated sprint performance, in women throughout menstrual phase. Data were analyzed for 39 participants randomized to either a CrM group (n=19) or PL group (n=20). CrM supplementation did not appear to influence HRV values as no significant differences were seen in HRV values at rest or post-exercise. For repeated sprint outcomes, there was a significant phase × supplement interaction (p=0.048) for fatigue index, with the greatest improvement seen in luteal phase in the CrM group (-5.8 ± 19.0%) compared to changes in the placebo group (0.1 ± 8.1%). Performance and recovery were reduced in the LP for both groups. Though not statistically significant, the data suggests that CrM could help counteract sprint performance decrements in the LP. This data can help inform CrM loading strategies for active females, demonstrating potential benefits in the LP.
ACKNOWLEDGEMENTS

I would like to thank the Department of Exercise and Sport Science faculty at the University of North Carolina at Chapel Hill for providing me the opportunity to receive a quality graduate level education in Exercise Physiology.

To my committee, Dr. Hackney and Dr. Hirsch, thank you for dedicating your time and insight to guide me through this process. I am grateful to learn from such influential and intelligent researchers.

I want to thank the participants in this study for their commitment and willingness to participate in this study.

This project would not have been successful without the help of the team around me. To Sam Moore, Noah Patterson, Maggie Hostetter, Jillian Vordick, Hannah Cabre and Lacey Gould; each of you was an integral part in the development and execution of this project. From the many hours spent writing and re-writing to the early mornings spent collecting data, your support and assistance was unwavering, and I could not have asked for a better team around me. I am inspired by each of you and know I am a better researcher and person because of y’all.

To my cohort, I could not have imagined a better group of people to share my graduate school experience with. It was a privilege to not only learn alongside you but to learn from each of you. To my family and friends, thank you for being my biggest encouragers and for pushing me to pursue my passions; I would not be where I am today without you.
Thank you to my advisor and mentor Dr. Smith-Ryan. You saw potential in me that I didn’t know I had and pushed me beyond what I thought I was capable of. You practice the perfect balance between high standards and abundant grace. With your guidance I have become a confident researcher, scientist, and woman. You exceeded my expectations as an advisor and mentor and allowed me to accomplish more than I could have anticipated. I will forever be grateful for my experience at Carolina as your student.
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<table>
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<tbody>
<tr>
<td>AGAT</td>
<td>Arginine-glycine aminotransferase</td>
</tr>
<tr>
<td>ANCOVA</td>
<td>Analysis of Covariance</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
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<tr>
<td>AP</td>
<td>Average Power</td>
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<tr>
<td>AWC</td>
<td>Anaerobic Working Capacity</td>
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<tr>
<td>CK</td>
<td>Creatine Kinase</td>
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<tr>
<td>Cr</td>
<td>Creatine supplementation</td>
</tr>
<tr>
<td>CrM</td>
<td>Creatine Monohydrate</td>
</tr>
<tr>
<td>DXA</td>
<td>Dual-energy x-ray absorptiometry</td>
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<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
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<tr>
<td>FI</td>
<td>Fatigue Index</td>
</tr>
<tr>
<td>FP</td>
<td>Follicular Phase</td>
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<tr>
<td>HF</td>
<td>High frequency power</td>
</tr>
<tr>
<td>HR</td>
<td>Heart Rate</td>
</tr>
<tr>
<td>HRV</td>
<td>Heart Rate Variability</td>
</tr>
<tr>
<td>IUD</td>
<td>Intrauterine Device</td>
</tr>
<tr>
<td>LF</td>
<td>Low frequency power</td>
</tr>
<tr>
<td>LP</td>
<td>Luteal Phase</td>
</tr>
<tr>
<td>MC</td>
<td>Menstrual Cycle</td>
</tr>
<tr>
<td>NSAID</td>
<td>Non-steroidal Anti-Inflammatory Drugs</td>
</tr>
<tr>
<td>OC</td>
<td>Oral Contraceptive</td>
</tr>
<tr>
<td>PCr</td>
<td>Phosphocreatine</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td>PP</td>
<td>Peak Power</td>
</tr>
<tr>
<td>RMSSD</td>
<td>Square root of the mean squared difference of successive RR intervals</td>
</tr>
<tr>
<td>SDNN</td>
<td>Standard deviation of normal-to-normal RR intervals</td>
</tr>
<tr>
<td>TCR</td>
<td>Total Muscle Creatine</td>
</tr>
<tr>
<td>TPP</td>
<td>Time to Peak Power</td>
</tr>
</tbody>
</table>
### DEFINITION OF TERMS

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Rate Variability</td>
<td>The fluctuation in normal-to-normal RR intervals calculated and displayed in comparable units.</td>
</tr>
<tr>
<td>DXA</td>
<td>Body composition device that uses X-rays to quantify bone mass</td>
</tr>
<tr>
<td>Creatine Loading</td>
<td>5-7 days of creatine supplementation consisting of 20g taken per day in 5g doses.</td>
</tr>
<tr>
<td>Placebo</td>
<td>Non-caloric powder (Crystal Light)</td>
</tr>
<tr>
<td>VO&lt;sub&gt;2&lt;/sub&gt; Max</td>
<td>Maximal oxygen consumption</td>
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CHAPTER I: INTRODUCTION

Each year an estimated $400 million dollars is spent by consumers on creatine supplementation (Cr). Cr is regarded as one of the most effective supplements for exercise performance. Though a large body of research exists examining the effects of Cr, the majority of this research has been conducted in males. Reports indicate that females are more likely to use dietary supplements compared to males, emphasizing the need to target supplement research to better understand specific interactions of dietary supplements and female physiology.

The most widely used and studied form of supplemental Cr, creatine monohydrate (CrM), has been shown to increase the amount of creatine stored in skeletal muscle. As a result of improving cellular metabolism, Cr supplementation has been suggested to improve exercise recovery by helping to maintain pH, augmenting glycogen storage, and decreasing inflammation. A main contributor to skeletal muscle metabolism during exercise and exercise recovery is the depletion and replenishment of muscle glycogen. Males and females exhibit similar increases in total Cr post-supplementation, females exhibit higher levels of intramuscular Cr, as well as lower levels of creatinine, suggesting sex-divergent responses to Cr. Though Cr improves mechanisms that implicate accelerated exercise recovery, little research has been done on prolonged recovery post-exercise. More research is needed to understand the impact of Cr on post-exercise recovery outcomes, specifically as it relates to females.
Heart rate variability (HRV) is the variation in time between successive RR intervals\textsuperscript{11}. These measurements are broken up into two categories, time and frequency domains\textsuperscript{11}. Time domain indices represent overall HRV, while frequency domain indices can provide specific representations of parasympathetic and sympathetic activity by high frequency power (HF) and low frequency power (LF) respectively\textsuperscript{12}. A higher overall HRV is an indicator of self-regulatory capacity and efficiency\textsuperscript{13,14}. Exercise recovery can be characterized from HRV, represented by the balance and fluctuation between the parasympathetic and sympathetic nervous system (autonomic function)\textsuperscript{15}. The onset of exercise induces vagal withdrawal, increasing heart rate and sympathetic drive as heart rate approaches 100 beats per minute\textsuperscript{15}. The termination of exercise activates a rise in parasympathetic activity. Both of these divisions of the autonomic nervous system contribute to heart rate, a common variable used to quantify exercise recovery\textsuperscript{15}. Heart rate recovery has been shown to be related to measures of HRV, indicating parasympathetic influence in the efficiency of recovery\textsuperscript{15}. When measured after exercise, HRV values represent this trend demonstrating a general decrease\textsuperscript{16–18}.

With increasing popularity of HRV amongst in devices used to track athletic performance and recovery, it is important to understand the potential utility of HRV application to a variety of situations and specifically how it can be applied to recreationally active women. Existing data report small differences in HRV across the menstrual cycle, with a slight decrease the luteal phase\textsuperscript{19,20}, or no change\textsuperscript{21}. During exercise recovery HRV decreases immediately post exercise, with a slow increase over a 30 minute time period following submaximal exercise (likely due to onset parasympathetic tone)\textsuperscript{15}. Creatine metabolism could potentially vary based on menstrual phase due to the suppressive effect of estrogen on glycolytic enzymes\textsuperscript{22}, fluctuation in creatine kinase (CK)\textsuperscript{23} as well as the effect of estrogen levels on the rate limiting enzyme in creatine
synthesis, arginine-glycine aminotransferase (AGAT). Cr has the potential to influence recovery by altering cellular metabolism by increasing intramuscular PCr concentrations, helping to maintain pH, augmenting glycogen storage, as well as decreasing inflammation. After exercise the body is working to restore homeostasis which is heavily influenced by autonomic activity; the sympathetic nervous system is dominant to allocate energy where it is most necessary. If creatine increases the efficiency of the body to elicit the recovery process, reflected by parasympathetic drive, HRV values such as RMSSD could see significant changes post-exercise. To our knowledge, HRV recovery rates have yet to be examined during exercise recovery in different menstrual phases, nor with Cr supplementation.

**Purpose**

1) The primary purpose of this study was to evaluate the effects of five days of creatine loading on exercise recovery outcomes from a repeated sprint ability (RSA) test during the follicular and luteal phases compared to placebo.

   a) Recovery was determined from HRV variables, including standard deviation of normal-to-normal RR intervals [(SDNN),(ms)], square root of the mean squared difference of successive RR intervals [(RMSSD),(ms)], measures throughout a 15-minute supine recovery period (5 min, 10 min, 15 min) following an exercise test before and after five days of creatine loading (4 × 5 grams of creatine monohydrate) across the follicular and luteal menstrual phases.

   b) Peak power per sprint (PP) and fatigue index (FI) throughout a 10 × 6-sec sprint test was evaluated before and after five days of creatine loading during the follicular and luteal phases compared to a placebo.
2) The secondary purpose of this study was to evaluate the potential differences in resting HRV [SDNN intervals (ms), RMSSD (ms)] during a 30-minute baseline assessment before and after creatine loading and across the menstrual cycle.

Research Questions

1) Do SDNN and RMSSD measures of HRV differ during a 15-minute exercise recovery period performed before and after a five-day creatine loading phase?

2) Does menstrual phase have an effect SDNN and RMSSD measures of HRV during a 15-minute exercise recovery period?

3) Is peak power per sprint and fatigue index improved during an RSA test following a five-day creatine loading phase in the follicular and luteal phases?

4) Do SDNN and RMSSD measures of HRV differ during a 30-minute baseline rest period prior to exercise with creatine loading and/or across the menstrual cycle?

Research Hypotheses

1) A five-day creatine loading phase will improve (increase) SDNN and RMSSD measures during exercise recovery post-supplementation compared to pre-supplementation and placebo.

2) Menstrual phase will not affect SDNN and RMSSD measures of HRV during a 15-minute exercise recovery period.

3) Peak power and fatigue index per sprint will improve during an RSA test after a creatine-loading phase compared to placebo.

4) No changes will be seen in HRV at rest.

Delimitations

1. Participants were generally healthy females between the ages of 18 and 40 years
2. This study consisted of five laboratory visits

3. Participants completed a familiarization/control visit during the early follicular phase.
   Each participant was then tested twice in the follicular phase and twice in the luteal phase, prior to and after a 5-day creatine loading phase.

4. Participants were eumenorrheic

5. Participants were randomized into starting in the follicular phase or the luteal phase.

6. Participants were randomized into a placebo or creatine group

7. The study was randomized and double blind.

**Limitations**

1. Participants were recruited at the University of North Carolina at Chapel Hill and in the surrounding areas, therefore, selection of participants was not truly random.

2. Menstrual cycle phase was tracked by calendar-based counting, allowing more room for phase identification error.

**Assumptions**

*Theoretical*

1. Participants provided accurate answers to screening questions, including health history, exercise and nutrition status, and dietary food intake.

2. Participants adhered to pre-assessment guidelines, including fasting and abstinence from caffeine, alcohol, and tobacco prior to testing.

3. Participants adhered to the supplementation protocol.

4. Participants exerted maximal effort during the exercise test.
Statistical

1. The sample of participants that was used in the study are from a population that is normally distributed.

2. Treatment groups and menstrual cycle phase was randomly assigned.

3. The variability of the sample was approximately equal.

Significance of Study

Cr supplementation appears to improve recovery from exercise in men\textsuperscript{26}. To date, the mechanistic potential for Cr to enhance recovery is well supported; but has yet to be evaluated in women\textsuperscript{6,23}. Due to the significant physiological differences women experience compared to men particularly related to the menstrual cycle, it is expected that exercise recovery is likely affected. This is represented in the literature by the difference in the storage and metabolism of creatine in the muscle\textsuperscript{22}. HRV can be used to quantify exercise recovery and provides a deeper physiological insight from its representation of parasympathetic and sympathetic tone. To our knowledge no studies exist examining the effect of Cr on exercise recovery in women, and how it differs across the menstrual cycle. The aim of this study is to assess the effect of Cr on exercise recovery outcomes in women both in the follicular and luteal phase; these outcomes may help understand the specific benefits of Cr for women and the potential differences in these effects between menstrual phases during exercise recovery.
CHAPTER II: LITERATURE REVIEW

Introduction

Creatine monohydrate is a well-known and heavily researched ergogenic aid. Although many misconceptions about creatine supplementation exist, extensive research has provided evidence that it is a safe and effective supplement to aid in exercise performance improvements, \(^2,28\). To date, a large majority of the research has focused solely on the effects in males\(^7,29\). In recent literature, females have been included due to potential physiological differences between males and females, and to better understand sex-based variability in the use of creatine. Creatine supplementation has consistently proven to enhance recovery in men\(^26\), enhancing glycogen replenishment\(^30\), reducing muscle damage as well as improved inflammatory markers such as plasma CK and lactate dehydrogenase\(^24,31\). To date, the implications for use of creatine for recovery in women has yet to be evaluated, particularly when accounting for the menstrual cycle. Heart rate variability reflects the modulation between the parasympathetic and sympathetic nervous system. During exercise the sympathetic nervous system is predominately active; once exercise ceases, the parasympathetic nervous system is predominately active to begin recovery\(^32\). Measures of HRV can reflect how efficiently someone can shift from sympathetic to parasympathetic dominance during and after exercise\(^11,32\). The hormonal changes that occur throughout the menstrual cycle have implications on measures of HRV because of the role of estrogen in modulation of cardiovascular function\(^33,34\). Given the fluctuation of estrogen levels throughout the menstrual cycle, HRV measures have shown to be different depending on
menstrual cycle phase\textsuperscript{20,33,35}. The aim of this literature review is first to present the current literature on HRV as it relates to recovery, and subsequently to describe the effects of creatine supplementation on exercise capacity and recovery.

**Heart Rate Variability**

HRV is the fluctuation in RR intervals, or variation in inter beat intervals (IBIs). It represents the interaction between the sympathetic and parasympathetic nervous systems\textsuperscript{13,36}. Measurements of HRV can be reported using various outcome variables and have been reported to be helpful in determining cardiac function and efficiency, as well as being predictive of disease/disease risk. A change in HRV is often indicative of a change in cardiac function, and specific measurements, such as frequency-domain measurements (Hz)\textsuperscript{11} can help identify underlying mechanisms of change. Frequency-domain measurements include the low frequency (LF) band (0.04-0.15Hz), which reflects the oscillatory rhythm of baroreceptor activity\textsuperscript{12} and high frequency (HF) band (0.15-0.40Hz), which is commonly referred to as the respiratory band, and represents vagal activity\textsuperscript{36}. A ratio of these two frequencies can be used to estimate the relationship between sympathetic and parasympathetic activity\textsuperscript{11,36}. Although most commonly used in clinical settings, HRV can be a beneficial measure in more than just clinical settings due to its representation of underlying physiological mechanisms of autonomic function. A healthy HRV reflects a heart with an efficient self-regulating capacity, in other words, a higher variability is advantageous\textsuperscript{11,13,37}. With advancements in technology, the use of HRV is becoming more wide-spread and applicable to general population.

**HRV Technology**

The “gold standard” for the measurement of HRV is considered to be electrocardiogram (ECG)\textsuperscript{11,13,38}, however, it is less accessible and cost effective for use in general populations, as
well as for use outside a laboratory setting. Heart rate monitors allow for a more feasible data
collection period due to portability and relative low cost. Several validation studies have
employed gold standard ECG measures in comparison to wearable heart rate monitors, reporting
no significant between-device differences from the same subject\(^\text{39-41}\). In addition to the ability to
measure HRV using portable monitoring devices, new technology allows for real-time results of
HRV to be seen on a smartphone application, making this method widely accessible. Polar S810i
and Polar H7/10 monitors have been observed to be reliable for HRV measurements, when
compared to ECG\(^\text{38,39,41,42}\). The Polar H7 chest strap measures were taken over a period of five-
minutes in a seated position\(^\text{42}\), short-term periods of HRV have shown to be a valid method of
assessing HRV with ICC values ranging from 0.92-0.97 at a 95% confidence interval\(^\text{43}\). In order
to get an accurate measurement the first 5 seconds of the data was discarded to allow for a
stabilization period\(^\text{42}\). All HRV measures were strongly correlated with ECG (r = 0.99 (0.98;
1.00)\(^\text{42}\). From the smartphone application, Elite HRV© (Elite HRV Inc, Asheville NC, USA),
measures were shown to be consistent and accurate compared to Kubios HRV values\(^\text{44,45}\). The
literature suggests overall agreement and suggestion of portable devices for assessing HRV\(^\text{38}\).

**HRV and Recovery**

Representing qualities of parasympathetic and sympathetic activity, HRV can be used to
quantify recovery and efficiency after exercise. As it relates to HRV recovery is how quickly
HRV and HR can return to a resting state post exercise; and efficiency is defined as the ability of
the heart to quickly modulate between sympathetic and parasympathetic dominance\(^\text{13,36}\) HRV
decreases when the “fight-or-flight” response is activated, as well as when a disturbance in
homeostasis, such as the onset of exercise\(^\text{11,27}\). HRV responses can vary based on activity level of
the individual, as well as type and intensity of exercise\(^\text{46}\). After intense bouts of resistance
training, HRV has been shown to decrease up to 30 minutes post exercise, indicating a decrease parasympathetic (vagal) modulation\textsuperscript{47,48}. A drop in HRV after maximal resistance training is shown to take up to 48 hours to return back to baseline levels\textsuperscript{49}. In aerobic exercise, HRV was evaluated after an eight-minute treadmill run at 6km/h, recovery HRV measures were taken between 1-4 minutes and 5-8 minutes during a seated recovery period. At both recovery measures, HF values were significantly decreased, indicating an increase in sympathetic dominance\textsuperscript{50}. Another study evaluating HRV after an 8 minute step test 70\% of maximal effort showed a decreased RMSSD and HF after exercise; although both gradually increased throughout a 30 minute recovery period, they never reached pre-exercise resting values\textsuperscript{15}. A study done on 27 highly training mountain cyclists (21 males, 6 females) looked at HRV after a maximal aerobic test, as well as during intermittent sprint recovery of a maximal effort sprint test (4 sets of 4 × 30-sec max; 90 sec rest; separated by 25-40 min dependent on blood pH levels). HRV measures were taken following the interest recovery period at the beginning of each sprint set. When evaluated after post hoc analysis compared to the first sprint set, significant decreases in RMSSD, HF and LF values HRV indices decreased significantly in the third and fourth sets\textsuperscript{16}. Attenuated sympathetic dominance post-exercise increases the risk for a cardiac events and may delay recovery\textsuperscript{47}. It may be advantageous to spend less time with a reduced HRV post-exercise, with data suggesting that the quicker the return to baseline represents parasympathetic modulation which can facilitate recovery from exercise\textsuperscript{47,51}.

A shift to parasympathetic modulation may allow for enhanced nutrient metabolism, aiding in muscle recovery and energy restoration\textsuperscript{27}, which would be advantageous for recovery. The body shifts into parasympathetic modulation when predominantly in a resting state-represented by a decrease in heart rate and an increase in digestion, storage and absorption of
nutrients. The overall goal of the parasympathetic nervous system is storage and conservation of energy, as well as regulation of digestion and other bodily functions. Since HF indices of HRV are thought to represent vagal modulation, higher values post exercise, as well as a higher value of an overall estimate such as RMSSD, would be indicative of a quicker shift to parasympathetic post-exercise. An increase in these values could potentially indicate an increase in recovery mechanisms. To our knowledge there is no literature evaluating HRV in relation to specific mechanism of overall recovery, as well as recovery in untrained populations.

HRV in Women

Reported sex differences in HRV suggest that the female autonomic system is characterized by parasympathetic dominance, while the male autonomic system is generally characterized by sympathetic dominance. Due to hormonal fluctuations throughout the menstrual cycle, HR and HRV are shown to respond differently according to menstrual phase. In young healthy females, resting HR was significantly higher in the luteal phase compared to the follicular phase. Resting HRV in healthy females ages 18-25 years were evaluated on days two, 10, and 21 of their menstrual cycle. RMSSD values were significantly decreased from day two (31.87 ± 21.22 ms) to day 21 (23.27 ± 19.50 ms, p=0.03). Although not statistically significant, measurements taken on the 21st day were characterized by an increase in low frequency band (LF) and a decrease in high frequency band (HF) values. Another study of resting HRV in young healthy females, during both the follicular and the luteal phases, found mean LF components to be significantly higher in the luteal phase (p<0.001), and mean HF components to be significantly lower in the luteal phase compared to follicular phase. When accounting for breathing frequency, shown to influence frequency domain values, logarithmic HF values showed a significant decrease across the five different time points (early follicular,
late follicular, ovulatory, midluteal and late luteal) throughout the menstrual cycle \( (p=0.03)^{21} \).

The same study found that after ovulation, SDNN decreased significantly\(^{21}\). To our knowledge, no literature exists comparing HRV after exercise in different menstrual phases.

**Menstrual Cycle**

A typical menstrual cycle can range from 26-35 days\(^{55}\). The menstrual cycle can be separated into two main phases, the follicular and the luteal phase, separated by ovulation. Each of these phases is characterized by a fluctuation in estrogen, progesterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH)\(^{56,57}\). In a standard 28-day cycle (Figure 1), menstruation (days 1-5) begins the cycle in the early follicular phase (days 0-7); in the phase hormones levels are the lowest\(^{56,58,59}\). The late follicular phase (days 7-14) is when a spike in estrogen, LH and progesterone occurs, in the days before ovulation (day 14). The early luteal phase (days 15-21) is characterized by a large increase in progesterone, as well as an increase in estrogen\(^{55,58,59}\). In the mid luteal phase (day 21) estrogen and progesterone levels peak, and decline in the late luteal phase (days 22-28)\(^{59}\).
Implications for Exercise

This unique physiology presents potential for different responses to exercise in different menstrual phases. Studies showing decrements in exercise performance, seen in the early follicular phase could be due to the low levels of estrogen and progesterone\textsuperscript{57}. The lack of anabolic and neuroexcitatory effects of estrogen\textsuperscript{60,61} may contribute to exercise decrements when levels are low. Most of the extensive research done on estrogen in relation to muscle strength has been done in mice, showing a 10-20\% difference in leg muscle strength in mice whose ovaries were not removed versus ovary in-tact mice; suggesting the presence of estrogen has a positive effect on muscular strength\textsuperscript{61}. A similar study showed exposure to estrogen showed 7\% greater strength in rodent muscles when compared to strength of muscles devoid of various other hormones\textsuperscript{61}. The potential effects of fluctuations in estrogen on muscle strength, suggests possible changes in performance throughout the menstrual cycle. Some literature suggests a slight decrease in performance during the early-follicular phase; however these effects are exercise dependent \textsuperscript{57,62}.

The literature on high-intensity and sprint exercises in regards the menstrual cycle is inconclusive. In a sample of 14 eumenorrheic females, exercise performance defined by peak power output and fatigue index, was evaluated across three phases of the menstrual (follicular, midcycle and luteal) \textsuperscript{63}. The study found that peak power recovery was not significantly different between follicular (Mean ± Standard Error, 86 ± 3\%), midcycle (81 ± 3\%), and luteal (89 ± 3\%) phases; there was also no significance in fatigue index for power recovery between follicular (105 ± 6\%), midcycle (91± 6\%) and luteal (101 ± 4\%)\textsuperscript{63}. In contrast, in six active eumenorrheic women, average work, power, and recovery after 10 six-second maximal sprints performed on a cycle ergometer demonstrated a significant increase in average work from the follicular phase
(38.3 ±3.1 W) to the luteal phase (39.3 ± 3.4 W), but no significant differences in peak power or blood lactate levels\textsuperscript{64}. A study of 17 eumenorrheic females examined the effects of menstrual phase on anaerobic capacity, using a cycle ergometer test consisting of four eight-second maximal sprints, showed no significant differences in absolute power between early follicular, late follicular, and luteal phases\textsuperscript{65}. These studies utilize smaller sample size. In study of 100 healthy women, power output was lower in the follicular phase (532.3 ± 88.7 W) compared to the luteal phase (566.0 ± 86.7 W) and fatigue index was greater in the follicular phase (55 ± 12%) compared to the luteal phase (47 ± 12\%)\textsuperscript{66}.

**Creatine Supplementation**

*Female Specific*

Initial evidence suggests there may be a divergent response to creatine supplementation between males and females\textsuperscript{6}. Across the menstrual cycle females exhibit a shift in muscle metabolism according to menstrual phase\textsuperscript{67}. Due to differences in hormone levels, skeletal muscle metabolism presents differently in males versus females\textsuperscript{6,67}. Studies examining intramuscular glycogen and phosphocreatine (PCr) levels in males after anaerobic exercise showed that males exhibited a significant depletion in PCr and glycogen levels\textsuperscript{67}. Whereas in women, an attenuated reduction in glycogen is seen after exercise compared to males; this could be due to the suppressive effect of estrogen on glycolytic enzymes, which is seen most tangibly in the luteal phase when estrogen levels are at highest\textsuperscript{67}. Fluctuation in creatine kinase (CK) activity, a key enzyme in creatine synthesis, is also seen throughout different phases of the menstrual cycle, suggesting a fluctuation in the ability to connect ATP generation and consumption\textsuperscript{22,68}. A rate limiting step in creatine synthesis is the expression of arginine-glycine aminotransferase (AGAT)\textsuperscript{6,22,23}; AGAT has been shown to be influenced by estrogen and
testosterone; estrogen levels decrease AGAT levels, while testosterone results in an increase, potentially affecting the endogenous synthesis of creatine as a result of fluctuations in estrogen. Females also often exhibit lower exogenous creatine intake and thus synthesis compared to males. Some evidence also suggests sex-differences in creatine metabolism may be attributed to differences in muscle size; with males typically having more skeletal muscle compared to females. Existing data examining muscle biopsies of the vastus lateralis reported 10% higher concentrations of creatine in females, compared to males. However, after undergoing a creatine loading phase, males and females possess similar increases in intramuscular total muscle creatine (TCR) as well as increases in PCr. Current evidence suggests a potentially sex-divergent response to creatine. To date, we are unaware of any data evaluating the effect of creatine supplementation across the menstrual cycle.

Anaerobic Sprint Performance

Creatine supplementation in females has been shown to improvement in strength performance, muscular power and sport performance; creatine supplementation has also been shown to be particularly effective in improving anaerobic exercise performance. Theoretically, creatine supplementation supports anaerobic performance due to its ability to increase intramuscular total creatine and PCr, producing ATP for short, high-intensity bouts of exercise and increasing PCr resynthesis rate. Specifically, sprint performance in both males and females has resulted in improvements as a result of creatine supplementation, represented by anaerobic working capacity (AWC) or peak power. After a five day creatine-loading phase (20 grams per day), 10 physically active women showed significant improvements in critical power from pre-supplementation (8.5 ± 1.5 kJ) to post-supplementation (10.5 ± 3.3 kJ) during two bouts of cycling. When 20 meter sprint performance was examined in 33
amateur female soccer players, after six weeks of supplementation, including a five-day loading phase (20 grams per day), players improved their sprint time by 3.3%. Anaerobic cycling performance was examined in 12 females after a four-day creating loading phase (20 grams per day) resulted in a significant increase in maximum power (774 ± 165 W) compared to the placebo group (746 ± 163 W). In men, similar improvements have been reported; 25 healthy male soccer players, evaluated after one-week creatine loading (0.3 g/kg per day) reported significant improvements in average power from pre (453.5 ± 76.2 W) to post-supplementation (531.2 ± 94.2 W). Recreational maximal sprints prior to and after a six week creatine electrolyte supplementation period resulted in a significant increase in maximum power (774 ± 165 W) compared to the placebo group (746 ± 163 W). In men, similar improvements have been reported; 25 healthy male soccer players, evaluated after one-week creatine loading (0.3 g/kg per day) reported significant improvements in average power from pre (453.5 ± 76.2 W) to post-supplementation (531.2 ± 94.2 W). Recreational maximal sprints prior to and after a six week creatine electrolyte supplementation period resulted in a significant increase in average power from pre (453.5 ± 76.2 W) to post-supplementation (531.2 ± 94.2 W). The average increase anaerobic performance across the literature shows a 5-15% improvement in multiple sprint performance after creatine supplementation in both males and females.

Exercise Recovery

Creatine supplementation has the potential to improve exercise recovery through altering cellular metabolism by increasing intramuscular PCr concentrations, helping to maintain pH, augmenting glycogen storage, as well as decreased inflammation. An increase in PCr concentrations can sequester hydrogen ions produced while breaking down ATP, potentially maintaining pH levels for a longer time to fatigue during exercise. A study done in 15 female athletes showed significant improvements in physical working capacity at fatigue threshold during a cycle ergometer test after five days of creatine loading (195 ± 34.6 W) compared to a placebo group (146.3 ± 22.3 W), suggesting beneficial effects of creatine in delaying fatigue. Repeated isometric contractions in eight healthy males were performed before and after a five-
day creatine loading phase and muscle biopsies were taken to examine skeletal muscle PCr resynthesis after induced muscular contractions. After taking creatine, total Cr and PCr resynthesis increased. In healthy males, 16 weeks of creatine supplementation and resistance training resulted in improved cell membrane stabilization and increased satellite cell number, resulting in an increase in muscle fiber recovery, measured via muscle biopsy. A study examining healthy males found when measured after a repeated sprint test, inflammatory markers were significantly reduced in the creatine group compared to the placebo group, suggesting an anti-inflammatory effect of creatine. When examined in males, creatine has demonstrated a significant reduction in CK post-exercise, however female data with creatine supplementation and exercise recovery is limited. As a result of differences in muscle metabolism, sex differences may be highlighted in exercise recovery.

Conclusions

HRV represents the regulation between the sympathetic and parasympathetic nervous system. Overall values of HRV represent cardiac efficiency and can be used to quantify exercise recovery status. After exercise HRV decreases and slowly returns back to baseline levels. Literature suggests resting HRV differs in females throughout the menstrual cycle, exhibiting a decrease in the luteal phase compared to the follicular phase, and an increase in LF frequency, thought to represent sympathetic activity. Creatine monohydrate is widely known to improve exercise performance and increase muscle creatine content. The impact of creatine supplementation on exercise recovery is less clear, particularly for women. While the mechanisms of creatine supplementation are supported by the literature in males, the effects on exercise recovery in women have yet to be evaluated despite the knowledge of differing mechanisms of storage and metabolism.
CHAPTER III: METHODOLOGY

Subjects/participants

52 women enrolled in this study; 15 dropped out due to time constraints and becoming ineligible after enrolling (Figure 3). Data were analyzed for 39 participants (Mean ± Standard Deviation: Age: 24.6 ± 5.9 years, Height: 172.5 ± 42.3 cm, Weight: 65.1 ± 8.1 kg, PBF: 27.4 ± 5.8 %, Estimated VO$_2$max: 39.0 ± 6.0 ml/kg/min) randomized to either a CrM group (n=19) or PL group (n=20). Participants using birth control (oral contraceptives, IUDs and vaginal rings) were included. Of the total sample 29 women had a natural menstrual cycle (21-35 days), 12 were on a hormonal or copper IUD or a vaginal ring, and 11 were on a monophasic oral contraceptive. Participants who completed at least one phase (FP or LP) or pre- and post-supplementation visits were included in analysis. Participants were active (exercising at least 3 days a week) and normally menstruating, with a BMI between 18.5-29.9 kg/m$^2$. Participants were excluded from the study if they were: 1) amenorrheic; 2) had experienced a musculoskeletal injury in the past three months; 3) were using any ace-inhibitors, beta-blockers statin drugs; 4) were pregnant; 5) were using creatine or had used creatine within the 8 week prior to screening call; 6) were unwilling to abstain from taking NSAIDs throughout the duration of the study; and 7) were unwilling to consume less than 200mg of caffeine per day during the study. All procedures in this study were approved by the University’s Institutional Review Board in concordance with the Declaration of Helsinki, and all participants provided written informed consent prior to enrollment.
Experimental Design

This was a randomized double-blind, placebo-controlled design. Prior to enrollment participants were screened via phone as well as were asked to complete a written informed consent document approved by the University’s Institutional Review Board. This study included five laboratory visits (Figure 2), each visit aligned with either follicular or luteal phases of the menstrual cycle. After enrollment participants completed a baseline visit at the start of their menstrual cycle (days 0-2). Participants arrived at least eight hours fasted to each visit and having refrained from vigorous exercise for at least 48 hours. Participants were asked to refrain from caffeine consumption 24 hours before each visit and consume below 200mg per day throughout the duration of the study, to avoid any interaction with the erogenicity of creatine. Besides caffeine consumption, subjects were asked to maintain their normal diet pattern throughout participation in the study. A urine sample was taken to determine pregnancy and hydration (1.002-1.025) via urine specific gravity. Anthropometric measures were collected followed by a 30-minute resting HRV measure, body composition measurements, functional movements assessment, a repeated sprint ability test and finish with a 15-minute recovery test measuring HRV. After the baseline visit, participants were randomly assigned to return in either follicular or luteal phases. Participants were randomly assigned to either a creatine (Cr; 20 g/day of creatine monohydrate for five days) or placebo (PL, 20 g/day of maltodextrin for five days). Participants began the five-day supplementation phase in either the follicular phase (beginning on days 2-4) or the luteal phase (beginning on days 16-18). Each visit followed the same protocol as the baseline visit. Upon completion of visit two, participants received their assigned supplement to start the five-day loading phase. Participants returned within 2 days from loading to complete visit three/five. Depending on group visit three/five took place in days 7-9.
(follicular) or days 21-23 (luteal) of the menstrual cycle, respectively. After visit three/five, participants completed a minimum four-week washout period before completing the protocol in their next designated menstrual phase.

**Body Composition**

*Dual-Energy X-Ray Absorptiometry*

At the baseline/control visit participants completed a full-body DXA scan (GE Lunar iDXA, GE Medical Systems Ultrasound & Primary Care Diagnostics, Madison, WI, USA). All participants were confirmed not pregnant from a urine pregnancy test. Participants were positioned supine in the center of the scanning table and be asked to remove all metal, thick clothing, and heavy plastic to reduce interference with the scan. The scans were automatically analyzed by the software (encore Software Version 16), but specific regions of interest were adjusted by the technician.

**Heart Rate Variability**

HRV was measured using a heart rate monitor strapped to the participant’s chest (Polar H10, Polar Electro Oy), which has been previously compared to ECG values of HRV showing no significant difference between the two measures\(^{41,42,80,81}\). A 30-minute baseline HRV reading was obtained with participants laying supine. Participants wore the monitor for the duration of the exercise test and 15-minutes post-exercise laying supine\(^{81}\). HRV data was taken throughout the 15-minute period \(^{11,13,36,37}\). Recordings were uploaded from the HR monitor to Kubios Software (Biosignal Analysis and Medical Imaging Group, Department of Physics, University of Kuopio, Kuopio, Finland)\(^{45}\). The software automatically detects R-wave intervals and were set to correct any artifacts caused by missed or ectopic beats. Outcomes included standard deviation of normal-to-normal RR intervals in milliseconds (SDNN), and root mean square of successive RR
intervals (RMSSD), all of which are recommended to estimate overall HRV within short-term recordings (less than 24 hours)\textsuperscript{11}. Outcomes were evaluated as a whole as well as in five-minute segments to compare the potential change throughout the 15-minute period.

**Submaximal Exercise Test**

Maximal oxygen consumption (VO2max) was estimated using a single-stage, treadmill (4FRONT, Woodway, Woodway USA, Inc., Waukesha, WI, USA) based submaximal CRF assessment. Participants performed a four-minute warm-up prior to beginning the VO2max assessment. Heart rate (HR) was monitored during the warm-up, VO2max assessment, and cool down period using a Polar chest strap heart rate monitor and watch (Polar Electro, Lake Success, NY, USA). The warm-up consisted of walking on the treadmill at a self-chosen speed between 3.22 to 7.24 kilometers per hour (2.0 to 4.5 miles per hour, respectively) at a 0\% grade to elicit a HR within 50 to 70\% of their age predicted HR maximum (HR max= 207 – 0.7 x age) (20). Following completion of the warm-up protocol, participants continued to walk at the same speed for four minutes at a 5\% grade. Heart rate was recorded during the last 15 seconds of the last two minutes of the test (minute 3 and 4) and averaged to determine the final, average HR. A cool-down was completed at a 0\% grade as the last stage of the CRF assessment\textsuperscript{82}. To estimate VO2max, the following equation was used:

\[
\text{VO2max} = 15.1 + 21.8 \times \text{Speed (mph)} - 0.327 \times \text{Heart Rate (bpm)} - 0.263 \times \text{Speed} \times \text{Age (year)} + 0.00504 \times \text{Heart Rate} \times \text{Age} + 5.98 \times \text{Gender (0 = female, 1 = male)}
\]
Repeated Sprint Ability

Participants completed an RSA test on a friction-loaded cycle ergometer (Monark 894E, Stockholm, Sweden) as previously described by Roelofs et al. To warm-up the participants cycled for five minutes at 50 rpm against a resistance of 0.5kg, followed by two 30 second bouts of cycling at a resistance of 1.5kg, keeping the cadence between 85-115 rpm. Each of these warm-up sprints was followed by a 60 second passive recovery. Once the warm-up was completed, the participants began the RSA test. The test consisted of 10 six-second maximal sprints with 30 seconds of passive recovery. The load applied to each sprint was 65 g/kg of body mass. Peak power (PP), time to peak power (TPP), fatigue index (FI), and average power (AP) were recorded.

Supplementation

Supplementation was conducted in a randomized, double-blind design. Participants were randomly assigned to the order for when they will consume five consecutive days of supplementation or placebo, using a computer-generated allocation sequence (Random Allocation Software) for follicular and luteal phases, respectively. Starting the day after their visit, participants were instructed to consume four 5 g doses of creatine monohydrate (Creapure® AlzChem, Trostberg GmbH, Germany) or 5 g doses of maltodextrin in powder form per day for five consecutive days. Participants were asked to consume each individual 5 g dose with 6-8 ounces of water at regular intervals (every 3-4 hours), and to take each dose around the same time each day. To track compliance participants were asked to log each dose and to return individual dosing packets to the laboratory at the end of each loading phase. A minimum of four weeks was allotted before returning to the laboratory to complete visits four and five to allow for a supplement wash-out period.
Dietary Intake

To account for normal dietary intake, a three-day diet log was given to the participant at visits one; they were instructed to return the completed diet log at visit two. Participants were instructed to record their normal diet, as detailed as possible, for two nonconsecutive weekdays and one weekend day. Using a nutrition analysis software (The Food Processor, version 10.12.0, Esha Research, Salem, OR, USA), diet logs were evaluated for average calories (CAL; kcal), carbohydrate (CHO; g), fat (FAT; g), protein (PRO; g) and relative protein (g/kg body mass) intake.

Statistical Analysis

Separate ANCOVAs, covaried for fitness level from estimated VO₂ max were used to compare HRV values (SDNN, RMSSD [ms]) between supplement groups. Separate mixed factorial ANOVAs [2 x 2; treatment (CrM vs. PL) x (follicular vs. luteal)] were used to compare the change scores between groups for all primary outcomes (AP [W], PP [W], tPP [ms], FI [%]); in the event of a significant interaction, post hoc comparisons were analyzed using Bonferroni pairwise comparisons. For each sprint, change scores from baseline to post-supplementation were calculated for PP and TP. Change scores (post-pre) were calculated for total AP and FI in the FP vs. LP. A series of two-way (2 x 10; treatment x sprint) mixed model ANOVAs were used to compare change scores between treatments for each sprint. The Bonferroni method was used to evaluate post hoc comparisons. Analyses were performed using SPSS software (Version 20.0; IBM, Armonk, NY, USA), and 95% confidence intervals were calculated and plotted in Microsoft Excel (Version 2011, Microsoft Corporation; The Microsoft Network, LLC, Richmond, WA, USA). Statistical significance was set a priori at α≤0.05
CHAPTER IV: MANUSCRIPT I
THE EFFECTS OF CREATINE MONOHYDRATE LOADING ON RECOVERY IN HEALTHY WOMEN THROUGHOUT THE MENSTRUAL CYCLE

Introduction

Each year an estimated $400 million dollars is spent by consumers on creatine supplementation (Cr). Cr is regarded as one of the most effective supplements for exercise performance. Though a large body of research exists examining the effects of Cr, the majority of this research has been conducted in males. More recently, data suggests that Cr supplementation may be more beneficial in females due to hormonal fluctuations in creatine kinase and varied baseline levels of phosphocreatine (PCr) between males and females. Additionally, reports indicate that females are the largest group of dietary supplement consumers, emphasizing the need to better understand sex-specific effects of dietary supplements and female physiology.

The most widely used and studied form of supplemental Cr, creatine monohydrate (CrM), has been shown to increase the amount of phosphocreatine (PCr) stored in skeletal muscle. As a result of improving cellular metabolism, CrM supplementation has led to improvements in exercise performance and recovery by helping to maintain pH, augmenting glycogen storage and decreasing inflammation. Males and females exhibit similar increases in total PCr post-supplementation, while females have exhibited higher levels of intramuscular PCr, as well as

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lower levels of creatinine, suggesting sex-divergent responses to CrM supplementation\textsuperscript{6}. Though CrM improves mechanisms that implicate accelerated exercise recovery, little research has evaluated the effects on post-exercise recovery. Heart rate variability (HRV) is the variation in time between successive RR intervals\textsuperscript{11}. These measurements are broken up into two categories, time and frequency domains\textsuperscript{11}. Time domain indices represent overall HRV, while frequency domain indices can provide specific representations of parasympathetic and sympathetic activity by high frequency power (HF) and low frequency power (LF) respectively\textsuperscript{12}. Time domain indices are commonly presented as the standard deviation of normal-to-normal RR intervals (SDNN). Exercise is shown to positively influence HRV; when compared to sedentary individuals, endurance trained individuals exhibit a higher resting HRV\textsuperscript{18}. Exercise recovery can be characterized from HRV, represented by the balance and fluctuation between the parasympathetic and sympathetic nervous system (autonomic function)\textsuperscript{15}. During exercise recovery HRV decreases immediately post exercise, with a slow increase over a 30 minute time period following submaximal exercise (likely due to onset parasympathetic tone)\textsuperscript{15}. In healthy individuals, HRV was shown to significantly increase at rest before exercise as well as during a recovery period after exercise following a ten-week aerobic training program\textsuperscript{85}.

Existing data report small differences in HRV across the menstrual cycle, with a slight decrease, or no change\textsuperscript{21}, in the luteal phase, compared to the follicular\textsuperscript{19,20}. Creatine metabolism may vary based on menstrual phase due to the suppressive effect of estrogen on glycolytic enzymes\textsuperscript{22}, fluctuation in creatine kinase (CK)\textsuperscript{23}, as well as the effect of estrogen levels on the rate limiting enzyme in creatine synthesis, arginine-glycine aminotransferase (AGAT)\textsuperscript{6,22,23}. CrM supplementation has the potential to influence recovery by increasing intramuscular PCR concentrations, helping to maintain pH, augmenting glycogen storage, as well as decreasing
inflammation. After exercise, restoration of homeostasis is heavily influenced by autonomic activity; the sympathetic nervous system is dominant to allocate energy where it is most necessary. If Cr increases the efficiency of the body to elicit the recovery process, reflected by parasympathetic drive, HRV values such as root mean square of successive differences (RMSSD) could see significant changes post-exercise.

To date, the mechanistic potential for Cr to enhance recovery is well supported; but has yet to be evaluated in women. Due to the significant physiological differences women experience compared to men, particularly related to the menstrual cycle, it is expected that exercise recovery will be affected in some way. This is represented in the literature by the difference in storage and metabolism of Cr in the muscle. HRV can be used to quantify exercise recovery and provides deeper physiological insight from its representation of parasympathetic and sympathetic tone. To our knowledge, HRV recovery rates have yet to be examined during exercise recovery in different menstrual phases, and with CrM supplementation. The aim of this study is to assess the effect of CrM supplementation on exercise recovery measured from HRV (SDNN, RMSSD) and repeated sprint outcomes (average power, peak power, fatigue index, time to peak power), in women both in the follicular and luteal phase; these outcomes may help understand the specific benefits of CrM supplementation for women and the potential differences in these effects between menstrual phases during exercise recovery.
Methods

Subjects

Fifty-two women enrolled in this study; 15 dropped out due to time constraints and becoming ineligible after enrolling. Data were analyzed for 39 participants (Mean ± Standard Deviation: Age: 24.6 ± 5.9 years, Height: 172.5 ± 42.3 cm, Weight: 65.1 ± 8.1 kg, PBF: 27.4 ± 5.8 %, Estimated VO$_2$max: 39.0 ± 6.0 ml/kg/min) randomized to either a CrM group (n=19) or PL group (n=20). Participants using birth control (oral contraceptives, IUDs and vaginal rings) were included. Of the total sample 29 women had a natural menstrual cycle (21-35 days), 12 were on a hormonal or copper IUD or a vaginal ring, and 11 were on a monophasic oral contraceptive. Participants who completed at least one phase (FP or LP) of pre- and post-supplementation visits were included in analysis. Participants were active (exercising at least 3 days a week) and normally menstruating, with a BMI between 18.5-29.9 kg/m$^2$. Participants were excluded from the study if they were: 1) amenorrheic (absence of menstruation); 2) had experienced a musculoskeletal injury in the past three months; 3) were using any ace-inhibitors, beta-blockers statin drugs; 4) were pregnant; 5) were using creatine or had used creatine within the 8 week prior to screening call; 6) were unwilling to abstain from taking NSAIDs throughout the duration of the study; and 7) were unwilling to consume less than 200mg of caffeine per day during the study. All procedures in this study were approved by the University’s Institutional Review Board in concordance with the Declaration of Helsinki, and all participants provided written informed consent prior to enrollment (Figure 2).

Experimental Design

This study was a randomized double-blind, placebo-controlled design. Prior to enrollment participants were screened via phone as well as completed a written informed consent document
approved by the University’s Institutional Review Board. This study included five laboratory visits (Figure 2), each visit aligned with either the follicular or luteal phases of the menstrual cycle. Each visit was conducted following a minimum of an eight hour fast and having refrained from vigorous exercise for at least 48 hours, and caffeine from 24 hours prior to each visit and kept to less than 200 mg for the duration of the study. Besides caffeine consumption, subjects were asked to maintain their normal diet pattern throughout participation in the study. A urine sample was taken to confirm non-pregnant status (HCG test) and hydration (1.002-1.025) via urine specific gravity. Anthropometric measures were collected followed by a 30-minute resting HRV measure, body composition measurements, a repeated sprint ability test and finish with a 15-minute recovery test measuring HRV. Baseline testing was completed at the start of their menstrual cycle (days 0-5). After baseline visit, participants were randomly assigned to return in either the follicular (beginning on days 2-8) or luteal phase (beginning on days 14-18); and were randomly assigned to either a creatine (Cr; 20 g/day of creatine monohydrate for five days) or placebo (PL, 20 g/day of maltodextrin for five days). The follicular phase for the participants on oral contraceptives was defined as their placebo or withdraw pills; and the luteal phase was considered the three weeks of active pills. Testing occurred prior to supplementation and within 2 following loading protocol. Participants completed a minimum four-week washout period between their menstrual cycle phase testing.

**Cycle tracking app**

Participants that had naturally occurring cycles (not on hormonal birth control) tracked their menstrual cycle by recording daily basal body temperature and menstrual symptoms into a tracking app (FertilityFriend) accessible to both the participant and researcher. This data was
used to indicate cycle start date, cycle length and ovulation date. This information was confirmed prior to testing sessions.

*Salivary Estrogen*

To account for menstrual cycle phase, estrogen concentration was determined using a 2.5mL passive drool saliva sample. Estrogen levels were determined using an ELISA assay for salivary estrogen. Participants were asked to avoid brushing their teeth for 45 minutes prior to sample collection to avoid sample contamination.

*Dual-Energy X-Ray Absorptiometry*

During the baseline visit, a body composition assessment was performed using a total body dual-energy x-ray absorptiometry (DXA) scan (Lunar iDXA; General Electric Medical Systems Ultrasound & Primary Care Diagnostics, enCORE Software Version 16, General Electric, Madison, WI, USA) to determine total body and regional fat mass (FM) and lean mass (LM). Participants were asked to wear light-weight clothing as well as remove their shoes before the measurement was taken. Height, weight, age, and ethnicity were entered into the DXA database prior to each scan. Participants were positioned in the center of the table in a supine position with their arms at their sides and palms facing down. A foam pad and strap at the feet were secured before the scan to ensure consistent foot positioning and orientation of the feet perpendicular to the scanning table throughout the duration of the scan. Reliability from a previous study from this lab showed for FM (intraclass correlation coefficient [ICC] = 0.99, standard error of measurement [SEM] = 0.46 kg), and lean mass (ICC = 0.99, SEM = 0.81 kg).

*Heart Rate Variability*

Heart rate variability was measured using a heart rate monitor strapped to the participant’s chest (Polar H10, Polar Electro Oy) to collect raw RR intervals, and was assessed
through an app (EliteHRV) for Standard Deviation of Normal-to-Normal RR intervals (SDNN) and Root Mean Square of Successive Differences (RMSSD). After each visit, recordings were uploaded from the app to Kubios Software (Biosignal Analysis and Medical Imaging Group, Department of Physics, University of Kuopio, Kuopio, Finland) for analysis. All readings were taken when the participant was laying supine on a table. A 30-minute resting HRV recording was taken at the beginning of each visit to determine a resting value. After completing the exercise protocol, a 15-minute recovery HRV reading was taken in five minutes increments and averaged to determine recovery. Each of the three recovery time points (5 minutes, 10 minutes, 15 minutes) were averaged to obtain average recovery. Additionally, the average of the first 5 minutes of recovery was obtained to determine immediate recovery; recovery times were also evaluated from 5-10 minutes, and 5-15 minutes. To evaluate the change in recovery from exercise, the resting value was compared against the average post-exercise recovery value and evaluated across menstrual cycle phase. Reliability for resting HRV (SDNN: ICC = 0.97, SEM = 6.4 ms, RMSSD: ICC = 0.70, SEM = 44.8 ms).

Submaximal Exercise Test

To obtain baseline fitness levels, maximal oxygen consumption (VO₂ max) was estimated using a single-stage, submaximal cardiorespiratory fitness assessment on a treadmill (4FRONT, Woodway, Woodway USA, Inc., Waukesha, WI, USA). Heart rate (HR) was monitored during the assessment period using a Polar chest strap heart rate monitor and watch. Participants completed a warm-up consisting of walking on the treadmill at a self-chosen speed between 3.22 to 7.24 kilometers per hour (2.0 to 4.5 miles per hour, respectively) at a 0% grade to elicit a HR within 50 to 70% of their age predicted HR maximum (HR max = 207 – 0.7 × age). Following completion of the warm-up protocol, participants walked at the same self-selected speed for four
minutes at a 5% grade. Heart rate was recorded at the end of each minute and HR of the last two minutes was averaged to determine the final, average HR. To estimate VO$_2$\text{max}, the following equation was used:

$$VO_2\text{max} = 15.1 + 21.8 \times \text{Speed (mph)} - 0.327 \times \text{Heart Rate (bpm)} - 0.263 \times \text{Speed} \times \text{Age (year)} + 0.00504 \times \text{Heart Rate} \times \text{Age} + 5.98 \times \text{Sex (0 = female)}$$

\textit{Repeated Sprint Ability}

Participants completed a repeated sprint test on a friction-loaded cycle ergometer (Monark 894E, Stockholm, Sweden). Following a two-minute warm-up cycling between 50-60 rpm against a resistance of 0.5kg, a subsequent warm up of two 30 second bouts of cycling between 85-115 rpm at a resistance of 1.5kg was completed, with 60 seconds of passive rest in between. After the warm-up, participants completed 10 six-second maximal sprints with 30 seconds of passive recovery in between. The resistance was adjusted to 65 g/kg of the participant’s weight. Peak power (PP), time to peak power (TPP), fatigue index (FI), and decline in PP along with HR and rate of perceived exertion (RPE) per sprint were calculated by manufacturer software recorded. Reliability for AP from our laboratory is reported at (Power: ICC = 0.97, SEM = 120.8 W, FI: ICC = 0.97, SEM = 4.0%).

\textit{Supplementation}

Supplementation was a randomized, double-blind design. Participants were randomly assigned to the order for when they consumed five consecutive days of supplementation or placebo, using a computer-generated allocation sequence (Random Allocation Software) for follicular and luteal phases, respectively. Starting the day of or the day after their visit, participants were instructed to consume $4 \times 5$ g doses of creatine monohydrate (20 grams total) (Creapure® AlzChem, Trostberg GmbH, Germany, GRAS Notice No GRN 931; Creatine
Monohydrate) or 4 × 5 g doses of a non-caloric powder for five consecutive days (20 grams total; crystal light). To achieve a double-blind design, the supplementation and placebo were identical in color and taste and were measured and packaged by an individual not directly involved with the distribution of the treatments. To create both treatments, a calibrated scale (Sartorius Portable Balance) was used to measure 5 g of creatine monohydrate in addition to 2 g of a non-caloric powder for the creatine group and 3.5 g of the non-caloric powder to form the placebo. Both treatments were distributed in opaque containers and were identical in texture; treatments were consumed orally. Participants were asked to consume each individual 5 g dose with 6-8 ounces of water at regular intervals and to take each dose around the same time each day. To track compliance participants were asked to log each dose and to return individual dosing packets to the laboratory at the end of each loading phase (PL: 99.8%, CrM: 96.6%). A minimum of four weeks was allotted before participants returned for their second randomized session (between visits 3 and 4). Participants were asked to continue their cycle tracking throughout the 4-week washout period; and were tentatively scheduled for their pre-supplementation visit in the next designated phase. Throughout this washout period, participants were asked to continue normal activity, diet, and keep caffeine consumption below 200mg. The supplement for the next cycle was distributed at the following pre-supplementation visit (visits 2 and 4).

**Dietary Intake**

To account for normal dietary intake, three-day diet logs were given to each participant after their first visit; they were instructed to return the completed diet log at the end of the loading phases. Participants were instructed to record their normal diet, as detailed as possible, for two nonconsecutive weekdays and one weekend day. Using nutrition analysis software (The
Food Processor, version 10.12.0, Esha Research, Salem, OR, USA), diet logs were evaluated for average calories (CAL; kcal), carbohydrate (CHO; g), fat (FAT; g), protein (PRO; g) and relative protein (g/kg body mass) intake (PL: CAL- 1831.5 ± 106.7 kcal, CHO- 222.9 ±15.8 g, PRO- 90.7 ± 7.3 g, FAT- 65.2 ± 4.6 g, relative PRO- 1.4 ± 0.1 g/kg; CrM: CAL- 1771.1 ± 106.7 kcal, CHO- 208.8 ±15.8 g, PRO- 84.0 ± 7.3 g, FAT- 66.3 ± 4.6 g, relative PRO- 1.3 ± 0.1 g/kg). When evaluated by paired sample t-tests, there were no significant differences between CAL (p=0.987), CHO (p=0.972), PRO (p=0.395), FAT (p=0.791) and relative protein intake (p=0.670) between the PL and CrM groups.

Statistical Analysis

Separate ANCOVAs, covaried for fitness level from estimated VO2 max were used to compare HRV values (SDNN, RMSSD [ms]) between supplement groups. Separate mixed factorial ANOVAs [2 × 2; treatment (CrM vs. PL) × (Δ FP vs. Δ LP)] were used to compare the change scores between groups for all primary outcomes (AP [W], PP [W], tPP [ms], FI [%]); in the event of a significant interaction, post hoc comparisons were analyzed using Bonferroni pairwise comparisons. For each sprint, change scores from baseline to post-supplementation were calculated for PP and TP. Change scores (post-pre) were calculated for total AP and FI in the FP vs. LP. A series of two-way (2 × 10; treatment × sprint) mixed model ANOVAs were used to compare change scores for each sprint between treatments. The Bonferroni method was used to evaluate post hoc comparisons. Analyses were performed using SPSS software (Version 20.0; IBM, Armonk, NY, USA). Statistical significance was set a priori at α≤0.05.
Results

Standard Deviation of Normal-to-Normal RR intervals (SDNN)

Average recovery change (0-15min)

There was no significant phase × supplement interaction (p=0.552). In the PL group, values decreased in both the FP (-1.0 ± 2.0 ms) and the LP (-2.8 ± 2.9 ms); whereas the CrM group displayed an increase in both the FP (2.1 ± 2.0 ms) and the LP (3.2 ± 3.0 ms) (Table 2). There was no main effect for phase (p=0.355), with minimal change seen during the FP (Δ0.5 ± 1.4 ms) and the LP (Δ0.2 ± 2.1 ms). There was no main effect for supplement (p=0.095). There was a non-significant difference (-4.6 ± 1.8 ms) between PL and CrM groups (Figure 4).

Immediate Recovery (first 5min)

There was no significant phase × supplement interaction (p=0.293). In the PL group there was an increase from FPpre (77.3 ± 5.8 ms) to FPpost (80.0 ± 5.1 ms) and an increase from LPpre (69.5 ± 4.9 ms) to LPpost (74.3 ± 4.7 ms) (Table 1). Whereas in the CrM group there was a decrease from FPpre (78.6 ± 6.0 ms) to FPpost (74.1 ± 5.2 ms) and a decrease from LPpre (65.7 ± 5.0 ms) to LPpost (59.8 ± 4.9 ms). There was no main effect for phase (p=0.382) with minimal differences seen between FPpre and FPpost (Δ0.9 ± 3.8 ms) and between LPpre and LPpost (Δ0.5 ± 4.1 ms). There was no main effect for supplement (p=0.299) but there was a small non-significant difference between the PL and the CrM group (5.7 ± 5.4 ms).

Rest vs. Average Recovery

There was no phase × supplement interaction (p=0.057); collapsed across groups LPpost resulted in the smallest difference (39.1 ± 1.7 ms) compared to LPpre (73.8 ± 5.0 ms), FPpre (73.3 ± 5.3 ms) and FPpost (71.5 ± 5.2 ms). There was no main effect for phase (p=0.093). There
was no main effect for supplement (p=0.368). There was a non-significant difference (-5.7 ± 6.3 ms) between PL and CrM groups.

Resting

When pre-supplementation phases were compared, there was no significant differences between FPpre and LPpre (4.4 ± 34.8 ms; p=0.439). When compared across all four phases and by supplement group, there was no phase × supplement interaction (p=0.352). There was a main effect for phase (p=0.030), with a decrease between FPpre and FPpost (-2.4 ± 4.3 ms) and between LPpre and LPpost (-6.6 ± 4.8 ms) (Figure 5).

Root Mean Square of Successive Differences (RMSSD)

Average recovery change (0-15min)

There was no phase × supplement interaction (p=0.890). There was a greater decrease in the CrM group from LPpre (12.0 ± 3.4 ms) to LPpost (8.4 ± 2.1 ms), compared to the PL group from LPpre (15.6 ± 3.3 ms) to LPpost (15.1 ± 2.0 ms) (Table 3). There was no main effect for phase (p=0.155). The greatest difference was seen between FPpost and LPpost (2.5 ± 2.1 ms). There was no main effect for supplement (p=0.113). There was a non-significant difference between the PL and CrM group (-17.7 ± 10.9 ms).

Immediate Recovery (first 5min)

There was no significant phase × supplement interaction (p=0.542). There was no main effect for phase (p=0.842). FPpre (16.7 ±2.7 ms) and FPpost (16.0 ± 2.7 ms) values were higher compared to LPpre (13.9 ± 2.2 ms) and LPpost (12.2 ± 1.6 ms) values (Table 3). There was no main effect for supplement (p=0.563). There was a small difference (2.0 ± 3.4 ms) between PL and CrM groups.
Rest vs. Average Recovery

There was no phase × supplement interaction (p=0.590); collapsed across groups, the smallest difference was seen in the PL (65.6 ± 7.9 ms) and in the CrM group (67.1 ± 8.2 ms), in LPpost. There was no main effect for phase (p=0.168). The greatest difference was seen between FPpre and LPpost (12.7 ± 4.7 ms). Similar differences were seen between FPpre and FPpost (4.3 ± 4.0 ms) and between LPpre and LPpost (4.5 ± 5.5 ms). There was no main effect for supplement (p=0.312). There was a non-significant difference (-9.7± 9.5 ms) between PL and CrM groups.

Resting

When pre-supplementation phases were compared, there was no significant differences (p=0.104) between FPpre and LPpre (8.5 ± 32.0 ms). When compared across all four phases and by supplement group, there was no phase × supplement interaction (p=0.331). There was no main effect for phase (p=0.079). There was no main effect for supplement (p=0.566). There was a small non-significant difference between PL and CrM groups (-5.4 ± 9.4 ms).

Exercise Performance

Total Average Power

There was no significant phase × supplement interaction (p=0.293) for the change in AP across menstrual cycle phase (Table 4). There was no main effect for phase (p=0.406) or supplement group (p=0.284). Though not significant, across both groups the difference in AP was higher in the FP compared to the LP (782.3 ± 930.9 W); and higher in the CrM group compared to the PL group (1002.0 ± 921.0 W).
Fatigue Index

For FI, there was a significant phase × supplement interaction (p=0.048) (Figure 6). When decomposing the model, there were no significant differences across phase for PL (p=0.848) or CrM (p=0.143) groups. In the PL group there was a slight decrease in the FP (-3.6 ± 5.9%) and a smaller change in the LP (0.1 ± 8.1%). In the CrM group there was an increase in the FP (4.1 ± 19.6%) and a decrease in the LP (-5.8 ± 19.0%) (Table 4). There was a significant main effect for supplement (p=0.048); the PL group exhibited a greater decrease in FI (-1.7 ± 2.2 %) compared to the CrM group (-0.8 ± 2.3 %), although not significantly from each other (p=0.781). FI resulted in an increase in the FP (0.2 ± 2.3%) and a decrease in LP (-2.8 ± 2.3%) (p=0.366). When collapsed across supplement group there was a non-significant increase in FI in the FP (0.2 ± 2.3%, p=0.102) and a decrease in LP (-2.8 ± 2.3%, p=0.211).

Peak Power

There was no significant phase × supplement × sprint interaction (p=0.685). There were no significant phase × supplement (p=0.385), sprint × supplement (p=0.530), or time × sprint interactions (p=0.596). There was a main effect for phase (p=0.026) and sprint (p<0.001). Pairwise comparisons for phase demonstrated FPre was significantly lower than LPpost (-19.2 ± 8.79 W; p=0.039), LPpost was significantly greater than LPpre (22.9 ± 7.6 W, p=0.005) (Figure 7) (Table 4). Pairwise comparisons for sprint demonstrated a significant decrease in PP in sprints one through four compared to sprint 10 (p<0.001) and for sprint five, compared to sprint 10 (p=0.005). No significant differences were seen between bout 10 and bout six (p=0.066) or bouts seven through nine (p=0.999) (Figure 8).
Time to Peak Power

There was no three-way interaction for phase × sprint × supplement (p=0.232), and no two-way phase × supplement (p=0.953) or sprint × supplement interaction (p=0.664). There was a significant phase × sprint interaction (p<0.001), although no significant differences resulted between tPP per sprint in FPpre and LPpre (p=0.348) or between sprint FPpost and LPpost (p=0.999). Additionally, there were main effects for phase (p=0.002) and sprint (p>0.001). Pairwise comparisons demonstrated a significantly greater tPP in FPpost compared to FPpre (Δ544.64 ± 93.3 ms; p<0.001). FPpre was also lower than LPpost (Δ503.1 ± 183.1 ms) but it was not significant (p=0.063) (Table 4). Pairwise comparisons showed a significant decrease in tPP between sprints three, five (Δ679.3 ± 157.8 ms, p=0.009) and 10 (Δ571.5 ± 135.0 ms, p=0.011). There was also a significant decrease between sprint nine to 10 (Δ423.8 ± 111.6 ms, p=0.034). There were no main effects for supplement (p=0.668) (Figure 9).

Discussion

Previous research supports the potential for Cr supplementation to enhance recovery, through hydrogen ion buffering and pH regulation, increasing intramuscular PCr, and promoting greater glycogen storage. Given physiological differences that could uniquely impact Cr utilization in women, such as CK fluctuations throughout the cycle, suppression of glycolytic enzymes by estrogen, and increased sympathetic activity in the luteal phase, there is potential for sex-specific differences in exercise and recovery outcomes. In the current study, CrM appeared to have no significant effect on recovery measured from HRV. In the 15min post exercise recovery, a slight improvement was seen in the CrM group in both phases (FP and LP) in SDNN values. For immediate recovery, the CrM group exhibited a lower HRV (MD FP: -4.4 ms; MD LP: -5.0 ms) compared to the PL (MD FP: 2.5 ms; MD LP: 4.0 ms). CrM
supplementation did not significantly influence resting HRV values, but when resting values were compared to recovery values, the smallest change between rest and recovery was demonstrated in LPpost (MD: 39.1 ms). HRV appeared to be impacted by MC with lower SDNN (4.4 ± 34.8 ms) and RMSSD (8.5 ± 32.0 ms) outcomes in the LP, regardless of supplementation, suggesting a delayed recovery. Across both groups, there was a decrease in performance in the LP, represented by a decrease in AP and a larger FI. There was a significant positive effect on FI with CrM supplementation demonstrated by a decrease in the LP (-5.8 ± 19.0 %), representing greater fatigue resistance compared to the PL group (0.1 ± 8.1 %).

Recovery

To date, little data exists characterizing the response of SDNN and RMSDD over an exercise recovery period, however it is known that HRV is decreased in the five to thirty minutes following high-intensity exercise in men\(^{15,88,89}\). Previous research in men using a similar repeated sprint exercise stimulus has demonstrated a decrease in the natural logarithm of standard deviation of normal-to-normal intervals (LnSDNN), HRV immediately post-exercise (Mean ± SD: 1.62 ± 0.39 ms) followed by a gradual rise after 10 minutes (1.69 ± 0.52 ms) and 30 minutes (2.81 ± 0.53 ms)\(^{17}\). A study in active men and women examining post-exercise HRV reported a similar trend when evaluating total power (ms\(^2\)) after high-intensity exercise; total power (the sum of frequency-domain measures) as lower when measured across 7-27 minutes of recovery (6.1 ± 0.7 ms\(^2\)) compared to measurements across 30-50 minutes (6.76 ± 0.8 ms\(^2\))\(^{85}\). In the present study, instead of a gradual increase, HRV remained elevated immediately after exercise (0-5 min) and began to decrease by minute 10 of recovery, and then gradually increased by minute 15 of recovery, regardless of supplementation. Within the CrM group RMSSD values were lower compared to the PL group (MD: -17.7 ± 10.9 ms), however, this decrease could be
explained by the greater increase in power output observed in the CrM group (Table 2). While there was no significant phase × supplement interaction, average recovery over 15 minutes in the PL group went down in both the FP (-1.0 ± 2.0 ms) and the LP (-2.8 ± 2.9 ms); whereas the CrM group displayed an increase in both the FP (2.1 ± 2.0 ms) and the LP (3.2 ± 3.0 ms) from pre-to post-supplementation. Existing data is unclear on whether a specific menstrual phase effects performance or recovery differently, and to our knowledge no data on post-exercise HRV exists in relation to menstrual cycle. This study suggests HRV may be lower in the LP, compared to the FP. Although not significant, CrM supplementation provided a slight improvement in exercise recovery HRV values across both phases of the MC.

Resting

Little data exists evaluating the impact of CrM on resting HRV, with none evaluating women. One previous investigation in male bodybuilders reported attenuated time-domain values following four weeks of CrM (178.37 ± 60.8 ms) compared to placebo 194.31 ± 36.1 ms)\textsuperscript{90}. The current study reported no significant effect of CrM on resting HRV compared to PL (-5.4 ± 9.4 ms). Since creatine is an energy buffer for short-term energy systems, utilized primarily in high intensity exercise, it is unsurprising that it would not significantly impact resting values due to the difference in primary energy system usage. Data examining HRV at rest in different menstrual phases suggests a higher sympathetic dominance at rest in the LP when compared to the FP, with a significant relationship to estradiol-17 and progesterone concentrations\textsuperscript{19,53}. When healthy eumenorrheic females ages 18-25 were evaluated during a five minute rest period for time-domain values, both SDNN and RMSSD values were lower in the LP (SDNN: 0.03 ± 0.03, RMSSD: 23.3 ± 19.5 ms) compared to the FP (SDNN: 0.04 ± 0.02 ms, RMSSD: 31.9± 21.2 ms)\textsuperscript{20}. The present study resulted in similar effects, with a significant
decrease in SDNN across the menstrual cycle, with the largest decrease seen in the LP (-6.6 ± 4.8 ms). Though not significant, the current study showed lower RMSSD values in the LP compared to the FP (-8.5 ± 32.0 ms). This data aligns with current literature suggesting a lower HRV at rest in the LP, possibly due to high levels of estrogen and progesterone\textsuperscript{19}.

**Performance**

Short-term creatine supplementation is widely associated with improved anaerobic exercise. CrM supplementation has resulted in improvements in AP and PP over 15 sec sprints in trained males.\textsuperscript{91} A similar study in trained males examining sprint performance, with short 10 sec rest periods, resulted in a significant increase in AP from pre- to post-supplementation in the CrM group (79.7 ± 43.6 W) compared to the PL group (44.1 ± 21.3 W). In a sample of female athletes, improvements in repeated sprint times were reported for the CrM group from pre (1.75 ± 0.10 s) to post-supplementation (1.68 ± 0.11 s) compared to the PL group pre- (2.06 ± 0.98 s) and post-supplementation (2.02 ± 0.65 s)\textsuperscript{92}. Other anaerobic performance indices have been improved following CrM supplementation in women\textsuperscript{70,78,93,94}; anaerobic working capacity was significantly improved following CrM loading (10.5 ± 3.3 kJ) compared to PL (8.6 ± 1.2 kJ)\textsuperscript{75}. To date, we are aware of no other study that has accounted for the menstrual cycle with CrM supplementation. In the current study there was no significant increase in AP between groups, though the CrM group exhibited a higher AP compared to the PL group (MD: 1002.0 ± 921.0 W), which is well beyond the measurement error (120.8 W) and can be considered a real change. Non-significant improvements in PP were also demonstrated in the present study for the CrM group compared to the PL group, aligning with previous data in men and women supporting improvements in anaerobic performance following CrM supplementation. Existing data examining anaerobic performance across the menstrual cycle, without supplementation, suggests
no significant differences between MC phases\textsuperscript{63,65}. In the current study there were no significant differences in AP between phases, however AP was lower in the LP compared to the FP for both supplement groups. Additionally, improvements in AP in the LP resulted for the CrM (MD: 180.8 ± 66.0 W) group, compared to PL (MD: 171.8 ± 64.3 W), although they were not significantly different.

\textit{Fatigue Index}

Along with improved anaerobic performance, Cr supplementation could help improve the onset of fatigue during exercise. A study using a similar repeated sprint protocol in untrained males showed no significant differences in FI during repeated sprint exercise following CrM supplementation (MD: 2.68 ± 0.39\%) pre to post-supplementation\textsuperscript{8}. Another study in trained men resulted in no significant improvements in FI per sprint, however the CrM group exhibited a lower FI (average: 25.8\%) compared to the PL group (31\%)\textsuperscript{95}. A study examining FI differences in different menstrual phases reported the lowest FI in the FP (52.0 ± 3.0\%) compared to midcycle (54.0 ± 2.0\%) and LP (54.0 ± 3.0\%), with no statistical difference between phases\textsuperscript{63}. In the current study, greater improvements were seen in the LP in the CrM group (-5.8 ± 19.0\%), compared to the PL group (0.1 ± 8.1\%). Additionally, FI appeared to be impacted by the MC, with a lower FI in the FP (0.2 ± 2.3\%) compared to LP (-2.8 ± 2.3\%). The outcomes of this study suggest that an increase in fatigue, possibly seen in the LP, can be improved with short-term CrM supplementation.

\textit{Limitations}

Limitations of this study should be noted. This study used the counting method and use of cycle tracking from an app, which could allow for inconsistencies in phase/day determination depending on participant adherence to daily tracking, which may have impacted accurate
identification of MC phase. It should be noted that participants were asked to track their MC for at least one month prior to testing, and a familiarization session completed at the baseline visit was completed during menstruation which allowed for greater tracking accuracy. The sample size was smaller than anticipated for this study; the dropout rate in this study (n=15; 28%) was higher than expected than our typical dropout with female participants (10%), which may have impacted statistical significance. Post hoc sample analysis suggested that the study was adequately powered. A secondary aim of this study, due to high rates of contraceptive use, was to gather pilot data on the impact of hormonal contraception on the presented outcomes; thus, women using select hormonal contraception (i.e., Monophasic oral contraception and intrauterine device), in addition to naturally cycling women were recruited and enrolled. Due to a lower sample size from each of those groups additional stratification for statistical analyses was not conducted. Physiologically this should not have an impact on the selected outcomes, as all women were tested in similar hormonal phases, regardless of contraception type.
CHAPTER V: CONCLUSION

There appeared to be a clinically meaningful effect of CrM supplementation on some performance outcomes, particularly in the LP, shown by a 5% decrease in FI in the CrM group compared to a less than 1% change in the PL group. Though findings were not statistically significant, the data suggests that CrM could help counteract performance decrements in the LP. This data can help inform CrM loading strategies for active females. If an event is during the individuals’ LP a five-day CrM supplementation protocol prior to the start of LP (before ovulation) could help optimize supplementation and improve performance. CrM supplementation did not appear to influence HRV values. Across both groups resting HRV values were lower in the LP, suggesting the possibility of diminished recovery from exercise in this phase; this could also be attributed to the higher PP obtained during the cycling test in the CrM group. Although CrM supplementation did not appear to improve recovery HRV, this data suggests women should pay particular attention to other exercise recovery strategies in the LP, due to the lower HRV values reported in that phase. It should be noted that recovery HRV results of this study differ from previous research done in men; recovery HRV in men shows an immediate drop then a gradual increase over time up to 30 minutes after exercise. In this study, HRV remained elevated immediately after exercise, then the drop was seen after 10 minutes, then a gradual increase after 15 minutes. Future studies should examine the differences in immediate HRV response to anaerobic exercise in men vs. women to better understand potential sex-based differences in recovery. More research in women is needed to examine the effect of a longer
CrM supplementation period on performance and recovery values across different hormonal profiles. It appears that a short-term loading phase may help reduce fatigue for women, particularly in their high hormone phase. The impact of hormonal contraception on these outcomes should be explored further.
Table 1. Mean ± Standard Error demographics for creatine (CrM) and placebo (PL) group

<table>
<thead>
<tr>
<th>Supplement Group</th>
<th>Age (yrs)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>Percent Body Fat (%)</th>
<th>Estimated VO₂ Max (ml/kg/min)</th>
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</thead>
<tbody>
<tr>
<td>CrM (n=19)</td>
<td>25.5 ± 7.2</td>
<td>164.6 ± 6.1</td>
<td>66.2 ± 9.2</td>
<td>26.9 ± 6.1</td>
<td>38.3 ± 7.3</td>
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<tr>
<td></td>
<td>7.2</td>
<td>6.1</td>
<td>9.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>23.8 ± 4.3</td>
<td>167.0 ± 4.8</td>
<td>64.1 ± 7.0</td>
<td>27.9 ± 5.7</td>
<td>39.7 ± 4.6</td>
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<td>PL (n=20)</td>
<td>4.3</td>
<td>4.8</td>
<td>7.0</td>
<td></td>
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</tr>
</tbody>
</table>

* Birth control type: IUD or vaginal ring: (CrM n=2, PL n=4) Oral contraceptive: (CrM n=5, PL n=3), Natural Cycle: (CrM n=12, PL n=13).
Table 2. Mean ± Standard Error for standard deviation of normal-to-normal RR intervals (SDNN) at rest and after exercise for pre-and post-supplementation in the follicular (FP) and luteal (LP), separated by supplement group

<table>
<thead>
<tr>
<th></th>
<th>Creatine (n=19)</th>
<th>Placebo (n=20)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Follicular</td>
<td>Luteal</td>
</tr>
<tr>
<td>Resting (ms)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>120.6 ± 7.4</td>
<td>112.6 ± 7.4</td>
<td>103.3 ± 7.2</td>
</tr>
<tr>
<td>Placebo (n=20)</td>
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<td></td>
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<tr>
<td>Follicular</td>
<td>7.4</td>
<td>7.4</td>
</tr>
<tr>
<td>Luteal</td>
<td>37.0 ± 3.0</td>
<td>33.7 ± 2.4</td>
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<tr>
<td>5 minutes Post-Exercise (ms)</td>
<td>65.1 ± 5.1</td>
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<td>10 minutes</td>
<td>78.0 ± 6.0</td>
<td>73.6 ± 5.2</td>
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<tr>
<td>Post-Exercise (ms)</td>
<td>19.6 ± 2.0</td>
<td>18.3 ± 1.9</td>
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<tr>
<td>15 minutes</td>
<td>19.8 ± 2.1</td>
<td>19.1 ± 2.2</td>
</tr>
<tr>
<td>Post-Exercise (ms)</td>
<td>25.5 ± 3.2</td>
<td>24.7 ± 3.4</td>
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<tr>
<td>5 minutes Post-Exercise (ms)</td>
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</table>

Data are presented as raw values. Significance was based on analyses of covariance (ANCOVA), covarying for an estimated VO₂max of 39.03ml/kg/min
Table 3. Mean ± Standard Error for square root of the mean squared difference of successive RR intervals (RMSSD) at rest and after exercise for pre-and post-supplementation in follicular (FP) and luteal (LP), separated by supplement group

<table>
<thead>
<tr>
<th></th>
<th>Creatine (n=19)</th>
<th>Placebo (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Follicular pre</td>
<td>Follicular post</td>
</tr>
<tr>
<td>Resting</td>
<td>98.9 ± 7.3</td>
<td>93.1 ± 8.1</td>
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<tr>
<td>Average Recovery 5 minutes Post-Exercise</td>
<td>12.2 ± 3.0</td>
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<td></td>
<td>3.0 ± 3.4</td>
<td>2.7 ± 3.4</td>
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<td>Post-Exercise  10 minutes</td>
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<tr>
<td>Post-Exercise  15 minutes</td>
<td>9.8 ± 2.7</td>
<td>12.3 ± 3.5</td>
</tr>
<tr>
<td>Post-Exercise  15 minutes</td>
<td>9.3 ± 3.0</td>
<td>9.3 ± 4.1</td>
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</table>
Table 4. Mean ± Standard Error for Total Average Power, Fatigue Index, Peak Power and Time to Peak Power for pre-and post-supplementation in follicular (FP) and luteal (LP), separated by supplement

<table>
<thead>
<tr>
<th></th>
<th>Creatine (n=19)</th>
<th>Placebo (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Follicular pre</td>
<td>Follicular post</td>
</tr>
<tr>
<td><strong>Total Average</strong></td>
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<td></td>
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<tr>
<td>Power (W)</td>
<td>3160.5 ± 246.5</td>
<td>5116.5 ± 5</td>
</tr>
<tr>
<td>Fatigue Index (%)</td>
<td>29.2 ± 2.6</td>
<td>37.9 ± 3.5*</td>
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<tr>
<td>Peak Power (W)</td>
<td>492.9 ± 21.9</td>
<td>508.1 ± 21.7</td>
</tr>
<tr>
<td>Time to Peak Power (ms)</td>
<td>1663.5 ± 181.6</td>
<td>2265.6 ± 207.3</td>
</tr>
</tbody>
</table>

* indicates statistical significance from pre- to post-supplementation (p ≤ 0.005)
**FIGURES**

**Figure 1.** Representation of hormone levels throughout a 28-day menstrual cycle.\(^{59}\)
Figure 2. Study Design
Figure 3. CONSORT (Consolidated Standards of Reporting Trials) diagram.
Figure 4. Average recovery standard deviation of normal-to-normal RR intervals (SDNN) across all four time points (Follicular Pre, Follicular Post, Luteal Pre, Luteal Post) in the PL group vs. CrM group.

Figure 5. Resting standard deviation of normal-to-normal RR intervals (SDNN) across all four time points (Follicular Pre, Follicular Post, Luteal Pre, Luteal Post) in the PL group vs. CrM group. *indicates statistical significance between pre and post supplementation across both groups.
Figure 6. Fatigue index changes across all four time points (Follicular Pre, Follicular Post, Luteal Pre, Luteal Post) in the placebo (PL) group vs. Creatine (CrM) group. * indicates significant phase × supplement interaction (p<0.05). + indicates a significant main effect for supplement.
Figure 7. Peak Power per sprint changes across all four time points (Follicular Pre, Follicular Post, Luteal Pre, Luteal Post) in the placebo (PL) group vs. creatine (CrM) group. No statistical significance was reported.
**Figure 8.** Peak Power Per Sprint (W) between placebo (PL) and creatine (CrM) group. * indicates statistical significance (p<0.05) between sprints.

**Figure 9.** Time to Peak Power Per Sprint (ms) between placebo (PL) and creatine (CrM) group. * indicates statistical significance (p<0.05) between sprints.
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