The Power-Duration Relationship is Just as Reproducible in Females as Males, Despite the Presence of the Menstrual Cycle

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The Power-Duration Relationship is Just as Reproducible in Females as Males,
Despite the Presence of the Menstrual Cycle

Jessica Joy Linde

A thesis submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Master of Science

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ABSTRACT

The Power-Duration Relationship is Just as Reproducible in Females as Males, Despite the Presence of the Menstrual Cycle

Jessica Joy Linde
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Master of Science

PURPOSE: To investigate the effect of the menstrual cycle (MC) on exercise performance across the power-duration relationship (PDR). We hypothesized females would exhibit greater variability in the PDR across the MC than males across a similar timespan, with critical power ($P_{CRIT}$) and Work prime ($W'$) being lower during the early follicular phase than the late follicular and mid-luteal phases.

METHODS: Eumenorrheic, endurance-trained females ($n = 10$, age = 24.1 ± 5.59) performed multiple constant-load-to-task-failure and maximum-power tests at three time points across the MC (early follicular, late follicular, mid-luteal phases). Endurance-trained males ($n = 10$, age = 29.5 ± 9.18) performed the same tests approximately 10 days apart to mimic the time between the phases of the MC.

RESULTS: No differences across the PDR were observed between MC phases ($P_{CRIT}$: 175.66 ± 34.97 W, $P = 0.632$, CV = 1.28 ± 0.97 %) ($W'$: 7916.53 ± 2316.69 J, $P = 0.283$, CV = 13.56 ± 6.93 %). $P_{CRIT}$ was similar for males and females ($11.82 ± 1.44$ W • kg$^{-1}$ vs. $11.20 ± 1.82$ W • kg$^{-1}$, respectively) when controlling for leg lean mass. However, $W'$ was larger ($P = 0.048$) for males ($617.28 ± 130.10$ J • kg$^{-1}$) than females ($505.24 ± 137.66$ J • kg$^{-1}$).

CONCLUSION: These findings indicate that researchers do not need to account for MC phase when conducting performance research on female subjects. Nevertheless, factors, such as body size and leg lean body mass, do limit exercise performance in males and females. As such, previous studies looking at factors limiting exercise performance in males may not always apply to females.

Keywords: menstrual cycle, power-duration relationship, critical power, $\dot{V}O_{2\text{MAX}}$, sex differences
ACKNOWLEDGEMENTS

When I decided to come to BYU, I planned to continue my collegiate track career and learn more about the human body until I obtained my master’s degree and then attend medical school. While not everything went according to plan, I got more out of my two years in Provo than I originally pictured. I fell in love with research, finding my calling to be within the field of female endocrinology. I built relationships here that I hope last forever. There are many people who helped me during this journey. Below are a few specific people I would like to thank.

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INTRODUCTION

Despite the National Institute of Health Revitalization Act of 1993 from the United States government, which promoted the inclusion of females in clinical research, females are often excluded from exercise performance research to this day (6). Historically, very few females participated in sports until Title IX was passed, which resulted in most of the research in this field being performed on males. Additionally, uncertainty regarding the impact of the female menstrual cycle (MC) on exercise performance continues to exclude females from this research. Consequently, relatively little is known about what factors specifically affect exercise performance in females and if they vary throughout the MC.

The MC is generally discussed in terms of the ovarian cycle which contains 3 parts: the follicular phase, ovulation and the luteal phase (34). During the follicular phase, an ovarian follicle matures. This phase starts with menstruation, which is defined as the discharge of blood and endometrial lining from the uterus. During ovulation, which is determined by a rise in luteinizing hormone, the follicle ruptures and an egg is released. Ovulation marks the transition from the follicular to luteal phase. During the luteal phase, degeneration of the follicle occurs.

Throughout the MC, sex hormones fluctuate, eliciting changes in physiological systems throughout the body. Specifically, estrogen has been shown to have many effects on the vasculature and blood components. Estrogen plays a role in activating endothelial-derived nitric oxide, leading to vasodilation (5) and has a long-term genomic effect through increasing nitric oxide synthase expression (24). High total and free estradiol levels were associated with high hematocrit (29). With nitric oxide being related to vascular health and blood flow (14), and hematocrit being related to the oxygen delivery and the maximum rate of oxygen consumption
(\dot{V}O_{2\text{MAX}}) (20), it seems possible that MC-induced variations in estrogen could impact aerobic exercise capacity and performance.

The most comprehensive way to measure exercise tolerance is by examining the whole power-duration relationship (PDR), which is defined as the maximum duration that a given power output can be sustained (4). The relationship between power output and duration is hyperbolic, with the maximum duration of exercise decreasing exponentially beyond a certain intensity. The hyperbolic PDR is mathematically described by two parameters: the asymptote critical power (P_{CRIT}) (18, 26, 30) and the curvature constant W prime (W') (18, 26, 30).

From a physiological standpoint, the curvilinear nature of the PDR is thought to reflect the ability of the body to compensate for exercise-induced disturbances to homeostasis at different power outputs (4). While any exercise disturbs metabolic homeostasis, the body can compensate for smaller disturbances to homeostasis by increasing factors like \dot{V}O_{2} to eventually reach steady-state or equilibrium conditions. P_{CRIT}, which is primarily fueled aerobically, is thought to represent the largest exercise-induced metabolic disturbance that is compensable (i.e., maximal metabolic steady-state) (18, 26, 30). During exercise above P_{CRIT}, disturbances to homeostasis progressively accrue and impair function throughout the exercise until reaching a catastrophic, intolerable point that causes task failure. The amount of work and disturbance to metabolic homeostasis that can be tolerated when working above P_{CRIT} before task failure is known as W' (18, 26).

Some of the most influential components determining an individual’s capacity to perform exercise across the PDR are an individual’s cardiovascular system, \dot{V}O_{2\text{MAX}}, P_{CRIT}, and fatigability (4). To date, very few studies have investigated the possibility of sex differences in the shape of the PDR, described by P_{CRIT} and W'. To our knowledge, no studies have examined
the impact of the MC on the PDR. With $P_{\text{CRIT}}$ and $W'$ being strongly influenced by cardiovascular function and fatigue development, it seems possible that $P_{\text{CRIT}}$ and $W'$ vary throughout the MC, leading to either improved or decreased performance.

A few previous studies have examined the impact of the MC on exercise tolerance but have yielded inconsistent results. This inconsistency of findings is potentially due to focusing on exercise tolerance at only one specific intensity, such as $\dot{V}O_{2\text{MAX}}$ or lactate threshold, or by not accurately determining the exact phase of the MC.

Despite the changes in physiology present because of the MC, the impact of the MC on exercise tolerance is equivocal, with some studies reporting an effect of the MC on performance (1, 12, 28) and others reporting no effect of the MC on performance (22, 33, 42). Some of the disagreement between studies may be due to error in how researchers determined the phases of the MC. MC phase has generally been identified by menstrual mapping (i.e., determining MC phase by time elapsed since the start of menstruation) (1, 33). However, Wideman et al. (41) found that when conducting a study merely based on menstrual mapping and verifying by progesterone concentrations, only 18% of females actually ovulated on the hypothesized day when counting forward from the onset of menstruation. Consequently, Schaumberg et al. (36) recommend using a more precise method consisting of menstrual mapping, urinary ovulation prediction, and serum/plasma hormone measurement.

The present investigation collected data from eumenorrheic, endurance-trained females who performed multiple constant-load exercise tests to task failure and maximum power tests at 3 time points across the MC: the early follicular phase (i.e., menstruation), the late follicular phase and the midluteal phase. Data was also collected from endurance-trained males who performed the same tests approximately 10 days apart to act as a time-control replicating the
time between the phases of the MC. We sought to determine the degree to which the MC affects the PDR and its reproducibility. We hypothesized that when implementing certain methodological approaches to reduce error and ambiguity regarding the determination of MC phase (i.e., menstrual mapping, urinary ovulation prediction, and serum/plasma hormone measurement) the PDR would have an increased variability throughout the MC within females when compared to males with $P_{CRIT}$ and $W'$ being lower during the early follicular phase than during the late follicular and midluteal phases.

**METHODS**

**Participants and Ethical Approval**

The study protocol was approved by the Institutional Review Board at Brigham Young University (F20201-213) and conformed with the ethical principles of the *Declaration of Helsinki*. All subjects provided written informed consent prior to participating in the study.

Twenty young, healthy subjects (Females: $n = 10$, age $= 24 \pm 6$, Males: $n = 10$ age $= 30 \pm 9$) were recruited (Table 1). Subjects underwent a general health screening and were included in the study if they were endurance-trained, which was defined as running approximately 10 miles or biking approximately 30 miles per week for the previous 3 months. Endurance-trained individuals were chosen for this study in order to minimize the risk of a training effect associated with repeated measurements. To ensure subjects were endurance-trained, subjects completed a 7-day recall of exercise with the International Physical Activity Questionnaire (IPAQ) (7). According to the IPAQ, all subjects qualified to be in the high activity category. Subjects were also given an accelerometer to wear on the lateral side of their nondominant wrist for seven days. Subjects were excluded if they met any of the following: history of cardiovascular disease or other heart problems, history of metabolic disease, history of
smoking or illicit drug use, currently on medication that affects the cardiopulmonary system or a high body mass index (BMI > 30).

Females were included if they had been eumenorrheic for greater than 6 months and had been recording the dates of menstruation for greater than 6 months. The average MC duration for the female subjects was 29 ± 3 days. Females were excluded if they met any of the following: currently pregnant, currently on any type of birth control, birth control use in the last 12 months, history of an irregular MC (i.e., MC lasting less than 21 or more than 35 days) (35) or have a diagnosed menstrual disorder (e.g., endometriosis, polycystic ovary syndrome, dysmenorrhea).

**Experimental Design**

Subjects reported to the laboratory eight times, completing two familiarization visits and six experimental visits to measure $P_{CRIT}$, $W'$, and $P_{MAX}$ (maximum power output). The familiarization visits were conducted on any day of the MC. The experimental visits were completed at 3 time points across the MC for females and approximately 10 days apart for males. Experimental visits were conducted during the early follicular, late follicular and midluteal phases of the MC. Female subjects started experimental visits dependent on where they currently were in their MC after familiarization visits were completed. Blood draws and ovulation tests were performed in order to confirm phase of MC. Subjects were instructed not to perform moderate to vigorous physical activity or consume caffeine or alcohol 12–24 hours before testing (Figure 1). Accuracy of all measurements was within ± 1 as results were recorded in whole numbers.
Experimental Protocol

Visit 1: Familiarization

Ramped Maximum Exercise Test and \( \dot{V}{O_2}_{\text{MAX}} \) Protocol. Subjects performed a ramped maximum cycle-ergometer (Excalibur Sport, Lode, Groningen, Netherlands) exercise test to determine maximal oxygen consumption (\( \dot{V}{O_2}_{\text{MAX}} \)) and ramped maximum cycle-ergometer exercise test power output (\( P_{\text{RAMP}} \)) (9). Subjects initiated cycling at a predetermined pedal rate (85–95 revolutions per minute [RPM]) on an electronically braked cycle ergometer. The ramped maximum exercise test started at 50 watts and increased by 1 watt every 3 seconds until task failure. Task failure was determined when the subject’s cadence fell > 5 RPM below the predetermined pedal rate for more than 5 seconds despite strong verbal encouragement. The last power output each subject was able to complete before task failure was considered their \( P_{\text{RAMP}} \).

During the exercise test, gas exchange data (e.g., \( \dot{V}{O_2} \), carbon dioxide production [\( \dot{V}{CO_2} \)], respiratory exchange ratio [RER]) (32) as well as heart rate was obtained breath-by-breath, using a Quark PFT Ergo metabolic cart (COSMED, Italy), which was calibrated before exercise according to the manufacturer’s recommendation. Each subject’s \( \dot{V}{O_2}_{\text{MAX}} \) was defined as the maximum \( \dot{V}{O_2} \) achieved during the test after taking a 30-second average of the breath-by-breath data. Heart rate was measured by a Polar heart rate monitor and logged by the metabolic measurement system. Each subject’s maximum heart rate was defined as the highest heart rate achieved during the test after taking a 30-second average of the breath-by-breath data.

\( \dot{V}{O_2}_{\text{MAX}} \) Verification Test. A \( \dot{V}{O_2}_{\text{MAX}} \) verification test was conducted 30 minutes after the ramped maximum exercise test. This test was conducted to verify that the \( \dot{V}{O_2} \) achieved during the ramped maximum exercise test was the true \( \dot{V}{O_2}_{\text{MAX}} \) of each subject (31). This test
was completed at 105% of each subject’s $P_{\text{RAMP}}$ (32). The highest heart rate and $\dot{V}O_2$ subjects achieved during either test before task failure was considered their true $\dot{V}O_2_{\text{MAX}}$.

**Body Composition.** Assessment of body composition including body fat percentage, lean mass and leg lean mass of each subject was measured with dual energy X-ray absorptiometry (Lunar iDXA, GE Healthcare, Chicago, IL, USA) (37). Anthropometric measurements of the subjects are reported in Table 1.

**Visit 2: Familiarization**

Maximum Power Output ($P_{\text{MAX}}$) Protocol. Subjects performed a 5-minute warm-up at 40% $P_{\text{RAMP}}$. When 5 seconds of the warm-up remained, subjects increased their pedaling frequency to a sprint ($\geq 120$ RPM). Once each subject reached 120 RPM, the bike automatically increased the resistance to a predetermined torque (Nm), dependent on the subject’s weight (kg) and a torque factor of 0.67 (Nm • kg$^{-1}$). A 7-second timer on the computer automatically started once the resistance increased. The subjects were encouraged to maintain the highest pedaling frequency possible and were instructed to remain seated throughout the 7-second test. The maximum power output reached during the test was considered the subjects’ $P_{\text{MAX}}$. After the first test, the subjects completed a 2-minute active recovery at 40% $P_{\text{RAMP}}$. When 5 seconds of the active recovery remained, the subjects performed another 7-second test as explained previously. The highest power output achieved during the two tests was considered their true $P_{\text{MAX}}$.

Critical Power ($P_{\text{CRIT}}$) Protocol. All $P_{\text{CRIT}}$ tests were conducted as follows: Subjects began a 5-minute warm-up at 40% $P_{\text{RAMP}}$. This was followed by a 5-minute rest interval. Subjects then completed a 3-minute warm-up at 40% $P_{\text{RAMP}}$. Following the warm-up, wattage was increased to a certain percentage (between $\sim$80% and $\sim$100%) of each subject’s $P_{\text{RAMP}}$. Subjects cycled at a predetermined pedal rate until task failure (cadence < 5 RPM below
predetermined pedal rate for more than 5 seconds despite strong verbal encouragement). During these tests, subjects were blinded to the time elapsed. After the test, subjects performed a cooldown for 5 minutes at 40% $P_{RAMP}$. The time that each subject was able to cycle at the given intensity was recorded. Each subject’s $\dot{V}O_2$ was recorded during this test.

Two $P_{CRIT}$ familiarization tests were conducted during this visit; the first test 30 minutes after the $P_{MAX}$ test and the second test 30 minutes after the first $P_{CRIT}$ test. For the first $P_{CRIT}$ familiarization test, subjects performed at 80% of their $P_{RAMP}$. Dependent on time-to-task failure during the first test, the percentage was manipulated by increasing or decreasing the wattage above or below 80% in order to achieve a range of durations between tests.

**Testing Phase Selection and Menstrual Cycle Verification**

Three phases across the MC were examined in this study, which included the early follicular, late follicular and midluteal phases. Two visits for each phase were conducted within 24–48 hours of each other. Females were assigned to start Phase 1 testing during one of the 3 phases of their MC. Phase 1 testing was dependent on the female’s nearest MC phase (i.e., early follicular, late follicular or midluteal phase) after the completion of familiarization testing.

A combination of factors, including menstrual mapping, urinary ovulation prediction and plasma hormone measurement, was considered when determining testing days (36). Menstrual mapping and ovulation testing was used to determine when the testing should be conducted pre- and postovulation. Ovulation tests were performed daily, starting on Day 7 (22, 36) of their MC, and testing concluded after a luteinizing hormone (LH) spike was detected. At-home ovulation testing kits (Quantitative Ovulation Predictor, Easy Healthcare Corporation, Burr Ridge, IL, USA) which measured LH concentration, were given to females at the end of Visit 2. Females downloaded the “Premom” mobile application and followed testing instructions as given by
manufacturer. LH values were reported to the researcher. Blood draws were performed on the first day of each testing phase, using plasma to measure estrogen and progesterone concentrations, to confirm females were in the correct MC phase.

Male subjects completed testing visits approximately 10 days apart to mimic the time between phases of the MC, as females would be performing testing near Days 2, 12 and 22 of their MC. The testing of the male subjects acted as a time control to determine the repeatability and natural variation in the measurements.

**Phase 1: Visits 3-4**

**Visit 3: Blood Draw and Critical Power Testing**

**Blood Draw Protocol.** Following a 4-hour fast, a sample of approximately 12 ml of venous blood was collected by a certified phlebotomist who drew from the antecubital area after the subject performed 20 minutes of seated rest.

**Blood Analysis.** Utilizing the microhematocrit method (25), hematocrit was measured in triplicate by using capillary tubes (Micro-Cal Heparinized Hematocrit Capillary Tubes, Chase Scientific Glass, Inc., Rockwood, TN, USA) filled with venous blood (IEC Micro-MB Centrifuge, International Equipment Company, Needham Heights, MA, USA), which was spun according to centrifuge manufacturer recommendations. A microcapillary reader (Micro-Capillary Reader, International Equipment Company, Needham Heights, Massachusetts, USA) was used to measure hematocrit. The average of the 3 values was recorded. Hematocrit values are reported in Table 3.

Hemoglobin was measured in triplicate by using microcuvettes (HemoCue Hb 801 Microcuvettes, HemoCue AB, Angelholm, Sweden) filled with venous blood and placing them
in a HemoCue Analyzer (HemoCue Hb 801 Analyzer, HemoCue AB, Angelholm, Sweden). The average of the 3 values was recorded. Hemoglobin values are reported in Table 3.

Estradiol and progesterone concentrations were measured using plasma, which were obtained postcentrifugation and stored at −80°C until analysis was performed. Total concentrations of 17β-estradiol and progesterone were measured in duplicate using hormone-specific, enzyme-linked immunoassay kits (Item No. 501890 and Item No. 582601, respectively; Cayman Chemical, Ann Arbor, MI, USA). The coefficient of variation (CV) for the ELISA kits, as provided by the manufacturer, were 8–12% for 17β-estradiol and 5–8% for progesterone. Hormonal profiles were determined “acceptable” if an increase in 17β-estradiol was observed from the early follicular phase to late follicular phase and a peak in progesterone was observed during the luteal phase. Sex hormone concentrations are reported in Table 2.

**PCRIT Tests.** After blood draws were complete, subjects performed two PCRIT tests with a 30-minute rest between tests. Protocol for the PCRIT tests can be found under the section ‘Critical Power (PCRIT) Protocol’. Dependent on time-to-task failure during familiarization visits, the constant load was completed at approximately 80% PRAMP for the first test and approximately 90% PRAMP for the second test.

**Visit 4: PMAX and PCRIT Testing**

Subjects performed a PMAX test. Protocol for this test can be found under the section ‘Maximum Power Output (PMAX) Protocol’. After the PMAX test was complete, subjects performed two PCRIT tests. Protocol for these tests can be found under the section ‘Critical Power (PCRIT) Protocol’. The PMAX test and first PCRIT test were followed by 30-minute rest periods. Dependent on time-to-task failure during Visit 3 and the familiarization visits, the constant load
was completed at approximately 85% P\textsubscript{RAMP} for the first P\textsubscript{CRIT} test and approximately 95% P\textsubscript{RAMP} for the second P\textsubscript{CRIT} test.

\textit{Phases 2 and 3: Visits 5–8}

\textbf{Repeated Measures}. Phase 2 (Visits 5–6) and Phase 3 (Visits 7–8) repeated the procedures outlined in Phase 1. For female subjects, these 2 phases were completed during the 2 remaining MC phases in order to observe the PDR during each phase of the MC (i.e., early follicular, late follicular, midluteal).

\textbf{P\textsubscript{CRIT} and W' Calculation}

To calculate P\textsubscript{CRIT}, a requirement of 3–5 constant load tests at different percentages of the subjects’ P\textsubscript{RAMP} must be completed (26). In this study, four P\textsubscript{CRIT} tests per testing phase were performed. The duration of each test had to be completed between approximately 2 and approximately 15 minutes (18, 26, 30). Additionally, the longest duration test had to be at least 5 minutes longer than the shortest duration test to qualify as an accurate assessment of P\textsubscript{CRIT} (26).

The four powers and corresponding durations for each phase were entered into a critical power calculator on exphyslab.com (21) which calculated P\textsubscript{CRIT} and W' with 3 different models including a hyperbolic model, a linear model and a 1/time model. The model chosen to report was specific to each subject and was determined by selecting the model with the lowest sum of error. To examine sex differences, we also normalized P\textsubscript{CRIT}, W' and P\textsubscript{MAX} by leg lean mass.

\textbf{Statistical Analysis}

A mixed-model ANOVA (2 groups with 3 repeated measures) was performed to determine the impact of biological sex and MC phase (or equivalent elapsed time for males) on P\textsubscript{CRIT}, W' and P\textsubscript{MAX}. In the event of a significant F-statistic, post hoc analysis with planned comparisons (t-tests) were performed. The reproducibility of measurements was determined with
intraclass correlation (ICC) and CV. Independent-samples t-test was used to compare the means of subject characteristics for male and female subjects. The alpha level for the statistical procedures was set to $P \leq 0.05$. All data are expressed as the mean ± standard deviation of $n = 10$ subjects in each group unless stated otherwise.

**RESULTS**

**Effect of the MC on the PDR**

Seven of the 10 female subjects presented a regular hormonal profile, defined as having high estrogen concentration during the late follicular phase and high progesterone concentration during the midluteal phase. One female did not have an increase in progesterone during the midluteal phase while all 3 of the females who did not present a regular hormonal profile did not have an increase in estrogen during the late follicular phase. When analyzing the PDR parameters while only using the 7 females with a regular hormonal profile, it was found that the results below remained unchanged.

**Effect of Sex and the MC on $P_{CRIT}$**

Ten female subjects performed multiple time-to-task failure tests to determine $P_{CRIT}$ and $W'$ at 3 time points across the MC: early follicular (i.e., menstruation), late follicular and midluteal phases. In female subjects the average time between Phases 1 and 2, and Phases 2 and 3 was $10.71 \pm 4.52$ and $10.86 \pm 4.26$ days, respectively. The median time between Phases 1 and 2 and Phases 2 and 3 in male subjects (Phase 1–Phase 2: 8 days, Phase 2–Phase 3: 10 days) was similar to females (Phase 1–Phase 2: 9.5 days, Phase 2–Phase 3: 10.5 days). Due to scheduling conflicts, 3 females had to report during a second MC. Consequently, the average days lapsed between Phase 1 and Phase 2 was $11.05 \pm 4.11$ days and the average days lapsed between Phase 2 and Phase 3 was $19.31 \pm 19.65$ days.
As illustrated in Figure 2A, mixed-model ANOVA for P\textsubscript{CRIT} revealed a main effect of sex (P = 0.002), indicating that, on average, females (175.66 ± 34.97 W) exhibited significantly lower P\textsubscript{CRIT} than males (222.86 ± 22.84 W). No main effect of phase (P = 0.632) or sex-by-phase interaction (P = 0.836) was observed. When P\textsubscript{CRIT} was normalized for leg lean mass, no main effect of phase (P = 0.609) or sex (P = 0.405) or a sex-by-phase interaction (P = 0.850) were observed (Females: 11.20 ± 1.82 W • kg llm\textsuperscript{-1} vs. Males: 11.82 ± 1.44 W • kg llm\textsuperscript{-1}). As illustrated in Figure 2C, the coefficient of variation for multiple P\textsubscript{CRIT} determinations were similar for males and females (1.40 ± 0.78 % and 1.28 ± 0.97 %, respectively, P = 0.756). Intraclass correlation showed excellent reliability for males and females (0.966, P < 0.001; and 0.992, P < 0.001, respectively). Importantly, there was no main effect of phase (P = 0.386) or sex (P = 0.303) or a sex-by-phase interaction (P = 0.413). The percent P\textsubbox{MAX} at which P\textsubscript{CRIT} was achieved (Females: 32.70 ± 6.01% vs. Males: 29.85 ± 6.01%). Additionally, there was no main effect of phase (P = 0.667) or sex (P = 0.336) or sex-by-phase interaction (P = 0.963) for the percentage of P\textsubbox{RAMP} at which P\textsubscript{CRIT} was achieved (Females: 69.35 ± 4.14% vs. Males: 70.95 ± 2.92%).

To determine if there was a learning effect between phases, a one-way repeated measures ANOVA was performed for female and male groups in the chronological order of visits. P\textsubscript{CRIT} was similar across all times for males (P = 0.609) and females (P = 0.897).

**Effect of Sex and the MC on W'**

As illustrated in Figure 3A, mixed-model ANOVA for W' revealed a main effect of sex (P = 0.002), indicating that females (7916.53 ± 2316.69 J) exhibited a significantly lower W' than males (11737.46 ± 2886.13 J). No main effect of phase (P = 0.283) or sex-by-phase interaction were observed (P = 0.487). When W' is normalized for leg lean mass (Figure 3B), the
main effect of sex persisted ($P = 0.048$), with females ($498.78 \pm 137.26 \text{ J} \cdot \text{kg}^{-1} \cdot \text{lm}^{-1}$) exhibiting a lower mass-specific $W'$ than males ($617.28 \pm 130.10 \text{ J} \cdot \text{kg}^{-1} \cdot \text{lm}^{-1}$). However, no significant main effect of phase ($P = 0.285$) or sex-by-phase interaction ($P = 0.444$) were observed. The coefficient of variation for calculated $W'$ was similar for males and females (Males: $10.09 \pm 5.14 \%$, Females: $13.56 \pm 6.93 \%$, $P = 0.220$). Intraclass correlation showed moderate to good reliability among male and female subjects ($0.611, P < 0.001$; and $0.888, P < 0.001$, respectively).

One-way repeated measures ANOVA were performed for female and male subjects in the order of visits. $W'$ was similar for both males ($P = 0.271$) and females ($P = 0.995$) when ordered chronologically.

**Effect of Sex and the MC on $P_{\text{MAX}}$**

A main effect of sex ($P < 0.001$) on $P_{\text{MAX}}$ with females ($544.177 \pm 262.59 \text{ W}$) exhibited significantly lower $P_{\text{MAX}}$ than males ($760.20 \pm 106.69 \text{ W}$) (Figure 4A). No main effect of phase ($P = 0.631$) or sex-by-phase interaction ($P = 0.965$) was observed. When normalized for leg lean mass ($P_{\text{MAX}}: \text{W} \cdot \text{kg}^{-1} \cdot \text{lm}^{-1}$), the main effect of sex persisted ($P = 0.013$), with females ($34.97 \pm 5.07 \text{ W} \cdot \text{kg}^{-1} \cdot \text{lm}^{-1}$) exhibiting a lower $P_{\text{MAX}}$ than males ($40.08 \pm 4.19 \text{ W} \cdot \text{kg}^{-1} \cdot \text{lm}^{-1}$). The coefficient of variation for repeated $P_{\text{MAX}}$ determination was similar for males and females (Males: $3.26 \pm 3.33 \text{ W}$, Females: $5.64 \pm 3.32 \text{ W}$, $P = 0.127$). Intraclass correlation showed excellent reliability for males and females ($0.927, P < 0.001$; and $0.929, P < 0.001$, respectively). No learning effect was noted for $P_{\text{MAX}}$.

**Effect of Sex and the MC on $\dot{V}O_2$:**

Absolute $\dot{V}O_{2\text{MAX}} (\text{L} \cdot \text{min}^{-1})$ revealed a main effect of sex ($P < 0.001$) as females ($2.85 \pm 0.40 \text{ L/min}$) exhibited significantly lower absolute $\dot{V}O_{2\text{MAX}}$ values than males ($3.58 \pm 0.35$
L/min). However, when normalized by leg lean mass, no main effect of sex was observed (Males: 190.46 ± 22.71 ml • kg llm\(^{-1}\) • min\(^{-1}\) vs. Females: 182.43 ± 22.09 ml • kg llm\(^{-1}\) • min\(^{-1}\), P = 0.433).

The percentage of $\dot{V}O_2$ achieved during the time-to-task failure tests indicated a main effect of sex (P = 0.029), with a significant difference on Trial 1 (P = 0.029). Similar values were obtained for males and females at Trial 2 (P = 0.085), Trial 3 (P = 0.170) and Trial 4 (P = 0.486). No main effect of phase or sex-by-phase interaction was observed. The coefficient of variation for percentage of $\dot{V}O_2$\(_{MAX}\) reached during the time-to-task failure tests was similar for males and females (Males: 3.26 ± 3.33, Females: 5.64 ± 3.32, P = 0.127).

**Effect of Sex and the MC on Blood Components**

Hematocrit levels revealed a main effect of sex (P < 0.001), indicating that, on average, females (42.62 ± 1.88 %) exhibited significantly lower hematocrit than males (46.94 ± 3.46 %) but there was no main effect of phase (P = 0.963) or sex-by-phase interaction (P = 0.65) (Table 3).

Hemoglobin concentration revealed a main effect of sex (P < 0.001), indicating that, on average, females (14.63 ± 0.81 g/dl) exhibited significantly lower hemoglobin concentration than males (16.52 ± 1.05 g/dl). There was no main effect of phase (P = 0.820) or sex-by-phase interaction (P = 0.525) (Table 3).

**Effect of Sex on Body Composition**

Table 1 characterizes the female and male groups. Body mass (kg) was similar in both groups. Females exhibited significantly lower total lean mass (P < 0.001), leg lean mass (P = 0.002), and a higher body fat percentage (P < 0.001) than males.
DISCUSSION

The present study examined the influences that biological sex and the menstrual cycle (MC) have on the PDR by comparing critical power (\(P_{\text{CRIT}}\)), Work prime (\(W'\)) and maximum power output (\(P_{\text{MAX}}\)) in males and females over the course of the MC. Our data provide direct evidence that the MC does not influence the measurement of \(P_{\text{CRIT}}, W'\) and \(P_{\text{MAX}}\). As such, whole-body performance in severe and extreme exercise intensity domains exhibit similar reproducibility compared to males. While sex differences in absolute \(P_{\text{CRIT}}, W'\) and \(P_{\text{MAX}}\) were observed, some of these differences, such as \(W'\), were eliminated when normalizing for lean mass.

Effect of Sex and the MC on \(P_{\text{CRIT}}\)

\(P_{\text{CRIT}}\) is thought to represent the highest intensity that elicits compensable and sustainable disturbances to homeostasis (18, 26, 30). \(P_{\text{CRIT}}\) was extremely reproducible for both males and females. Overall, female exercise performance based upon \(P_{\text{CRIT}}\) was similar across the 3 phases of the MC evaluated (Figure 2A). Very little research has been conducted on the effect the MC has on the PDR during whole-body exercise performance. The lack of impact that the MC has on \(P_{\text{CRIT}}\) is consistent with results from Mattu et al. (22), who demonstrated that the MC had no observable impact on maximum lactate steady-state, another index of maximal metabolic steady-state (17). Thus, there appears to be no need to control for the MC, among eumenorrheic females, when studying \(P_{\text{CRIT}}\) during whole-body exercise.

Females exhibited significantly lower \(P_{\text{CRIT}}\) than their male counterparts, which is consistent with previous research (2). Normalizing \(P_{\text{CRIT}}\) by lean body mass is important in sex comparisons as males tend to have greater amounts of lean mass than females and females tend to have greater amounts of fat mass than males (3). Our subjects followed this trend (Table 1).
Previously, Ansdell et al. (2) normalized $P_{CRIT}$ by overall body mass and found that differences in $P_{CRIT}$ between sex were still evident. When normalizing by overall body mass in the current study, similar results were found. In the current study we also normalized variables of the PDR by leg lean mass to account for its activation during cycling interventions. Controlling for leg lean mass allowed us to control specifically for the lean mass driving the exercise performance being tested. We found that when normalizing by leg lean mass, $P_{CRIT}$ between sex was similar (Figure 2B). This indicates that sex differences in muscle quantity is the main source of the sex-related difference in absolute $P_{CRIT}$.

**Effect of Sex and the MC on $W'$**

$W'$ is thought to represent the amount of work and disturbance to homeostasis that can be tolerated when working above $P_{CRIT}$ (26). Contrary to our hypothesis, MC phase had no effect on $W'$ (Figure 3A). $W'$ exhibited moderate to good reliability among male and female subjects. Importantly, the coefficient of variation showed no difference between males and females (Figure 3C). Together, these data indicate that there is likely no need to control for the MC among eumenorrheic females when studying $W'$ during whole-body exercise.

Females exhibited significantly lower absolute $W'$ than their male counterparts, which is consistent with previous research (2). $W'$ is considered to be positively related to the cross-sectional area of the exercising muscle (19). Previous research found that when observing $W'$ normalized to overall body mass, males still exhibited a higher $W'$ than females (2). Given differences in body composition between males and females, we normalized $W'$ in relation to leg lean mass and found that males still exhibited a higher $W'$ than females (Figure 3B).

It is not clear what causes $W'$ to differ between male and female subjects. The exact determinants of $W'$ are also unclear. Vanhatalo et al. (39) demonstrated that while $W'$ is related
to phosphocreatine utilization and metabolite production, it is not related to fiber type
distribution. Zarzissi et al. (44) demonstrated that $W'$ is strongly correlated to the amount of
peripheral fatigue a person can tolerate before failure occurs, with prefatiguing exercise
decreasing $W'$. More research is needed to determine the cause and associated consequences of
the observed sex difference in $W'$ (16).

**Effect of Sex and the MC on $P_{\text{MAX}}$**

In this study, we defined $P_{\text{MAX}}$ as the greatest power output achieved for 1 second during
a 7-second sprint. MC phase had no effect on $P_{\text{MAX}}$ (Figure 4A). Overall, females and males
exhibited excellent reliability of $P_{\text{MAX}}$. Importantly, the coefficient of variation in $P_{\text{MAX}}$ did not
differ between male and female subjects (Figure 4C). Thus, there appears to be no need to
control for the MC among eumenorrheic females when studying $P_{\text{MAX}}$ during whole-body
exercise.

Females exhibited significantly lower absolute $P_{\text{MAX}}$ ($W$) and normalized for lean body
mass ($W \cdot \text{kg lm}^{-1}$) than their male counterparts, which is consistent with previous research (42).
As $P_{\text{MAX}}$ is strongly influenced by muscle mass, we normalized $P_{\text{MAX}}$ by leg lean mass, which
differed between males and females. Previous studies have looked at $P_{\text{MAX}}$ (42) in relation to
overall body mass and lean body mass and reported that males still had a higher $P_{\text{MAX}}$ than
females. We found that when normalizing for leg lean mass, females still exhibited a lower $P_{\text{MAX}}$
than males (Figure 4B). The cause of this difference in mass-specific maximal power is unclear,
yet differences in fiber type expression or neural recruitment patterns have been implicated (15).

**Further Implications and Considerations**

In exercise physiology research, females are not studied as often as males (6). Exclusion
of females in research is often attributed to insufficient data in regard to the impact of the MC on
exercise performance and the difficulty in implementing the complex methodology required to accurately control for the MC (11).

If the MC phase affected whole-body exercise performance, endurance performance for females would change throughout the MC, while endurance for males would remain more constant over an equal period of time. We found the MC did not affect $P_{\text{crit}}$ for female subjects, and that females exhibited the same levels of reproducibility for $P_{\text{crit}}$ as males across the same amount of time. While these findings show that PDR data are very reproducible over time, it is clear that researchers do not need to control for the MC when monitoring whole-body exercise performance in eumenorrheic females based upon $P_{\text{crit}}$. The findings of this study should only be applied to whole-body cycle exercise as earlier work (1, 10, 28) has found that specific performance of small muscle groups are affected by the cyclical changes of hormones throughout the MC.

In situations when researchers prefer to control for the MC (40), menstrual mapping should be accompanied by other measurements to verify the phase of the MC. Wideman et al. (41) found that when conducting a study merely based on menstrual mapping and verifying by progesterone concentrations, only 18% of females actually ovulated when counting forward from the onset of menstruation. Many operate on the assumption that ovulation, which separates the follicular from the luteal phase, occurs on Day 14 of the MC. However, in the current study, only 1 out of the 10 female subjects exhibited a clear LH surge on Day 14. In fact, some demonstrated an LH surge as early as Day 10 and as late as Day 20 of the MC. This is consistent with data from Soumpasis et al. (38) which found that there is a very wide range of days that ovulation can occur, and that Days 14–15 are just the average of that wide range.
One primary question that remains is: Does the MC affect the PDR in women who do not classify as eumenorrheic, including those who have menstrual disorders? In this study, we specifically recruited females without menstrual disorders. Nevertheless, menstrual disorders affect a large number of the female population, with endometriosis affecting approximately 15% of females (23), polycystic ovary syndrome affecting approximately 10% of females (43) and premenstrual dysphoric disorder affecting approximately 5% of females (13). As females with menstrual disorders make up approximately one-third of the female population, it is important to determine if the PDR relationship is affected by these disorders.

CONCLUSION

This study demonstrated that the PDR for cycling did not differ throughout the MC. Specifically, the MC had no effect on \( P_{\text{CRIT}} \), \( W' \) or \( P_{\text{MAX}} \). The accuracy (CV) and reproducibility (ICC) were similar between males and females. Additionally, we found differences in factors \( W' \) and \( P_{\text{MAX}} \) that limited the endurance in males and females, even when controlling for body composition, suggesting that existing research on males may not apply to females. Sex differences are not found when \( P_{\text{CRIT}} \) was normalized to lean leg mass. However, females perform at lower levels than males when observing absolute levels of \( W' \) and \( P_{\text{MAX}} \) that appear controlled by muscle mass. Collectively, when conducting whole-body exercise performance research on females, the phase of the MC does not need to be controlled. However, sex differences do highlight the importance of conducting sex-based research, rather than applying previously generated male-only data within the literature to females.
REFERENCES


**Table 1.** Subject characteristics and exercise performance measures

<table>
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<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>29.50 ± 9.18</td>
<td>24.10 ± 5.59</td>
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<tr>
<td>Height (cm)</td>
<td>175.30 ± 6.40</td>
<td>169.20 ± 5.96</td>
<td>0.041*</td>
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<tr>
<td>Body Mass (kg)</td>
<td>69.23 ± 6.58</td>
<td>63.87 ± 8.94</td>
<td>0.144</td>
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<tr>
<td>Total Lean Mass (kg)</td>
<td>55.39 ± 4.70</td>
<td>45.08 ± 5.79</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Leg Lean Mass (kg)</td>
<td>18.95 ± 1.64</td>
<td>15.74 ± 2.21</td>
<td>0.002*</td>
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<td>Body Fat (%)</td>
<td>16.70 ± 3.04</td>
<td>26.31 ± 5.58</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>$\dot{V}O_{2\text{MAX}}$ (L • min$^{-1}$)</td>
<td>3.58 ± 0.35</td>
<td>2.85 ± 0.40</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>$\dot{V}O_{2\text{MAX}}$ (ml • kg$^{-1}$ • min$^{-1}$)</td>
<td>52.20 ± 7.37</td>
<td>44.38 ± 7.31</td>
<td>0.028*</td>
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<tr>
<td>$\dot{V}O_{2\text{MAX}}$ (ml • kg lm$^{-1}$ • min$^{-1}$)</td>
<td>64.99 ± 7.62</td>
<td>63.45 ± 6.41</td>
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<tr>
<td>$\dot{V}O_{2\text{MAX}}$ (ml • kg llm$^{-1}$ • min$^{-1}$)</td>
<td>190.46 ± 22.71</td>
<td>182.43 ± 22.09</td>
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<tr>
<td>PRAMP (W)</td>
<td>313.90 ± 26.98</td>
<td>251.90 ± 39.62</td>
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<td>PRAMP (W • kg$^{-1}$)</td>
<td>4.57 ± 0.57</td>
<td>3.99 ± 0.71</td>
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<td>PRAMP (W • kg lm$^{-1}$)</td>
<td>5.69 ± 0.57</td>
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<td>PRAMP (W • kg llm$^{-1}$)</td>
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<td>HRMAX</td>
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<td>185.00 ± 7.47</td>
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<td>Step Count</td>
<td>15,746.71 ± 2,653.88</td>
<td>14,199.82 ± 3,506.54</td>
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Values are reported in mean ± S.D. *P < 0.05 female different from male; $\dot{V}O_{2\text{MAX}}$ absolute (L O$_2$ • min$^{-1}$): relative to body mass (ml O$_2$ • kg$^{-1}$ • min$^{-1}$); relative to kilograms of lean body mass (ml O$_2$ • kg$^{-1}$ • llm$^{-1}$ • min$^{-1}$); P$_{\text{RAMP}}$: maximum power output (W) completed during the $\dot{V}O_{2\text{MAX}}$ test; P$_{\text{RAMP}}$ (W • kg$^{-1}$) relative to kilograms of body mass; P$_{\text{RAMP}}$ (W • kg • lm$^{-1}$) relative to kilograms of lean body mass (lm); P$_{\text{RAMP}}$ (W • k$^{-1}$ • llm$^{-1}$): relative to kilograms of lean leg mass; HR$_{\text{MAX}}$: maximum heart rate during the $\dot{V}O_{2\text{MAX}}$ test
Table 2. Average female concentrations for 17β-estradiol and progesterone across the 3 tested phases of the menstrual cycle

<table>
<thead>
<tr>
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<th>EF</th>
<th>LF</th>
<th>ML</th>
<th>Phase</th>
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</thead>
<tbody>
<tr>
<td><strong>17β – Estradiol (pg/ml)</strong></td>
<td>45.57 ± 32.22</td>
<td>71.04 ± 42.14</td>
<td>116.22 ± 56.67</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td><strong>Progesterone (ng/ml)</strong></td>
<td>13.26 ± 7.61</td>
<td>14.45 ± 7.14</td>
<td>33.91 ± 17.31</td>
<td>&lt; 0.001*</td>
</tr>
</tbody>
</table>

Averages include subjects who followed the regular hormonal profile. Values are reported in mean ± S.D. *Statistically significant: P < 0.05. EF: Early Follicular; LF: Late Follicular; ML: Mid-Luteal
Table 3. Power-duration relationship results and physiological measures at 3 time points across the menstrual cycle for female subjects and 3 time points for male subjects

<table>
<thead>
<tr>
<th>Trial</th>
<th>Sex</th>
<th>Phase 1/EF</th>
<th>Phase 2/LF</th>
<th>Phase 3/ML</th>
<th>ICC.</th>
<th>C.V. (%)</th>
<th>Phase 1/EF</th>
<th>Phase 2/LF</th>
<th>Phase 3/ML</th>
<th>ICC.</th>
<th>C.V. (%)</th>
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<th>C.V. (%)</th>
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<tr>
<td>1</td>
<td>M</td>
<td>688.40 ± 162.81</td>
<td>765.10 ± 252.42</td>
<td>745.90 ± 246.77</td>
<td>0.730*</td>
<td>10.68 ± 7.27</td>
<td>0.412</td>
<td>0.171</td>
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<tr>
<td></td>
<td>F</td>
<td>630.30 ± 102.25</td>
<td>593.30 ± 92.09</td>
<td>668.40 ± 188.21</td>
<td>0.350*</td>
<td>11.29 ± 6.54</td>
<td>0.787</td>
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<tr>
<td>2</td>
<td>M</td>
<td>330.80 ± 47.28</td>
<td>339.70 ± 64.99</td>
<td>342.30 ± 74.36</td>
<td>0.739*</td>
<td>6.91 ± 4.69</td>
<td>0.457</td>
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<td>F</td>
<td>343.60 ± 56.76</td>
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<td>0.466*</td>
<td>9.65 ± 4.14</td>
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<td>594.30 ± 119.92</td>
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<td>0.665*</td>
<td>12.14 ± 7.60</td>
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<td>480.90 ± 181.01</td>
<td>565.80 ± 148.92</td>
<td>569.70 ± 155.49</td>
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<tr>
<td>4</td>
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<td>179.20 ± 23.38</td>
<td>164.30 ± 28.36</td>
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<td>F</td>
<td>154.00 ± 23.77</td>
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</table>

Values are reported in mean ± S.D. *Statistically significant: P < 0.05; EF: Early Follicular; LF: Late Follicular; ML: Midluteal; TTF: time-to-task failure; Peak VO₂ Reached: VO₂ values achieved during the time-to-task failure tests in relation to the VO₂MAX value reached during the ramped maximum exercise test; Int.: main effect of interaction; C.V.: coefficient of variation
**Figure 1. Methods Diagram.** This figure illustrates the timeline of when each test occurred throughout the study. All test percentages were held constant after the first phase of testing was completed. DEXA: dual energy X-ray absorptiometry; \( \dot{V}O_{2\text{MAX}} \): referring to the ramped maximum cycle-ergometer exercise test; \( P_{\text{MAX}} \): maximum power output reached during maximum 7-second sprint; TTF: time-to-task failure test.
Figure 2. Effect of Sex and Menstrual Cycle Phase on Critical Power (P_{CRIT}). A: Absolute P_{CRIT} B: P_{CRIT} normalized by leg lean mass (i.e., mass-specific P_{CRIT}). C: Coefficient of variation in absolute P_{CRIT}. *Significantly different than corresponding time point of male subject. Data are presented as mean ± S.D.
Figure 3. Effect of Sex and Menstrual Cycle Phase on Work-Prime ($W'$). A: Absolute $W'$ B: $W'$ normalized by leg lean mass (i.e., mass-specific $W'$). C: Coefficient of variation in absolute $W'$. *Significantly different than corresponding time point of male subject. Data are presented as mean ± S.D.
Figure 4. Effect of Sex and Menstrual Cycle Phase on Maximum Power Output (P_{MAX}). A: Absolute P_{MAX} B: P_{MAX} normalized by leg lean mass (i.e., mass-specific P_{MAX}). C: Coefficient of variation in absolute P_{MAX}. *Significantly different than corresponding time point of male subject. Data are presented as mean ± S.D.