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Does the Menstrual Cycle Influence Aerobic Capacity in Endurance-Trained Women?

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ABSTRACT

Purpose: The aim was to study if aerobic capacity varies during different phases of the menstrual cycle (MC) in endurance-trained female athletes. **Methods:** Ten endurance-trained eumenorrheic women performed a submaximal test followed by an incremental test until exhaustion three times during one MC, early follicular phase (EFP), late follicular phase (LFP), and midluteal phase (MLP). During the submaximal test, the respiratory exchange ratio (RER) and utilization of fat and carbohydrates were analyzed; and, during the incremental test, $VO_{2\ peak}$, maximal heart rate, utilization of fat and carbohydrates, and RER were analyzed. Lactate levels were analyzed at rest, during the submaximal test, and after the incremental test. The anaerobic threshold was determined at RER = 1. **Results:** No significant differences ($p < .05$) between the MC phases were seen in a maximal heart rate or VO_{2peak}. Similarly, VO_2, heart rate, RER, fatty acid oxidation, and carbohydrate oxidation at 70, 80, 90, and 100% of VO_{2peak} did not differ significantly between MC phases. There were no significant differences between these phases in resting lactate before the test or during the submaximal tests, though there was a significant difference in lactate concentration 3 minutes after the incremental test between the EFP and the LFP ($p = .043$). **Conclusion:** This study did not display variations in physiological parameters between EFP, LFP, and MLP, indicating similar aerobic capacity despite hormonal variations. This knowledge may be useful when planning for competition in aerobic events.

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Aerobic capacity; female sex hormones; incremental test; menstrual cycle; sports physiology

Knowledge of physiological responses is essential to optimize training and performance to the demands within sport. The majority of sport studies have been performed with male participants, who in contrast to female athletes do not show cyclic production of sex hormones (Costello et al., 2014). In recent decades, the number of female participants within sport has increased, and the relevance of the variation of the female sex hormones during the menstrual cycle (MC) for sport performance has been highlighted (Isacco & Boisseau, 2017).

The normal MC lasts between 21 and 35 days during which the estrogen and progesterone levels vary with a specific pattern. In addition to regulating the reproductive ability, these hormonal variations affect the cardiovascular, respiratory, and metabolic systems. Studies have also suggested that these hormones have an impact on sports performance (Lebrun & Constantini, 2013), but the hormonal effects on endurance have shown conflicting results, as some studies showed differences in endurance during the MC cycle (Godbole et al., 2016; Ross et al., 2017) whereas other studies showed no differences (Smekal et al., 2007; Vaiksaar et al., 2011). These results denoting a phase dispersed in another phase can, to some extent, be explained by the interaction between estrogen and progesterone since estrogen has been shown to counteract the effects of progesterone and vice versa (Isacco & Boisseau, 2017; Lebrun & Constantini, 2013; Oosthuyse & Bosch, 2010). The disperse results can also be attributed to the high intra- and interindividual variability in training status and estrogen and progesterone concentrations as well as by the pulsatile release of these hormones (de Jonge, 2003; Filicori et al., 1984; Rechichi et al., 2008; Vaiksaar et al., 2011).

Estrogen and progesterone can potentially have an impact on substrate utilization, with a tendency toward having tissue-specific counteracting effects, related to the concentration of the respective hormone (Oosthuyse & Bosch, 2010). Estrogen has been shown to increase the uptake and storing of glycogen in the liver and muscles, resulting in an increased fatty acid oxidation. Metabolic effects of progesterone include relative glucose intolerance and insulin resistance which has been displayed by an increase in circulating free fatty acids, lower RER, and lower blood lactate levels during submaximal training in the luteal phase (LP) and ovulation (Ashley et al., 2000; Devries et al., 2006). This has led to the speculation that the aerobic capacity might be higher during the LP (Lebrun & Constantini, 2013). An increase in fatty acid oxidation and decrease in oxidation of carbohydrates while running during the LP compared to the follicle phase (FP) has also been shown (Isacco et al., 2012), as well as a lower lactate response during the LP that has been attributed to the former findings (Jurkowski et al., 1981; McCracken et al., 1994). However, these results are not consistent between studies (Bemben et al., 1995; Galliven et al., 1997). Female athletes compete during their entire MC and, thus, it is of importance to elucidate whether the fluctuation of sex hormones can have an impact on their aerobic capacity during different phases of the MC. This study was designed to elucidate if the variations of the female sex hormones during three phases of the MC have an impact on aerobic capacity in endurance-trained women.

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Methods

Participants

The a priori power analysis for the outcome VO_{2peak} showed a power of 0.66, provided $n = 10$, $\alpha = 0.05$, true mean difference = 0.50, null hypothesis mean difference = 0.2, and SD = 0.4. By changing the true mean to 0.56, the power increases to 0.8. We recruited 15 participants, but we were unable to fulfill that goal within the time frame and resources and dropouts. The participants were recruited by advertisement at sports facilities, and by digital advertising targeted at eumenorrheic endurance-trained women. Fifteen healthy women volunteered to take part in this experimental study, and 10 of them completed all test procedures. All participants received written and oral information about the study, including the test procedure, before they gave their written consent for participation. The study conformed to the Code of Ethics of the World Medical Association (Declaration of Helsinki), and the Regional Ethical Review Board approved the study, Dnr 2011–236-31 M. The inclusion criteria were age 18–35 years old, healthy by own report, non-smoker, regular menstrual cycle (acceptable 21–35 days), physically active with a minimum of three times endurance training weekly, and regularly performed and without interruptions two consecutive months before the first test. All the participants fulfilled the inclusion criteria and had followed their personal endurance training of three times endurance training weekly. The participants were encouraged to follow their usual food and training routine during their participation in the study. They were tested three times during a MC and the results were compared individually. Exclusion criteria were irregular menstrual cycle and use of hormonal contraceptives and other female sex hormones that could affect the MC. The participants were also medically screened before each test to ensure that no medical problem or medication, sport supplements, or ergogenic aids would affect the cardiovascular system or the MC and contraindicate their participation (Fields M & Martha Delaney, 1990). The reasons for discontinuation were sports-related injuries ($n = 3$), upper airway infection ($n = 1$), and back pain ($n = 1$). None of these reasons were related to the conduction of the tests. The participant characteristic data and MC data as mean values and standard deviation (±SD) were age 28 ± 5 years, weight 69.9 ± 6.0 kg, height 170.9 ± 6.9 cm, body mass index (BMI) 23.9 ± 1.9 kg/m^2, MC length 27 ± 3 days, and ovulation day in MC 15 ± 3 days. The mean and SD for the test days in the three different phases in the MC were day 3 ± 1 in EFP, day 10 ± 1 in LFP, and day 22 ± 2 in MLP.

Study design

The participants performed a submaximal test followed by an incremental test to exhaustion three times during a MC: at early follicle phase (EFP), late follicle phase (LFP), and midluteal phase (MLP) (McNulty et al., 2020). The following parameters were analyzed during the submaximal test: RER and utilization of fat and carbohydrates; and parameters analyzed during the incremental test were VO_2 peak, maximal heart rate, utilization of fat and carbohydrates, and RER. Lactate levels were analyzed at rest, during the submaximal test, and 3, 5, and 10 minutes after the incremental test. The anaerobic threshold was set at RER = 1. The estimated test days were in EFP (low estrogen and low progesterone levels) at day 2–5, and in LFP (high estrogen and low progesterone levels) at day 9–11, and in MLP (high estrogen and high progesterone) at day 21–23, in a MC of 28 days (Isacco & Boisseau, 2017; Lebrun & Constantini, 2013). The test days were adjusted to longer or shorter MC length if necessary, and to days of ovulation. The participants were encouraged to observe their MC with a mobile application ($n = 9$) or to count days in their MC ($n = 1$) for at least two cycles before participation in the study. The number of days in the MC, preliminary time for ovulation, and preliminary first day of bleeding could then be estimated. After the second test occasion, the participants verified that their ovulation had occurred by an ovulation test. The third test occasion was then decided after both the first day of bleeding, the number of days in the MC, and of the ovulation day.

Methodology

Facilities and equipment

The current study was conducted at a sport laboratory with appropriate technical equipment, standardized and calibrated for the measurements. Body weight (kg) (Avery Berkel HL 120, United Kingdom), height (cm) measuring stick (Swemed, Sjöbloms Health Care Equipment AB, Sweden), and blood pressure (mm Hg) (HE AB, Boso, Germany). Hemoglobin (Hb) levels (g/l) were measured with capillary blood samples and analyzed with a Hemocue Hb201+ Hemoglobin (Ängelholm, Sweden). Hb and blood pressure were verified to be within reference values before the three tests and to make sure that no significant differences were observed between the three test occasions. The concentration of blood lactate (mmol/L) was measured in capillary blood samples and analyzed with Biosen C-line Clinic (EKF-diagnostics GmbH Barleben, Germany). The submaximal and incremental tests were conducted on a treadmill (Rodby RL 2500E, Rodby Vänge, Sweden) ($n = 6$) or on an electronically braked bicycle (Monark 839 E, Vansbro, Sweden) ($n = 4$), depending on the participants' habit of running versus cycling. The participants performed all three tests with the same equipment. The oxygen consumption (VO2), the production of carbon dioxide (VCO2), and the respiratory exchange ratio (VCO2/VO2) were determined continuously with an online respiratory gas analyzer (Oxigraf O2CPX, Oxigraf Inc., Sunnyvale, CA, USA), and monitored as 10 seconds average value with the Innovision Version 8.02 software (Innovision ApS, Glamsbjerg, Denmark). Heart rate was monitored with a Polar H7 chest transmitter (Oulu, Finland). Ovulation test (Tento Medical, Limhamn, Sweden) was used to detect the LH surge in urine with a sensitivity of 25 mlU/ml urine and with a test sensitivity of 99.5%. Day of ovulation was controlled with ovulation urinary tests ($n = 9$) and with rise of temperature ($n = 1$).

Test procedure

The test procedure included a submaximal running or cycling test, which after a short recovery was followed by a maximal

incremental test to determine VO_{2peak}. All tests were performed on a treadmill or electronically braked bicycle. The participants were given information on the test procedure and the importance of the same preparations before each test. This included not strenuous training two days before the tests, no alcoholic beverage the day before testing, no meal within two hours before the tests, and not to use smokeless tobacco or drink coffee 30 minutes before the tests. The three tests were performed at approximately the same time during the day for each participant (±2 hours at a.m. or p.m., respectively). The participants were informed that an ordinary training level should be performed during the test period, and this was ensured by the participants. Before each test the participants had to pass the general health screening, including resting blood pressure, resting Hb, and resting lactate level; and fill out a health formulary with questions on known diseases, use of medications or food supplements, and other ailments that could impact test results such as injures or pain from the musculoskeletal system or abdomine. Control of length and regularity of the MC was performed, and date of the last MC´s first day was noted. Height and weight were measured in similar training clothes and without shoes at each test. BMI was calculated from weight and length at the EFP.

The submaximal test and the incremental test

The test procedure started with a submaximal controlled warmup on the treadmill or on the bicycle followed by intervals of 5 minutes at three different workloads. Each workload was separated by a 1-minute rest period to determine capillary blood lactate concentration from a fingertip sample. This procedure was performed to investigate the participants' substrate utilization (RER, carbohydrate and fat oxidation) and lactate response during a submaximal workload based on fixed, determined levels, and also to decide the workload to start the incremental test. VO_2, RER, and HR were measured continuously. VO_{2peak} and maximal HR were calculated from the highest 30-second moving average. On the treadmill, the speed for the three intervals were chosen as 7, 9, and 11 km/h, with a 0-degree incline. The workload on the bicycle was chosen to 50, 100, and 150 watt (W), respectively, for the three intervals and the participants were encouraged to maintain a cadence of 70–80 RPM during the bicycle test. The incremental test started after a 5-minute rest period after the submaximal test. A speed on the treadmill and workload on the bicycle was chosen to achieve exhaustion within 5–12 minutes and based on the submaximal test parameters, the RER, heart rate, and lactate levels during the different submaximal workloads. A speed between 9 and13 km/h was chosen, where the inclination was increased by one degree for each minute to exhaustion, starting at 0 degree. The workload on the bicycle started between 80 and 125 W and was increased every minute with 20–30 W until exhaustion. The second and third test were conducted according to the same test protocol for each participant. As a recovery period after the test procedure, the participants walked on the treadmill at a speed of 5 km/h or cycled with a load of 50 W, and lactate levels were measured after 3, 5, and 10 min, respectively. The submaximal and maximal tests were performed on the same day to ensure the same female sex hormonal milieu during the MC.

Outcome measures

Blood lactate concentrations were measured at minute 5 (level 1), minute 11 (level 2), and minute 17 (level 3) during the submaximal test with three different workloads of 5 minutes per workload and 1 minute of rest in between, and at 3, 5, and 10 minutes after the incremental test. VO_2 and VCO_2 from the submaximal tests were inserted into the formula for fat oxidation $((1.6946 * VO_2) - (1.7012 * VCO_2))$ and for carbohydrate oxidation $((4.585 * VCO_2) - (3.2255 * VO_2))$ and calculated as averages (g/min) of the last 3 minutes of each 5-minute interval in the submaximal test (Wallis, 2005). This was also analyzed for RER. Values from the submaximal test were shown as carbohydrate oxidation, fat oxidation, RER, and lactate concentration for levels 1, 2, and 3 during submaximal work, respectively. For the incremental test, the VO_{2peak} was determined to the highest value of VO_2 seen at 30 s averages of consumed VO_2. From VO_{2peak}, the VO_2 ml per kg per min was calculated. The values for RER, carbohydrate oxidation, and fat oxidation were determined by linear interpolation from the working intensities 70, 80, 90, and 100% of VO_{2peak}, respectively. Using linear interpolation, heart rate, $VO_{2'}$ and workload level of the incremental test (the first minute set to level 1) at RER = 1.0 could be ascertained.

When RER exceeds 1.0, it can be used as an indication for when anaerobic metabolism occurs during exercise (RER-AT). RER-AT was defined as the value of VO_2, heart rate, and stress level for when RER was stabilized above 1.0 and not returned to levels below. Mean values for every 30 s of RER were used (Solberg et al., 2005). Heart rate, VO_2, and workload level at RER 1.0 were then outdrawn with linear interpolation (Tables 1–6).

Statistical analysis

Data were analyzed with the Statistical Package for the Social Sciences (IBM, SPSS version 25). Mean and standard deviation (SD±) are presented for the submaximal variables, as well as the incremental test variables at the different test points, EFP, LFP, and MLP, during a MC. Repeated measure analysis of variance (ANOVA) was used to examine the differences in aerobic capacity variables between the three phases in the MC. Significance was set at $p < .05$ and reported as precise p-values. The Mauchly test for sphericity was conducted. Lactate concentration at 10 and 15 minutes during the submaximal test did not meet the Mauchly's criteria for sphericity, and Greenhouse Geysers was used to ascertain p-value. If overall effects were significant, the Bonferroni and LSD post hoc tests were performed to determine differences between the three phases. All variables were tested for normal distribution with the Shapiro-Wilk test, and for variables that were not normally distributed (resting levels of lactate, lactate concentration at 5, 10, and 15 minutes during the submaximal test, fatty acid oxidation at 80% and 90% of VO_{2peak} during the incremental test, and workload level at RER = 1); the nonparametric Friedman test was used to determine if there were significant differences between these variables.

Table 1. Lactate levels at rest before test, at submaximal levels 1–3, and at three times after the incremental test, all measured at three different phases of the menstrual cycle.

	EFP	LFP	MLP		
Lactate level (mmol/l)	(m ± SD)	(m ± SD)	(m ± SD)	*P*	F
Before test	1.35 ± 0.37	1.28 ± 0.35**	1.17 ± 0.24	0.378	1.028
Submaximal level 1	1.61 ± 0.69**	1.47 ± 0.56**	1.48 ± 0.40	0.661	0.424
Submaximal level 2	1.60 ± 0.91**	1.53 ± 0.82**	1.45 ± 0.72**	0.428†	0.754
Submaximal level 3	2.45 ± 1.88**	2.28 ± 1.58**	2.32 ± 1.46	0.584†	0.403
After i. test, 3 min	12.74 ± 1.63	13.71 ± 1.46	12.80 ÷ 1.51	0.043*	3.768
After i. test, 5 min	13.02 ± 1.94	13.68 ± 1.67	12.90 ± 1.55	0.302	1.282
After i. test, 10 min	10.72 ± 2.34	11.46 ± 2.16	10.55 ± 1.74	0.125	2.340

Note: Level 1 = 7 km/h, 50W, level 2 = 9 km/h, 100W, level 3 = 11 km/h, 150W. EFP = early follicular phase, LFP=late follicular phase, MFP = midluteal phase, i. test = incremental test. *Significant $p < .05$ with LSD post hoc test between EFP and LFP, not significant with Bon Ferroni post hoc test. **Not normally distributed values were also analyzed with the nonparametric Friedman test and showed no significance ($p = .905$ at level 1, $p = .527$ at level 2, and $p = .905$ at level 3). † = Greenhouse Geisser.

Results

No significant differences between the three MC phases could be seen in resting lactate before the test nor in lactate during the submaximal tests. However, there was a significant difference in lactate concentration 3 minutes after the incremental test between the EFP and the LFP (Table 1 and Figure 1) when applying the LSD post hoc test, but not in the Bonferroni post hoc test.

No significant differences between the three MC phases could be seen in fatty acid oxidation during the submaximal test (Table 2). Neither did the fatty acid oxidation at 70, 80, 90. and 100% of VO_{2peak} during the incremental test show a significant difference between the MC phases.

No significant differences between the three MC phases could be seen in carbohydrate oxidation during the submaximal tests. Neither did the carbohydrate oxidation at 70, 80, 90,

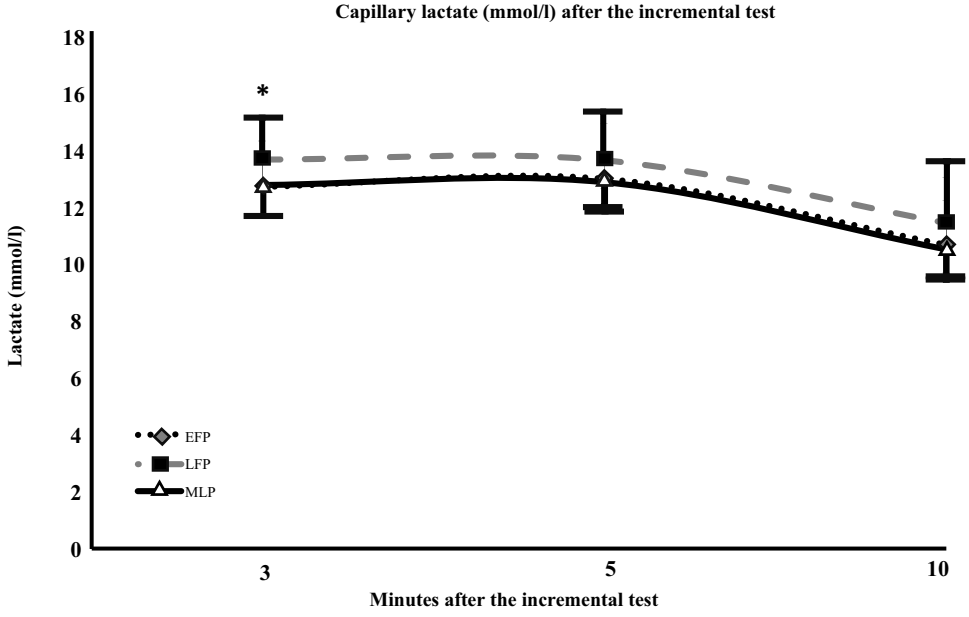

Figure 1. Blood lactate concentration 3, 5, and 10 min after incremental test showed a significant difference between early follicular phase (EFP) and late follicular phase (LFP) with the LSD post hoc test.

Table 2. Values of fat oxidation at submaximal levels 1–3, at 70, 80, 90, and 100% of VO_{2peak} during the incremental test, all measured at three different phases of the menstrual cycle.

	EFP	LFP	MLP		
Fat oxidation (g/min)	(m ± SD)	(m ± SD)	(m ± SD)	*P*	F
Submaximal level 1	0.43 ± 0.09	0.38 ± 0.14	0.42 ± 0.13	0.229	1.602
Submaximal level 2	0.38 ± 0.10	0.35 ± 0.15	0.39 ± 0.11	0.392	0.988
Submaximal level 3	0.34 ± 0.16	0.29 ± 0.20	0.27 ± 0.21	0.478	0.768
70% of VO_{2peak}	0.54 ± 0.18	0.49 ± 0.29	0.46 ± 0.21	0.412	0.933
80% of VO_{2peak}	0.36 ± 0.28	0.24 ± 0.30**	0.28 ± 0.25	0.286	1.341
90% of VO_{2peak}	0.09 ± 0.15**	0.03 ± 0.08**	0.06 ± 0.13**	0.358†	1.013
100% of VO_{2peak}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.378	1.028

Note: Level 1 = 7 km/h, 50W, level 2 = 9 km/h, 100W, level 3 = 11 km/h, 150W. EFP = early follicular phase, LFP = late follicular phase, MFP = midluteal phase. **Not normally distributed values were also analyzed with the nonparametric Friedman test and showed no significance at 80% of VO_{2peak} ($p = .122$), or at 90% of VO_{2peak} ($p = .311$). † = Greenhouse Geisser.

Table 3. Values of carbohydrate oxidation at submaximal levels 1–3 and at 70, 80, 90, and 100% of VO_{2peak} during the incremental test, all measured at three different phases of the menstrual cycle.

	EFP	LFP	MLP		
Carbohydrate oxidation (g/min)	(m ± SD)	(m ± SD)	(m ± SD)	*P*	F
Submaximal level 1	0.85 ± 0.32	0.93 ± 0.40	0.85 ± 0.35	0.488	0.746
Submaximal level 2	1.51 ± 0.45	1.51 ± 0.48	1.43 ± 0.45	0.427	0.892
Submaximal level 3	2.17 ± 0.70	2.18 ± 0.63	2.17 ± 0.69	0.996	0.004
70% of VO_{2peak}	1.86 ± 0.58	1.94 ± 0.77	2.00 ± 0.62	0.598	0.529
80% of VO_{2peak}	2.82 ± 1.03	3.04 ± 1.21	3.01 ± 1.07	0.579	0.563
90% of VO_{2peak}	4.53 ± 1.12	4.76 ± 1.41	4.86 ± 1.30	0.584	0.555
100% of VO_{2peak}	6.61 ± 0.99	7.15 ± 1.31	6.78 ± 1.28	0.103	2.587

Note: Level 1 = 7 km/h, 50W, level 2 = 9 km/h, 100W, level 3 = 11 km/h, 150W. EFP = early follicular phase, LFP = late follicular phase, MFP = mid luteal phase.

Table 4. Values of RER at submaximal levels 1–3 and at 70, 80, 90, and 100% of VO_{2peak} during the incremental test, all measured at three different phases of the menstrual cycle.

	EFP	LFP	MLP		
RER	(m ± SD)	(m ± SD)	(m ± SD)	*P*	F
Submaximal level 1	0.82 ± 0.02	0.83 ± 0.05	0.83 ± 0.03	0.823	0.196
Submaximal level 2	0.87 ± 0.03	0.88 ± 0.04	0.87 ± 0.03	0.316	1.230
Submaximal level 3	0.91 ± 0.05	0.91 ± 0.05	0.91 ± 0.06	0.991	0.009
70% of VO_{2peak}	0.85 ± 0.05	0.87 ± 0.08	0.86 ± 0.06	0,294	1.312
80% of VO_{2peak}	0.92 ± 0.07	0.93 ± 0.08	0.92 ± 0.07	0.664	0.420
90% of VO_{2peak}	1.01 ± 0.06	1.04 ± 0.06	1.02 ± 0.06	0.434	0.876
100% of VO_{2peak}	1.16 ± 0.06	1.17 ± 0.03	1.15 ± 0.04	0.570	0.581

Note: Submaximal level 1 = 7 km/h, 50W, submaximal level 2 = 9 km/h, 100W, level 3 = 11 km/h, 150W. EFP = early follicular phase, LFP = late follicular phase, MFP = midluteal phase.

and 100% of VO_{2peak} during the incremental test differ significantly between phases (Table 3).

No significant differences between the three MC phases could be seen in RER during the submaximal tests. Neither did RER at 70, 80, 90, and 100% of VO_{2peak} during the incremental test differ significantly between phases (Table 4).

No significant differences between the three MC phases could be seen in VO_2, heart rate, or workload at RER = 1 during the incremental test (Table 5).

No significant differences between the three MC phases could be seen in peak values neither in maximal heart rate, VO_{2peak} neither in l per min nor in ml per kg per min (Table 6).

Discussion

This experimental study examined whether there were differences in submaximal and maximal aerobic capacity between the EFP, LFP, and MLP phases in eumenorrheic, endurance-trained women. In summary, we found no significant differences between the three phases for VO_{2peak}, VO_{2peak} per kg body weight, maximum heart rate, or for RER, fat, or carbohydrate oxidation during submaximal or fatigue tests. Similarly, blood lactate concentrations during rest and submaximal work did not differ between the phases with one exception: lactate concentration 3 min after completion of the incremental test was higher in the LFP than the EFP.

Table 5. Values of VO_2, heart rate, and workload level (minute 1 set to workload level 1) at RER = 1.0 at the incremental test, all measured at three different phases of the menstrual cycle.

	EFP	LFP	MLP		
Values at RER = 1.0	(m ± SD)	(m ± SD)	(m ± SD)	*P*	F
VO_2 (ml/min)	3.10 ± 0.45	3.00 ± 0.42	3.06 ± 0.37	0.280	1.368
HR	172 ± 13	169 ± 13	173 ± 11	0.159	2.037
Workload level	5.6 ± 1.58	5.0 ± 1.33	5.2 ± 1.32**	0.156	2.066

Note: EFP = early follicular phase, LFP = late follicular phase, MFP = midluteal phase, HR = heart rate.
**Not normally distributed values were also analyzed with the nonparametric Friedman test and showed no significance ($p = .152$).

Table 6. Maximal heart rate and VO_{2peak} at the incremental test, all measured at three different phases of the menstrual cycle.

	EFP	LFP	MLP		
Peak values	(m ± SD)	(m ± SD)	(m ± SD)	*P*	F
Maximal HR	184 ± 8	184 ± 9	185 ± 8	0.283	1.353
VO_{2peak} (ml/min)	3.51 ± 0.48	3.55 ± 0.48	3.54 ± 0.47	0.620	0.490
$VO_{2peak/kg}$ (ml/kg x min)	50.16 ± 4.65	50.83 ± 4.41	50.40 ± 4.41	0.410	0.938

Note: EFP = early follicular phase, LFP = late follicular phase, MFP = midluteal phase.

The lack of significant differences between the different menstrual phases in aerobic capacity is consistent with most previous studies (De Souza MaM et al., 1990; Jurkowski et al., 1981; Lebrun & Constantini, 2013; Redman et al., 2003; Smekal et al., 2007; Smith et al., 2015). However, one exception, Lebrun et al. (Lebrun et al., 1995), found a lower VO_{2peak} during the LP compared to the FP. This could be due to methodological differences in test procedures during the measurements of VO_{2peak} with a faster increase in workload in their study. Another difference between studies is that they performed their tests during day 3–8 of the menstrual cycle, and in this study tests were performed on day 2–5 and 9–11 during the follicular phase. Test days for the luteal phase were more similar with day 18–23 in their study and day 21–23 in this study. Otherwise, there were no differences in age or training status of the participants. There is no other study using the same mode of testing as in these two studies that displaying a difference in VO_{2peak} why their results should be taken with caution. It should also be noted that newer studies using a test protocol similar to ours have also displayed the same results as in our study (Jacob Frandsen et al., 2020; Smekal et al., 2007). We also examined VO_2, heart rate, and stress level at RER = 1.0, which can be an indication of anaerobic threshold, with no differences in performance observed between the three menstrual phases. These results are consistent with most previous studies of the anaerobic threshold and MC (De Souza MaM et al., 1990; Redman et al., 2003; Smekal et al., 2007), with the exception of Bemben et al. (Bemben et al., 1995) who showed that the ventilatory anaerobic threshold occurred at a higher percentage level of VO_2 at the EFP compared to the MLP. They used similar test days as in this study for the EFP and MLP. Thus, this could not explain the difference in results. However, their results are also contradicted by newer studies that have results in line with ours (Bemben et al., 1995; De Souza MaM et al., 1990; Redman et al., 2003; Smekal et al., 2007).

Furthermore, our results showed no differences between MC phases neither in RER or fat and carbohydrate oxidation during the submaximal test, nor at 70, 80, 90, and 100% of VO_{2peak} during the fatigue test, respectively. In contrast, some previous studies have shown a higher fat oxidation and a lower RER during the LP compared with the FP (D'Eon CS et al., 2002; Redman et al., 2003). In the study by Redman et al. (Redman et al., 2003), the results from the incremental tests did not display any difference between the follicle and luteal phase except for the carbon dioxide output (VCO_2) and the respiratory exchange ratio (RER) that were lower during the LP (Redman et al., 2003). In most of the other studies, the mode of testing was different from ours or medically induced differences in hormonal levels were used to mimic the different phases of the menstrual cycle (D'Eon CS et al., 2002). When RER was used to calculate the fat and carbohydrate oxidation, no differences could be displayed between menstrual cycle phases (Devries et al., 2006).

Moreover, we found no differences between the phases for resting lactate or lactate response to submaximal work. However, a significantly higher lactate concentration in the blood 3 min after completion of the incremental test was observed in the LFP compared with the EFP in the ANOVA post hoc test with LSD correction, although this was not confirmed when using the more stringent Bonferroni post hoc correction. The tendency for an increased lactate response after exercise in the LFP is consistent with some previous studies (Jurkowski et al., 1981; McCracken et al., 1994), but contradicts other studies (Bemben et al., 1995; Galliven et al., 1997). This study also measured fat and carbohydrate oxidation via indirect calorimetry, where no differences between the different phases could be seen.

Taken together, the findings from this study as well as previous studies indicate that aerobic capacity is not affected by the MC, which may be useful knowledge for athletes and coaches in their preparations for aerobic competition. However, existing research is encumbered with methodological limitations.

Strengths and limitations

There are some limitations to this study that may have impacted our results such as detection of MC phases with a urinary ovulation detection kit and small sample sizes. We did not confirm the MC phases using hormonal blood tests, which is recommended as the gold standard (Janse De Jonge et al., 2019). Inaccurate measures of serum estrogen and progesterone concentrations may lead to incorrect phase detection, and it is suggested that up to 80% of studies of menstrual phases may have included participants with anovulatory or luteal phase deficient cycles, which could potentially mask differences in performance during the cycle (Janse De Jonge et al., 2019). In the present study, we used a combination of self-report of the first day of bleeding, a positive urinary ovulation test, and menstrual cycle mapping as the best available methods to confirm the MC phases. Although we did not have access to serum hormone measurements, the combination of three methods may provide higher accuracy of the menstrual phases than one single method (Schaumberg et al., 2017). These three methods were also considered to be practically applicable for the women who participated in the study.

The study included 15 participants, with 5 dropouts, which resulted in a low sample size ($n = 10$) that may have led to a lack of power to detect statistically significant differences in performance values between their menstrual phases. Further, it is known that diet can affect lactate (Berend MRB et al., 1994), RER (D'Eon CS et al., 2002), and fat and carbohydrate oxidation values (Ashley et al., 2000); hence, the women were encouraged to perpetuate their ordinary training and diet during the test period. Our participants had a wide interindividual range of lactate levels at fixed, determined load during the tests, which most likely reflected differences in training background. The inclusion criteria were physically active women with experience of endurance training (i.e., had performed endurance training for a long period), with a minimum of three times of endurance training weekly and without interruption for two consecutive months before starting to participate in the study. The participants´ experience of endurance training was controlled to fulfill the inclusion criteria in an interview before they started in the study to ensure that no one was untrained. Seven out of ten participants had more than one year experience of endurance training, and

three had about one year of experience. The aim of the current study was to compare the participants´ own test results three times during a menstrual cycle with different hormone levels, not to compare between the participants' test results. The general health screening before each test ensured that the tests were performed without any medication, injuries, or pain that might have had an impact on the test results.

Strengths of the study include tests performed at approximately the same time of day and with experienced test leaders. Urinary LH measurement provides a direct indication of ovulation using a minimally invasive method and, therefore is a commonly used method. However, this noninvasive method does not exclude luteal phase deficient cycles (Schaumberg et al., 2017). Although preferable, more invasive methods with blood samples are resource demanding and require the participants to give repeated blood samples in each MC. We ensured our urinary LH measurements by counting days of the MC during the test period, and also evaluated regularity and length of at least two MC before the start of the tests.

Varying hormone levels, estimated from ovulation and menstrual bleeding, did not impact aerobic capacity during different phases of the MC in this study. Future research using more accurate methods for MC phase verification could be more precise, and studies with larger sample sizes and a broader range of sports are also recommended.

What does this study add? Practical applications

In this study, the varying sex hormone levels of female athletes did not impact aerobic capacity during three phases of the MC. These findings could be useful knowledge for researchers in sport physiology, as well as coaches and female athletes planning for endurance performance and competition in aerobic events. Apart from within sports, this can also have an implication on occupational physiology as well as clinical physiology. Whether endurance training programs should be planned according to varying hormone levels during the MC needs further research before a consensus can be reached.

Conclusion

This study of 10 endurance trained, eumenorrheic women showed no significant differences in aerobic capacity between the three MC phases (EFP, LFP, and MLP), indicating similar aerobic capacity despite hormonal variations. This may be useful knowledge when planning for competition in aerobic events.

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References

Ashley, C. D., Kramer, M. L., & Bishop, P. (2000). Estrogen and substrate metabolism. *Sports Medicine*, *29*(4), 221–227. https://doi.org/10.2165/00007256-200029040-00001

Bemben, D. A., Salm, P. C., & Salm, A. J. (1995). Ventilatory and blood lactate responses to maximal treadmill exercise during the menstrual cycle. *The Journal of Sports Medicine and Physical Fitness*, *35*(4), 257–262.

Berend MRB, J. Z., Jones, N. A., Holliman, S. C., & Hackney, A. C. (1994). Effect of the menstrual cycle phase and diet on blood lactate responses to exercise. *Biology of Sport*, *11*(4), 241–248.

Costello, J. T., Bieuzen, F., & Bleakley, C. M. (2014). Where are all the female participants in sports and exercise medicine research? *European Journal of SportScience*, *14*(8), 847–851. https://doi.org/10.1080/17461391.2014.911354

D'Eon CS, T. M., Chipkin, S. R., Grow, D., Ruby, B. C., & Braun, B. (2002). Regulation of exercise carbohydrate metabolism by estrogen and progesterone in women. *American Journal of Physiology-Endocrinology and Metabolism*, *283*(5), E1046–E1055. https://doi.org/10.1152/ajpendo.00271.2002

de Jonge, X. A. K. J. (2003). Effects of the menstrual cycle on exercise performance. *Sports Medicine*, *33*(11), 833–851. https://doi.org/10.2165/00007256-200333110-00004

De Souza MaM, M. S., Rubin, K. R., & Maresh, C. M. (1990). Effects of menstrual phase and amenorrhea on exercise performance in runners. *Medicine and Science in Sports and Exercise*, *22*(5), 575–580. https://doi.org/10.1249/00005768-199010000-00006

Devries, M. C., Hamadeh, M. J., Phillips, S. M., Tarnopolsky & Mark A. (2006). Menstrual cycle phase and sex influence muscle glycogen utilization and glucose turnover during moderate-intensity endurance exercise. *The American Journal of Physiology*, *291*(4), R1120–R1128. https://doi.org/10.1152/ajpregu.00700.2005

Fields M, K. B., & Martha Delaney, M. A. (1990). Focusing the preparticipation sports examinaiton. *The Journal of Family Practice*, *30*(3), 304–312.

Filicori, M., Butler, J. P., & Crowley, W. F., Jr. (1984). Neuroendocrine regulation of the corpus luteum in the human. Evidence for pulsatile progesterone secretion. *The Journal of Clinical Investigation*, *73*(6), 1638–1647. https://doi.org/10.1172/JCI111370

Galliven, E. A., Singh, A., Michelson, D., Bina, S., Gold, P. W., & Deuster, P. A. (1997). Hormonal and metabolic responses to exercise across time of day and menstrual cycle phase. *Journal of Applied Physiology*, *83*(6), 1822–1831. https://doi.org/10.1152/jappl.1997.83.6.1822

Godbole, G., Joshi, A. R., & Vaidya, S. M. (2016). Effect of female sex hormones on cardiorespiratory parameters. *Journal of Family Medicine and Primary Care*, *5*(4), 822–824. https://doi.org/10.4103/2249-4863.201148

Isacco, L., & Boisseau, N. (2017). Sex hormones and substrate metabolism during endurance exercise. In A. Hackney (Ed.), *Sex hormones, exercise and woman* (pp. 35–58). Switzerland Springer.

Isacco, L., Duché, P., & Boisseau, N. (2012). Influence of hormonal status on substrateutilization at rest and during exercise inthe female population. *Sports Medicine*, *42*(4), 327–342. https://doi.org/10.2165/11598900-000000000-00000

Jacob Frandsen, N. P., Prats Quesada, J., José Amaro-Gahete, F., Ritz, C., Larsen, S., Dela, F., & Helge, J. W. (2020). Menstrual cycle phase does not affect whole body peak fat oxidation rate during a graded exercise

test. *Journal of Applied Physiology*, *128*(3), 681–687. https://doi.org/10.1152/japplphysiol.00774.2019

Janse De Jonge, X., Thompson, B., & Han, A. (2019). Methodological recommendations for menstrual cycle research in sports and exercise. *Medicine & Science in Sports & Exercise*, *51*(12), 2610–2617. https://doi.org/10.1249/MSS.0000000000002073

Jurkowski, J. E., Jones, N. L., Toews, C. J., & Sutton, J. R. (1981). Effects of menstrual cycle on blood lactate, O2 delivery, and performance during exercise. *Journal of Applied Physiology*, *51*(6), 1493–1499. https://doi.org/10.1152/jappl.1981.51.6.1493

Lebrun, C. M., McKenzie, D. C., Prior, J. C., & Taunton, J. E. (1995). Effects of menstrual cycle phase on athletic performance. *Medicine and Science in Sports and Exercise*, *27*(3), 437–444. https://doi.org/10.1249/00005768-199503000-00022

Lebrun, C. J., & Constantini, N. W. (2013). Female reproductive hormones on sports performance. In N. Constantini & A. Hackney (Eds.), *Endocrinology of physical activity and sport* (2nd ed., pp. 281–332). Humana Press.

McCracken, M., Ainsworth, B., & Hackney, A. C. (1994). Effects of the menstrual cycle phase on the blood lactate responses to exercise. *European Journal of Applied Physiology and Occupational Physiology*, *69*(2), 174–175. https://doi.org/10.1007/BF00609412

McNulty, K. L., Elliott-Sale, K. J., Dolan, E., Swinton, P. A., Ansdell, P., Goodall, S., Thomas, K., & Hicks, K. M. (2020). The effects of menstrual cycle phase on exercise performance in eumennorrheic women: A systemic review and meta-analysis *sports med*. *Sports Medcine*, *50*(10), 1813–1827. https://doi.org/10.1007/s40279-020-01319-3

Oosthuyse, T., & Bosch, A. N. (2010). The effect of the menstrual cycle on exercise metabolism. *Sports Medicine*, *40*(3), 207–227. https://doi.org/10.2165/11317090-000000000-00000

Rechichi, C., Dawson, B., & Goodman, C., (2008). Oral contraceptive phase has no effect on endurance test. *International Journal of Sports Medicine*, *29*(4), 277–281. https://doi.org/10.1055/s-2007-965334

Redman, L. M., Scroop, G. C., & Norman, R. J. (2003). Impact of menstrual cycle phase on the exercise status of young, sedentary women. *European Journal of Applied Physiology and Occupational Physiology*, *90*(5–6), 505–513. https://doi.org/10.1007/s00421-003-0889-0

Ross, J., Hecksteden, A., Fullagar, H. H. K., Meyer, T., & Lucía, A. (2017). The effects of menstrual cycle phase on physical performance in female soccer players. *PLoS One*, *12*(3), e0173951. https://doi.org/10.1371/journal.pone.0173951

Schaumberg, M. A., Jenkins, D. G., De Jonge, X. J., Emmerton, L. M., & Skinner, T. L. (2017). Three-step method for menstrual and oral contraceptive cycle verification. *Journal of Science and Medicine in Sport*, *20*(11), 965–969. https://doi.org/10.1016/j.jsams.2016.08.013

Smekal, G. V. D., Serge, P., Frigo, P., Tegelhofer, T., Pokan, R., Hofmann, P., Tschan, H., Baron, R., Wonisch, M., Renezeder, K., & Bachl, N. (2007). Menstrual cycle: No effect on exercise cardiorespiratory variables or blood lactate concentration. *Medicine & Science in Sports & Exercise*, *39*(7), 1098–1106. https://doi.org/10.1249/mss.0b013e31805371e7

Smith, J. R., Brown, K. R., Murphy, J. D., & Harms, C. A. (2015). Does menstrual cycle phase affect lung diffusion capacity during exercise? *Respiratory Physiology & Neurobiology*, *205*, 99–104. https://doi.org/10.1016/j.resp.2014.10.014

Solberg, G., Robstad, B., Skjønsberg, O. H., & Borchsenius, F. (2005). Respiratory gas exchange indices for estimating the anaerobic threshold. *Journal of Sports Science & Medicine*, *4*(1), 29–36.

Vaiksaar, S. J., Jürimäe, J., Mäestu, J., Purge, P., Kalytka, S., Shakhlina, L., & Jürimäe, T. (2011). No effect of menstrual cycle phase and oral contraceptive use on endurance performance in rowers. *The Journal of Strength & Conditioning Research*, *25*(6), 1571–1578. https://doi.org/10.1519/JSC.0b013e3181df7fd2

Wallis, G. A. (2005). Measurement of substrate oxidation during exercise by means of gas exchange measurements. *Internaitonal Journal of Sports Medicine*, *26*(S 1), S28–S37. https://doi.org/10.1055/s-2004-830512