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Menstrual and oral contraceptive cycle phases do not affect submaximal and maximal exercise responses

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To examine whether the menstrual or monophasic oral contraceptive cycle phases affect submaximal (oxygen uptake ($\dot{V}O_2$) kinetics, maximal lactate steady-state (MLSS)) and maximal ($\dot{V}O_{2max}$, time-to-exhaustion (TTE)) responses to exercise in healthy, active women. During the mid-follicular or inactive-pill phase and the mid-luteal or active-pill phase of the respective menstrual or oral contraceptive cycle, 15 non-oral contraceptive users (mean and standard deviation (SD) (±): 27 ± 6 years; 171 ± 5 cm; 65 ± 7 kg) and 15 monophasic oral contraceptive users (24 ± 4 years; 169 ± 10 cm; 68 ± 10 kg) performed: one $\dot{V}O_2$ kinetics test; one ramp-incremental test; two to three 30-minute constant-load cycling trials to determine the power output corresponding to MLSS ($MLSS_p$), followed by a TTE trial. The phase of the menstrual or oral contraceptive cycle did not affect the time constant of the $\dot{V}O_2$ kinetics response ($\tau\dot{V}O_2$) (mid-follicular, 20 ± 5 seconds and mid-luteal, 18 ± 3 seconds; inactive-pill, 22 ± 8 seconds and active-pill, 23 ± 6 seconds), $\dot{V}O_{2max}$ (mid-follicular, 3.06 ± 0.32 L min^{-1} and mid-luteal, 3.00 ± 0.33 L min^{-1}; inactive-pill, 2.87 ± 0.39 L min^{-1} and active-pill, 2.87 ± 0.45 L min^{-1}), $MLSS_p$ (mid-follicular, 181 ± 30 W and mid-luteal, 182 ± 29 W; inactive-pill, 155 ± 26 W and active-pill, 155 ± 27 W), and TTE (mid-follicular, 147 ± 42 seconds and mid-luteal, 128 ± 54 seconds; inactive-pill, 146 ± 70 seconds and active-pill, 139 ± 77 seconds) ($P > .05$). The rate of perceived exertion (RPE) at minute 30 of the $MLSS_p$ trials was greater in the mid-follicular phase (6.2 ± 1.5) compared with the mid-luteal phase (5.3 ± 1.4) for non-oral contraceptive users ($P = .022$). The hormonal fluctuations between the menstrual and oral contraceptive cycle phases had no detectable effects on submaximal and maximal exercise performance, even when RPE differed.

KEYWORDS
Follicular phase, luteal phase, maximal lactate steady-state, monophasic pill, oxygen uptake

1 | INTRODUCTION

Female endogenous (estrogen and progesterone) and exogenous (ethinyl estradiol and progestin) reproductive hormones fluctuate throughout the menstrual and oral contraceptive cycles, respectively. Physiological parameters linked to the cardiovascular system, respiratory function, and substrate metabolism may be impacted by the changes in estrogen or ethinyl estradiol throughout the menstrual or oral contraceptive cycles, respectively.[1-5] Briefly, estrogen is known to increase blood supply to the heart and muscles by enhancing vasodilation of coronary arteries and peripheral vascular

beds.[5-7] Additionally, higher estrogen concentrations in the mid-luteal phase have shown to increase pulmonary blood volume and pulmonary diffusion capacity compared with the follicular phase at rest and during exercise.[4] Importantly, during exercise, estrogen acts to spare glycogen stores and increase fat oxidation rates by promoting lipolysis in the muscles,[2,3,5,8] although this effect may be modulated by the concurrent presence of progesterone.[3,9] Interestingly, exogenous hormones in oral contraceptives have been reported to have greater receptor-level activities related to insulin response than endogenous ovarian hormones.[10] While also increasing reliance on lipids as a source of fuel at rest and during exercise,[11] an increase in stroke volume and preload, caused by increased plasma volume during the active-pill phase, may result in a greater cardiac output.[1] Collectively, such cardiovascular, respiratory, and metabolic adaptations due to high levels of endogenous or exogenous estrogen may be advantageous during submaximal and maximal aerobic exercise.

Despite the potential advantage of higher estrogen concentrations, there are inconsistent findings in the literature with regards to the effects of the menstrual cycle on submaximal and maximal responses to exercise.[12-21] In relation to submaximal exercise, only one study has examined the effect of menstrual cycle phase on oxygen uptake ($\dot{V}O_2$) kinetics in premenopausal women and found that $\dot{V}O_2$ kinetics was not affected by the menstrual cycle phase.[22] Interestingly, given the previously described effects of estrogen and ethinyl estradiol to spare glycogen stores and shift to a greater reliance on fat catabolism during exercise, no study has examined the effects of the menstrual or oral contraceptive cycle on the maximal lactate steady-state (MLSS) response. The workload at MLSS defines the highest intensity of exercise at which metabolic steady-state can be sustained. When exercising at this workload, oxidative processes are largely supported by glycogen degradation to pyruvate and oxidative phosphorylation. In relation to maximal performance, while the majority of studies have shown that maximal oxygen consumption ($\dot{V}O_{2max}$) is unaffected by menstrual cycle phase,[12-15] one study has found a greater absolute $\dot{V}O_{2max}$, but not relative $\dot{V}O_{2max}$, in the follicular phase compared with the luteal phase.[16] Additionally, findings are also conflicting when examining time-to-exhaustion (TTE) performance. Whereas some have shown no significant difference across the menstrual cycle phases,[16,17] Jurkowski et al[18] found TTE was doubled in the mid-luteal phase compared with the mid-follicular phase. Importantly, a major limitation to many of the studies examining submaximal and maximal performance is that the sample sizes were small (eg, 5-10 participants), which may be one of the reasons for the inconsistent findings in the literature.

The few studies that have examined the within-cycle effects of oral contraceptive use on cardiovascular responses to exercise have also reported conflicting data. For example, one study has shown no substantial influence of oral contraceptive phase on submaximal $\dot{V}O_2$,[23] while another study observed a significantly lower submaximal $\dot{V}O_2$ when exercising at the same absolute speed in the active-pill phase than the inactive-pill phase.[24] This lack of clarity and understanding regarding how the menstrual and oral contraceptive cycle phases might affect submaximal and maximal responses to exercise has led to an editorial questioning where the research-based evidence is on the effects of the menstrual cycle on performance.[25]

In general, even though individual studies have examined different components of submaximal and maximal responses to exercise in different phases of the menstrual cycle, no study has examined the menstrual and oral contraceptive cycle effects on a wide variety of responses to exercise in a single study. Therefore, the objective of this study was to determine whether the menstrual or monophasic oral contraceptive cycle phase affects submaximal ($\dot{V}O_2$ kinetics, MLSS) and maximal ($\dot{V}O_{2max}$, TTE) responses to exercise in healthy, active women.

2 | MATERIALS AND METHODS

2.1 | Participants

Thirty young, active women who indicated that they performed moderate to vigorous physical activity at least four times per week for a minimum of 30 minutes each bout volunteered to participate in the study. According to the classification levels proposed by Decroix et al[26], the present study included a heterogenous sample of recreationally trained, trained, and highly trained individuals, with the majority being trained or highly trained. This population was selected to minimize potential changes in the outcome measures being affected by large variability in responses between tests due to training effects. Fifteen participants were eumenorrheic (regularly menstruating) non-oral contraceptive users (mean and standard deviation (SD) (±): 27 ± 6 years.; 171 ± 5 cm; 65 ± 7 kg), and fifteen participants were regularly menstruating monophasic oral contraceptive users (24 ± 4 years; 169 ± 10 cm; 68 ± 10 kg) for at least six months. Non-oral contraceptive participants were excluded if they had anovulatory and/or irregular menstrual cycles (average cycle length less than 21 days or greater than 35 days), were pregnant, or used hormonal contraceptives within the 12 months prior testing. Oral contraceptive participants were excluded if they used any hormonal contraceptive methods other than the combined, cyclic, low-regular dose (between 20 and 35 μg of ethinyl estradiol and 100-200 μg of progestin) monophasic oral contraceptive pill (ie, progestin only pill, continuous pill, combined multiphasic pill, patch, ring, or intrauterine device). The oral contraceptive pill used was either a second-generation (n = 10) or third-generation (n = 5)

pill for an average of 45 ± 36 months (range: 6-108 months). All second-generation pills contained the progestin levonorgestrel and third-generation pills contained the progestin desogestrel. All participants were healthy, non-smokers, and not taking medications known to affect cardiovascular or hemodynamic responses to exercise. All procedures were approved by the Conjoint Health Research Ethics Board of the University of Calgary (REB17-2006). Informed consent was obtained from all participants included in the study.

2.2 | Procedures

The non-oral contraceptive group tracked their menstrual cycle for at least two months prior to the onset of testing to determine their average cycle length. Participants used the Clearblue Advanced Digital Ovulation kit (SPD Swiss Precision Diagnostics GmbH, Geneva, Switzerland) to verify the menstrual cycle phase. This ovulation test tracks the changes in luteinizing hormone (LH) concentration (greater than 40 mIU mL^{-1}) and estrone-3-glucuronide concentration, a principal urinary metabolite of estrogen that corresponds to the serum levels of estrogen,[27] compared with individual baseline hormone levels. According to the company, this ovulation kit is more than 99% accurate at detecting the LH surge, which occurs immediately prior to ovulation. Beginning at day 7, using the start of menses as day 1, non-oral contraceptive participants used the above kit daily, during their first urination of the morning, until peak fertility (ovulation) was achieved. The experimental protocol required six to seven visits where the testing procedures were completed in three to four visits in one phase of the menstrual or oral contraceptive cycle and repeated in the subsequent phase of the respective cycle. Figure 1 outlines the study protocol. The following testing procedures were completed on an electromagnetically braked cycle ergometer (Velotron; RacerMate): (a) one $\dot{V}O_2$ kinetics test followed by a ramp-incremental test to exhaustion (b) two to three 30-minute rides at a constant-load to determine the PO corresponding with MLSS ($MLSS_p$), followed by a two-minute recovery period, then a TTE trial. A minimum of 24 hours to a maximum of 48 hours was allowed between visits. For the oral contraceptive group, the testing order was randomized, with the first three to four visits completed in the inactive-pill phase (days 1-7 using the sugar pill or no pill, given the consumption of the first sugar pill is day 1) or active-pill phase (between days 17 and 24), and the final three visits completed during the subsequent phase. For the non-oral contraceptive group, the entire protocol was completed during one full menstrual cycle following ovulation verification. The first three to four visits were completed during the mid-follicular phase (days 5-10) and repeated during the mid-luteal phase (days 19-24), using the start of menses as day 1. All exercise trials were performed in the same room with standardized environmental conditions (20-22°C; 30%-40% ambient relative humidity) at a similar time of day (± 30 minutes). Participants were instructed to refrain

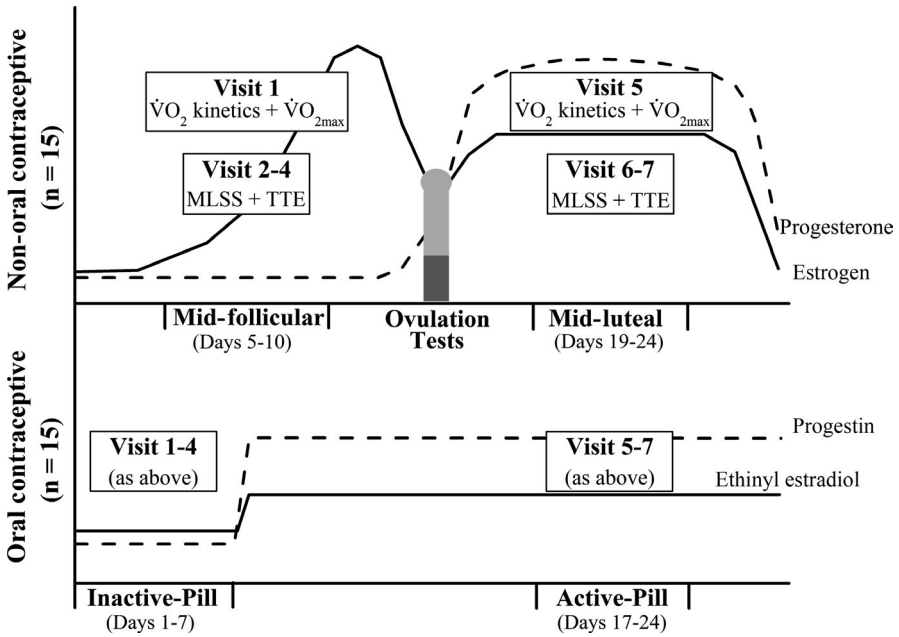

FIGURE 1 Study protocol. The non-oral contraceptive group underwent six to seven testing sessions scheduled during the mid-follicular, and mid-luteal phases of a single menstrual cycle. The oral contraceptive group underwent six to seven testing sessions during the inactive-pill and the active-pill phases of the monophasic oral contraceptive cycle. The testing order was randomized for the oral contraceptive group, while the non-oral contraceptive group completed the first three to four visits in the follicular phase and then repeated the protocol in three visits during the luteal phase. $\dot{V}O_2$: oxygen uptake; $\dot{V}O_{2max}$: maximal oxygen uptake; MLSS, maximal lactate steady-state; TTE, time-to-exhaustion

from caffeine and alcohol for at least 12 hours and consume the same meal three hours prior to testing. Participants were also asked to be consistent with their physical activity levels, avoiding any moderate to vigorous physical activity during the specific block of testing. During all trials, participants were blinded to the PO and elapsed time.

2.2.1 | $\dot{V}O_2$ kinetics

Participants performed three square-wave, step-transitions from 20 W (6 minutes) to 80 W (6 minutes). Participants were instructed to maintain a cadence of ~70 revolutions per minute (rpm) throughout the test. The 80 W PO was estimated to be an intensity that would allow for a sufficient increase in the $\dot{V}O_2$ response so as to increase the signal-to-noise ratio and ensure that all participants were within the moderate-intensity exercise domain. Correct assignment of the domain was confirmed from the estimation of the gas exchange threshold (GET) from the ramp-incremental test to exhaustion, and the lack of development of a $\dot{V}O_2$ slow component during data analysis. A near-infrared spectroscopy (NIRS) system was used to measure local muscle deoxygenation ([HHb]) throughout the test (see data collection).

2.2.2 | Ramp-incremental test

The $\dot{V}O_2$ kinetics test was transitioned into a ramp-incremental exercise test to volitional fatigue. Following 4 minutes of baseline cycling at 50 W, the PO increased 25 W•min^{-1}. Participants were instructed to cycle at their preferred cadence ranging between 75 and 95 rpm. The ramp-incremental test was ceased when participants dropped their cadence below 65 rpm despite strong verbal encouragement. Immediately after the trial, a sample of capillary blood was taken.

2.2.3 | MLSS determination trial

In the following two to three visits, participants performed 30-minute constant-load cycling trials. Each trial started with a 4 minutes baseline at 80 W, followed by an instantaneous increase to a predetermined PO. The PO for the first trial was determined using a mathematical equation capable of predicting the PO at MLSS with high accuracy.[28] $MLSS_p$ was defined as a change in capillary blood lactate concentration ([BLa]) of less than (or equal to) 1 mmol•L^{-1} from minute 10 to minute 30 of the trial.[29] The PO for the following trial was either increased or decreased by 10 W depending on whether [BLa] was less (or equal), or greater than 1 mmol•L^{-1} between minute 10 and 30 of the trial, respectively. Regardless, these 30-minute constant-load trials were repeated until the criterion for MLSS was satisfied. [BLa] was measured during the baseline (between minute 3-4) and every 5 minutes following the increase in PO. At the 10th and 30th minute, two measures of [BLa] were taken, and the average was used for analysis. For all the MLSS trials, participants were asked to cycle at the preferred cadence similar to the ramp-incremental test.

2.2.4 | Time-to-exhaustion trials

Following each 30-minute constant-load trial and after a two-minute recovery period cycling at 80 W, a TTE trial was performed at an intensity corresponding to 85% of the peak PO (PO_{peak}) previously recorded at the end of the ramp-incremental test during the first visit.[28] Participants were asked to cycle at a cadence similar to the ramp-incremental test. Immediately after the TTE trial, a sample of [BLa] was taken.

2.3 | Data collection

During the first visit of each phase, and before any other testing took place, body composition (height and weight) and percent body fat (dual-energy x-ray absorptiometry (DXA) system; Discovery QDR Series, Hologic, Inc) were assessed. Breath-by-breath gas exchange and ventilatory variables were measured using a metabolic cart (Quark CPET, COSMED). The system was warmed up for at least 30 minutes and calibrated before each test as recommended by the manufacturer. Heart rate (HR) was monitored using a HR chest strap (Garmin). Measures of [BLa] were taken by wiping the finger with an alcohol swab, performing a pin-prick, collecting a 20 μL blood with a capillary tube, and mixing it in an EKF pre-filled safe lock plastic tube for analysis (EKF Biosen C-Line Analyzer).

[HHb] of the quadriceps vastus lateralis muscle was measured using a frequency-domain multi-distance NIRS system (OxiplexTS, Model 92 505; ISS) as described elsewhere.[30] Briefly, the NIRS probe was placed on the belly at the distal portion of the vastus lateralis muscle. The probe was secured using a tightened black elastic strap to prevent movement and covered with an optically dense, black vinyl sheet to minimize the intrusion of extraneous light. An elastic tensor bandage was then loosely wrapped around the site, as not to constrict blood flow, but to further reduce movement and light intrusion. The probe stayed attached to the participant for the duration of testing. The NIRS measurements collected continuously for the entire duration of each test. Data were stored online at an output frequency of 2 Hz but reduced to 1-second bins for all subsequent analyses. The NIRS probe was composed of eight laser diodes operating at two wavelengths (λ = 690 and 828 nm; four at each wavelength) that pulsed in a rapid succession down a photomultiplier tube.

A rigid plastic NIRS probe (connected to laser diodes and photomultiplier tube by optical fibers) consisted of two parallel rows of light-emitter fibers and one detector fiber bundle; the source-detector separations for this probe were 2.0, 2.5, 3.0, and 3.5 cm for both wavelengths. The apparatus was calibrated following a 30-minute warm-up for each testing session, as per the manufacturer recommendations.

2.4 | Data and statistical analyses

2.4.1 | $\dot{V}O_2$ kinetics

$\dot{V}O_2$ data were edited on an individual basis by removing aberrant data points that lay three standard deviations outside of the local mean. During the step-transitions, data for each repetition of a similar protocol were linearly interpolated to 1-second intervals, time-aligned such that time zero represented the onset of the transition, and ensemble-averaged to yield a single averaged response for each participant for a given exercise protocol. These averaged responses were further time-averaged into 5-second bins. The on-transient responses for $\dot{V}O_2$ was modeled using the following equation:

$$Y_{(t)} = Y_{\text{bsln}} + \text{Amp} * \left(1 - e^{-\frac{t-\text{TD}}{\tau}}\right)$$

where $Y(t)$ represents the $\dot{V}O_2$ at any given time (t); Y_{bsln} is the steady-state baseline value of Y before an increase in work rate; Amp is the amplitude of the increase in Y above Y_{bsln}; and TD is the time delay (such that the model is not constrained to pass through the origin); τ is the time constant of the response (or the time required to attain 63% of the steady-state amplitude). $\dot{V}O_2$ data were modeled from the beginning of phase II (22 seconds) to 4 minutes (240 seconds), ensuring that during this period of time $\dot{V}O_2$ steady-state had been achieved.[31] The model parameters were estimated by least-squares nonlinear regression (Origin, OriginLab Corp.) in which the best fit is defined by minimization of the residual sum of squares and minimal variation of residuals around the Y-axis (Y = 0). The 99% confidence interval for the estimated time constant was determined after preliminary fit of the data with Y_{bsln}, Amp, and TD constrained to the best-fit values and the τ allowed to vary.

2.4.2 | Gas exchange and ventilatory data analysis for estimation of thresholds

During the ramp-incremental test, two experimenters independently inspected the test performed by each participant to identify GET and the respiratory compensation point (RCP) from the gas exchange and ventilatory variables that were plotted against $\dot{V}O_2$. The average of the two estimates from each experimenter was used if the difference was within 100 mL•min^{-1}. In those circumstances where the difference was greater than 100 mL•min^{-1}, two experimenters re-evaluated the profiles until a consensus was achieved. GET was determined as the point at which carbon dioxide production ($\dot{V}CO_2$) began to increase out of proportion in relation to $\dot{V}O_2$, coincidental with a systematic rise in the $\dot{V}_E$ (ventilation)-to-$\dot{V}O_2$ relation and end-tidal partial pressure of oxygen (PO_2), whereas the ventilatory equivalent of $\dot{V}CO_2$ ($\dot{V}_E/\dot{V}CO_2$) and end-tidal partial pressure of carbon dioxide (PCO_2) were stable.[32] RCP was identified as the point at which end-tidal PCO_2 began to fall after a period of isocapnic buffering. This point was confirmed by examining the $\dot{V}_E/\dot{V}CO_2$ and $\dot{V}_E/\dot{V}O_2$ relationships as well as by identifying the second breakpoint in the $\dot{V}_E$-to-$\dot{V}O_2$ relation.[33] The highest $\dot{V}O_2$ value computed from a 20-second rolling average during the ramp-incremental test was considered as $\dot{V}O_{2max}$. The same strategy was employed to determine $\dot{V}O_{2max}$ during each time-to-exhaustion trial. PO_{peak} was the highest PO value recorded at the end of the ramp-incremental test for each participant. The $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}_E$, and HR, at the 10th and 30th minute of the MLSS ride were calculated as the average of two minute data surrounding the 10th minute (9th-11th minute) and the last two minutes, respectively, of the 30-minute constant-load exercise.

The breath-by-breath $\dot{V}O_2$ data collected during the ramp-incremental, MLSS, and time-to-exhaustion trials were interpolated to 1-second intervals and then time aligned so that the onset of the test represented time "zero." For the ramp trial, the $\dot{V}O_2$ mean response time was individually calculated (Origin, OriginLab Corp.), as previously described,[34] to account for the lag time in the increase of $\dot{V}O_2$ at the onset of the ramp. Briefly, a double-linear model was fit from baseline to the established GET. The mean response time corresponded to the time delay between the onset of the ramp test and the intersection of the forward extrapolation of the baseline $\dot{V}O_2$ (slope constrained to "zero") and backwards extrapolation of the linear $\dot{V}O_2$-time relationship from GET.

2.4.3 | [HHb] during the $\dot{V}O_2$ kinetics test

The NIRS-derived [HHb] data were time aligned and ensemble- averaged to 5-second bins to yield a single response for each participant at each of the testing times. The [HHb] profile has been described to consist of a time delay at the onset of exercise (referred to as the calculated time delay), followed by an increase in the signal with an "exponential-like" time-course.[35] The calculated time delay (CTD) for the [HHb] response ($[HHb]_{CTD}$) was determined using second-by-second data and corresponded to the time, after the onset of exercise, at which the [HHb] signal begins a systematic increase

from its nadir value and was determined by calculating the $[HHb]_{CTD}$ from the averaged response of the three trials. The [HHb] data were modeled using the equation previously mentioned for the fitting of the $\dot{V}O_2$ kinetics response; the fitting window for the "exponential" response spanned from the end of the $[HHb]_{CTD}$ to 90 seconds into the transition. As described previously,[36] different fitting strategies result in minimal differences in estimates of the time course for the increase in [HHb]. Calculations of the [HHb]/$\dot{V}O_2$ ratio were similar to that previously described.[31] Briefly, the second-by-second [HHb] and $\dot{V}O_2$ data were normalized for each participant (0%-100% of the transition response). The normalized $\dot{V}O_2$ was left shifted to account for the phase I-phase II transition[37] so that the beginning of phase II $\dot{V}O_2$ coincided with the onset of exercise as detailed elsewhere.[35] An overall average [HHb]/$\dot{V}O_2$ ratio for the adjustment period during the exercise on-transient was derived for individually as the average of the ratio values from 20 to 120 seconds. The start point was selected to be 20 seconds to begin beyond the physiological $[HHb]_{CTD}$ derived from NIRS. An end point of 120 seconds was selected to ensure that the [HHb]/$\dot{V}O_2$ has reached a value of 1.0, indicating a steady-state in the matching of this response. The NIRS signal can often be limited by the amount of adipose tissue underneath the skin, and this can occur even in athletic women. In the cases where the [HHb] signal was of poor quality (n = 7), participants were removed from the analysis, resulting in a slightly smaller sample being used for the [HHb] data.

2.4.4 | Rating of perceived exertion

A 0-10 rating of perceived effort (RPE) scale was used to monitor perceptual responses to exercise. The scale was displayed to the participants immediately at the end of every ramp-incremental test and time-to-exhaustion trial, and during baseline and every five minutes during the MLSS rides.

2.5 | Statistics

Sample size was calculated based on means and SD for $\dot{V}O_{2max}$ (L min^{-1}) between cycle phases from previously published data.[16] Considering a type I error rate of 5% (2-tailed) and a power of 80%, the minimum sample size required for each group was eight participants. Despite the minimum

TABLE 1 Parameter estimates for $\dot{V}O_2$ and [HHb] kinetics responses

	Non-oral contraceptive		Oral contraceptive		*P*-values	
	Follicular	Luteal	Inactive-Pill	Active-Pill	Between Groups	Between Phases
$\dot{V}O_2$ Kinetics						
n	15		15			
$\dot{V}O_{2\ BSLN}$ (L min^{-1})	0.76 ± 0.10	0.77 ± 0.10	0.80 ± 0.10	0.76 ± 0.08	.677	.479
$\dot{V}O_{2\ AMPL}$ (L min^{-1})	0.58 ± 0.06	0.60 ± 0.06	0.57 ± 0.05	0.58 ± 0.05	.310	.296
$\dot{V}O_{2\ GAIN}$ (mL W^{-1})	9.7 ± 1.0	10.0 ± 1.01	9.5 ± 1.0	9.6 ± 0.8	.311	.310
TD $\dot{V}O_2$	15.3 ± 2.1	15.4 ± 2.8	14.5 ± 3.9	15.1 ± 4.1	.486	.697
τ$\dot{V}O_2$ (s)	19.6 ± 5.1[a]	17.8 ± 3.0[a]	22.2 ± 7.6	23.0 ± 5.5	.014	.722
[HHb] Kinetics						
n	12		11			
BSLN	16.1 ± 4.4	16.3 ± 4.9	15.4 ± 4.2	15.1 ± 3.6	.591	.881
AMPL	2.2 ± 1.2	2.4 ± 1.5	2.9 ± 2.2	2.5 ± 1.7	.544	.764
TD (s)	9.8 ± 4.9	9.0 ± 3.3	10.9 ± 3.5	13.8 ± 5.0	.286	.925
CTD[HHb] (s)	9.0 ± 3.8	9.2 ± 3.0	8.7 ± 3.5	7.2 ± 4.1	.394	.386
τ[HHb] (s)	8.8 ± 4.0	9.7 ± 3.4	8.1 ± 3.4	7.3 ± 4.5	.063	.301
τ'[HHb] (s)	18.8 ± 5.3	18.2 ± 3.8	19.6 ± 4.7	20.9 ± 4.1	.286	.350
[HHb]/$\dot{V}O_2$	1.01 ± 0.04	1.03 ± 0.05	1.03 ± 0.08	1.03 ± 0.05	.559	.321

Note: Data are presented as mean and SD (±).

Abbreviations: AMPL, amplitude; BSLN, baseline; CTD, calculated time delay for [HHb]; GAIN, functional gain. TD, time delay. τ, time constant of response. [HHb], deoxygenated hemoglobin concentration. τ[HHb], time constant of [HHb] response; τ'[HHb] , sum of τ[HHb] and CTD[HHb]; $\dot{V}O_2$, oxygen uptake.

[a]Significantly different compared with the oral contraceptive group.

number to detect differences was eight participants per group, in this study we decided to almost double the number of participants to minimize uncertainties related to having a small sample size, which has been a critique compared with some of the previous studies.[38] Data are presented as mean and SD (±). All statistics were performed using SPSS version 24 (SPSS, IBM). A mixed-model ANOVA was used to compare the variables of interest in the mid-follicular or inactive-pill phases of the menstrual or oral contraceptive cycle to the respective mid-luteal or active-pill phase, as well as between the non-oral contraceptive group and the oral contraceptive group. A Bonferroni post-hoc was applied when appropriate. Statistical significance was preset at $\alpha < 0.05$.

3 | RESULTS

There were no between-group differences or phase-related differences in age, height, and weight for the non-oral contraceptive and oral contraceptive group (refer to descriptive data in methods section). Non-oral contraceptive participants had a mean menstrual cycle length of 29 ± 2 days and ovulated on day 16 ± 3. The mean percent body fat was 21 ± 3% (average of both groups across both phases) (range: non-oral contraceptive, 16%-28%; oral contraceptive, 13%-27%).

3.1 | $\dot{V}O_2$ and [HHb] kinetics

A summary of the parameter estimates for the $\dot{V}O_2$ and [HHb] kinetics responses are presented in Table 1. Additionally, the group mean pulmonary $\dot{V}O_2$ response during the transition to moderate-intensity exercise for both the mid-follicular and mid-luteal phases of the menstrual cycle and the inactive-pill and active-pill phases of the oral contraceptive cycle are presented in Figure 2A,B, respectively. There were no differences related to the phase of the menstrual or oral contraceptive cycle for any of the $\dot{V}O_2$ or [HHb] kinetics responses (Table 1; Figure 2A,B). However, $\tau\dot{V}O_2$ was significantly smaller in the non-oral contraceptive group compared with the oral contraceptive group ($P = .014$) (Table 1).

3.2 | Responses during the ramp-incremental test

Physiological variables at maximal effort, GET, and RCP during the ramp-incremental test are presented in Table 2. There were no differences related to the phase of the menstrual or oral contraceptive cycle for any of these variables. Maximal [BLa] following the ramp test was significantly lower in the non-oral contraceptive group compared with the oral contraceptive group ($P = .034$) (Table 2).

3.3 | MLSS and time-to-exhaustion

Mean physiological variables for $MLSS_p$ and TTE are displayed in Table 3. During MLSS testing, there were no significant differences related to the menstrual or oral contraceptive cycle phases for $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}_{E,}$ PO, HR, and [BLa] from minute 10 to minute 30 (Figure 3A-D). PO at MLSS was significantly higher in the non-oral contraceptive group compared with the oral contraceptive group ($P = .013$) (Table 3). The respiratory exchange ratio (RER) was higher in the mid-follicular and inactive-pill phase compared with the respective mid-luteal and active-pill phase ($P = .035$). Moreover, the RPE at 30 minute was greater in the mid-follicular phase

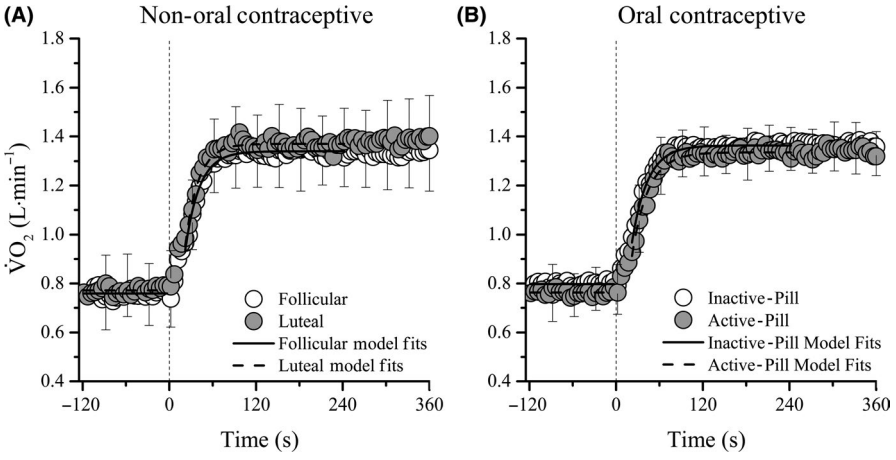

FIGURE 2 $\dot{V}O_2$ profile showing model of best-fit line for the group mean responses (with SD bars) of non-oral contraceptive group (A) and oral contraceptive group (B) during the transition to moderate-intensity exercise during the follicular or inactive-pill (open circles and solid black line) and luteal or active-pill (gray circles and dashed black line) phases of the respective menstrual and oral contraceptive cycles. Each dot represents the average $\dot{V}O_2$ within a 5-s time bin. Refer to Table 1 and results section for statistical significances

TABLE 2 Physiological performance measured during the ramp-incremental test

	Non-oral contraceptive (n = 15)		Oral contraceptive (n = 15)		*P*-values	
	Follicular	Luteal	Inactive-Pill	Active-Pill	Between Groups	Between Phases
PO_{peak} (W)	294 ± 27	289 ± 28	270 ± 36	271 ± 39	.088	.246
PO_{peak} (W kg^{-1})	4.5 ± 0.5	4.5 ± 0.4	4.3 ± 0.5	4.3 ± 0.5	.238	.161
$\dot{V}O_{2max}$ (L min^{-1})	3.06 ± 0.32	3.00 ± 0.33	2.87 ± 0.39	2.87 ± 0.45	.245	.304
$\dot{V}O_{2max}$ (mL kg^{-1} min^{-1})	47.1 ± 4.1	46.0 ± 3.9	45.5 ± 5.3	45.2 ± 4.3	.442	.103
HR_{max} (bpm)	182 ± 12	181 ± 11	186 ± 8	186 ± 9	.248	.977
$[BLa]_{max}$ (mmol L^{-1})	10.0 ± 1.4[a]	9.4 ± 1.3[a]	10.9 ± 2.1	11.0 ± 1.9	.034	.370
RPE	9.5 ± 0.8	9.8 ± 0.6	9.5 ± 1.1	9.4 ± 0.7	.773	.539
GET						
$\dot{V}O_2$ (L min^{-1})	1.88 ± 0.15	1.87 ± 0.16	1.81 ± 0.17	1.77 ± 0.18	.153	.180
HR (bpm)	137 ± 15	135 ± 16	141 ± 14	146 ± 14	.087	.311
RCP						
$\dot{V}O_2$ (L min^{-1})	2.60 ± 0.27	2.63 ± 0.29	2.46 ± 0.34	2.42 ± 0.31	.116	.945
HR (bpm)	165 ± 14	164 ± 16	167 ± 11	169 ± 13	.443	.774

Note: Data are presented as mean and SD (±).

Abbreviations: $[BLa]_{max}$, highest blood lactate value recorded at the end of the ramp test; GET, gas exchange threshold; HR, heart rate; HR_{max}, highest heart rate value recorded during ramp test; PO, power output; PO_{peak}, highest work rate achieved at the end of ramp test; RCP, respiratory compensation point $\dot{V}O_2$, oxygen uptake; $\dot{V}O_{2max}$, maximal oxygen uptake.

[a]Significantly different compared with the oral contraceptive group.

compared with the mid-luteal phase of the menstrual cycle ($P = .022$) but was not significantly different at any time point during $MLSS_p$ between the inactive- and active-pill phases of the oral contraceptive cycle (Table 3; Figure 3E,F). $\dot{V}O_2$, duration, and RPE at exhaustion during TTE were not different between the menstrual and oral contraceptive cycle phases. The PO at TTE was significantly higher ($P = .046$) while the [BLa] at the end of the trial was significantly lower ($P = .022$) for the non-oral contraceptive group compared with the oral contraceptive group (Table 3). Moreover, the [BLa] at the end of the trial was significantly higher in the mid-follicular phase compared with the mid-luteal phase of the menstrual cycle ($P = .040$) but was not significantly different between the inactive- and active-pill phases of the oral contraceptive cycle (Table 3). The $\dot{V}O_2$ at the end of the TTE was not different from the $\dot{V}O_{2max}$ observed during the ramp-incremental test ($P = .641$).

4 | DISCUSSION

The present investigation is the first to have examined both the menstrual and oral contraceptive cycle effects on a wide range of submaximal and maximal responses to exercise in a single study with a large sample size considering the physiological measures under evaluation. The key findings of the study were that the menstrual and monophasic oral contraceptive cycle phases did not affect: (a) the rate of adjustment of $\dot{V}O_2$ and [HHb] during the transition to moderate-intensity cycling exercise; (b) the PO and key physiological variables ($\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}_E$, HR, and [BLa] at minute 10 and 30) at MLSS; or (c) ramp-incremental maximal responses to exercise ($\dot{V}O_{2max}$, PO_{peak}, maximal HR, [BLa]) and TTE. RPE at minute 30 of the $MLSS_p$ trial and [BLa] at the end of the TTE trial were significantly different between the mid-follicular and mid-luteal phases of the menstrual cycle.

4.1 | Submaximal responses to exercise

4.1.1 | $\dot{V}O_2$ and [HHb] kinetics

The evaluation of the dynamic adjustment of oxygen utilization during the exercise on-transient provides valuable information about cardiovascular and metabolic adjustments during submaximal exercise. Given the known effects of the phases of the menstrual cycle on the O_2 transport system and metabolic responses to exercise,[6,7] it could be assumed that the $\dot{V}O_2$ kinetics might be affected by the phase of the menstrual cycle. Data from the present study indicate that, at least in a group of active women with relatively fast $\dot{V}O_2$ kinetics (~20 seconds), this is not the case. Surprisingly, only one previous study with a small sample (n = 7) of young women has examined and shown that the menstrual cycle phase (early-follicular compared with mid-luteal phase) did not affect the $\dot{V}O_2$ or [HHb] kinetics response.[22] In addition to confirming

TABLE 3 Group mean responses at $MLSS_p$ and during TTE

	Non-oral contraceptive (n = 15)		Oral contraceptive (n = 15)		*P*-values	
	Follicular	Luteal	Inactive-Pill	Active-Pill	Between Groups	Between Phases
$MLSS_p$						
PO (W)	181 ± 30[a]	182 ± 29[a]	155 ± 26	155 ± 27	.013	1.000
$\dot{V}O_2$(L min^{-1})	2.58 ± 0.34	2.62 ± 0.35	2.39 ± 0.32	2.39 ± 0.29	.083	.436
$\dot{V}CO_2$ (L min^{-1})	2.37 ± 0.30	2.37 ± 0.32	2.19 ± 0.35	2.13 ± 0.27	.073	.464
$\dot{V}_E$(L min^{-1})	91.84 ± 15.80	95.76 ± 15.42	84.52 ± 12.50	85.88 ± 17.79	.121	.213
RER	0.92 ± 0.04[b]	0.90 ± 0.05	0.91 ± 0.04[b]	0.90 ± 0.05	.494	.035
$HR_{10\ min}$ (bpm)	162 ± 15	164 ± 15	166 ± 9	166 ± 10	.412	.448
$HR_{30\ min}$ (bpm)	168 ± 16	170 ± 16	173 ± 9	172 ± 9	.385	.408
$[BLa]_{10\ min}$ (mmol L^{-1})	4.8 ± 1.8	4.2 ± 1.4	5.4 ± 1.6	5.1 ± 2.0	.206	.050
$[BLa]_{30\ min}$ (mmol L^{-1})	5.1 ± 1.9	4.6 ± 1.6	5.9 ± 1.4	5.6 ± 2.1	.130	.226
$RPE_{10\ min}$	4.3 ± 1.3	4.1 ± 0.7	4.1 ± 0.8	3.8 ± 0.7	.414	.101
$RPE_{30\ min}$	6.2 ± 1.5[c]	5.3 ± 1.4	6.6 ± 1.4	6.3 ± 1.5	.171	.017
TTE						
PO (W)	250 ± 24[a]		229 ± 31		0.013	
Time (s)	147 ± 42	128 ± 54	146 ± 70	139 ± 77	.827	.405
$\dot{V}O_2$(L min^{-1})	3.01 ± 0.38	3.02 ± 0.36	2.89 ± 0.44	2.86 ± 0.42	.310	.919
[BLa] (mmol L^{-1})	8.0 ± 2.1[a,c]	6.8 ± 1.5[a]	9.0 ± 2.0	8.6 ± 2.0	.022	.037
RPE	9.7 ± 0.6	9.6 ± 0.8	9.7 ± 0.6	9.7 ± 0.5	.881	.190

Note: Data are presented as mean and SD (±).

Abbreviations: [BLa], blood lactate concentration; HR, heart rate; PO, power output; RER, respiratory exchange ratio; RPE, rate of perceived exertion; $\dot{V}O_2$, oxygen uptake; $\dot{V}CO_2$, carbon dioxide production; $\dot{V}_E$, ventilation.

[a]Significantly different compared with the oral contraceptive group.

[b]Significantly different compared with the respective luteal or active-pill phase.

[c]Significantly different compared with the luteal phase.

the similarities across phases of the menstrual cycle that were previously observed, this study also demonstrates that $\dot{V}O_2$ and [HHb] kinetics responses are unaffected by the inactive-pill and active-pill phases of the monophasic oral contraceptive cycle as well.

Based on the idea that blood flow adjustments within the microcirculation might have been affected by the phase of the menstrual cycle,[6,7] this study examined the dynamic adjustment of the [HHb] and $\dot{V}O_2$ responses to indirectly evaluate the matching of local blood flow to $\dot{V}O_2$. The small values for the [HHb]/$\dot{V}O_2$ ratio (~1.00) and the lack of difference between the menstrual and oral contraceptive cycle phases suggest that the matching of local oxygen delivery to oxygen utilization within the active muscles was similar in both groups and between cycle phases. The combination of a small [HHb]/$\dot{V}O_2$ ratio and relatively fast $\dot{V}O_2$ kinetics responses is in line with previous reports.[35,37] This study did not aim to explain the mechanisms that control the dynamics of oxidative phosphorylation; however, the present data indicate that the phase of the menstrual or oral contraceptive cycle does not play a major role in regulating the matching of local O_2 delivery to O_2 utilization.

4.1.2 | Maximal lactate steady-state

This is the first investigation to explore and report on the effects of the menstrual and oral contraceptive cycle phases on the MLSS response. The results indicated that the PO corresponding to MLSS and the physiological variables ($\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}_E$, HR, and [BLa]) were unaffected by the menstrual and oral contraceptive cycle phases. Estrogen has been linked to increased lipid and reduced carbohydrate oxidation during exercise.[2,3,5,8] Consistent with this effect, high estrogen levels in the mid-luteal compared with the mid-follicular phase have been associated with the sparing of muscle glycogen,[39] primarily due to changes in lipoprotein lipase and hepatic lipase activities, and altered secretion rates of lipolytic and glucoregulatory hormones such as growth hormone, insulin and glucagon.[3,8] Similarly, a lower RER in the luteal phase[8,17,18]

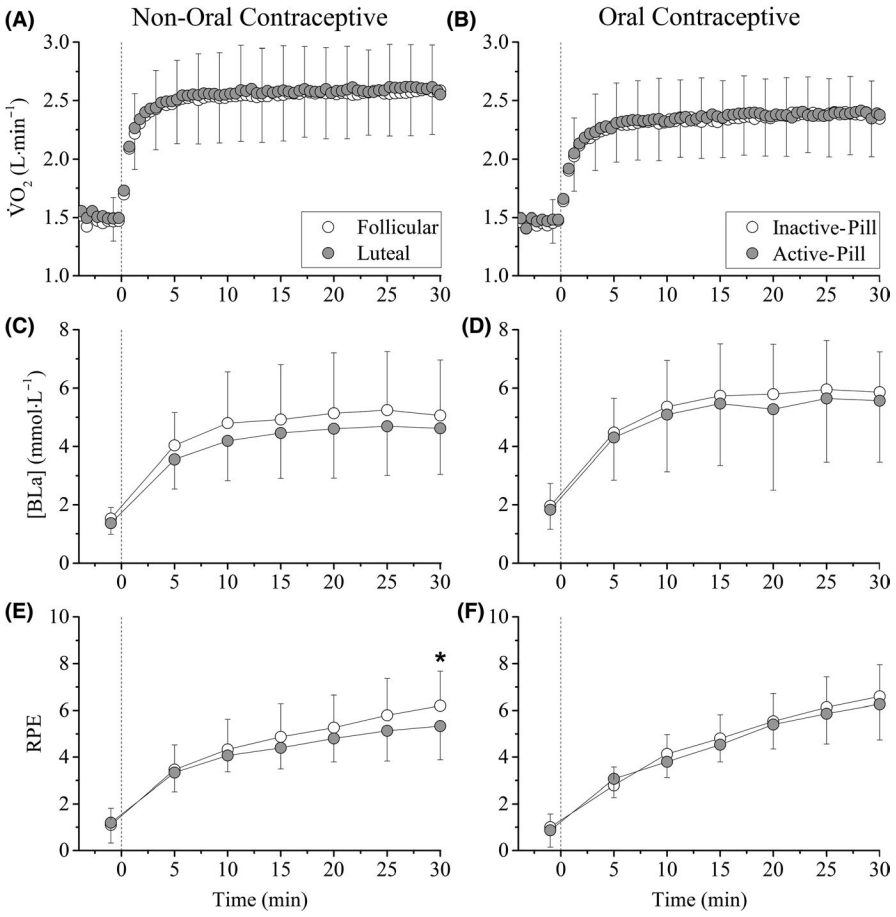

FIGURE 3 Group mean data (with SD bars) displaying $\dot{V}O_2$, blood lactate concentration, and RPE for non-oral contraceptive group (A, C, E) and oral contraceptive group (B, D, F) during the 30-minute constant-load trials at $MLSS_p$ during the follicular or inactive-pill (open circles) and luteal and active-pill (gray circles) phases of the respective menstrual or oral contraceptive cycle. Refer to Table 3 and results section for statistical significances

has been reported during submaximal exercise, which is indicative of a decreased reliance on glycolytic sources; however, not all studies support this outcome.[20] The findings of this investigation also showed RER was higher in the mid-follicular and inactive-pill phase compared with the respective mid-luteal and active-pill phase. Given the effect of estrogen on substrate metabolism, it is not surprising that some studies have shown lower [BLa] following submaximal exercise in the mid-luteal phase compared with the mid-follicular phase.[18,20] Similar to endogenous estrogen, oral contraceptive agents containing ethinyl estradiol have been reported to increase lipid oxidation and decrease carbohydrate oxidation during exercise.[11] Taken together, these findings support that MLSS, an intensity of exercise that predominately relies on the degradation of glycogen to pyruvate and subsequent oxidative phosphorylation, would be affected by the phase of the menstrual or oral contraceptive cycle. Although data from the present investigation cannot elucidate the potential mechanisms that may be responsible for the similar physiological responses at MLSS between the phases of the cycle, high levels of progesterone or progestin in the mid-luteal or active-pill phase, respectively, of the menstrual or oral contraceptive cycle may have played a role as progesterone has been proposed to have an antagonistic effect in the shift from glucose to fat oxidation.[3] In animal studies, it has been proposed that unlike estrogen, progesterone may increase glucose transport by upregulating the translocation of glucose transporter type 4 (GLUT4) and inhibit estrogens effects on increasing the activity of key enzymes in the fatty acid oxidation pathway (CPT1 and ß-3-hydroxyacyl-CoA dehydrogenase).[3]

An important observation was that the RPE at minute 30 of the MLSS ride was significantly higher in the mid-follicular phase compared with the mid-luteal phase of the menstrual cycle but did not differ between oral contraceptive cycle phases. Although there is minimal information about the influence of hormonal fluctuations on subjective responses to exercise, a large study (n = 117) found steeper increases in pain and RPE across an exercise bout in the early-follicular phase compared with the late-follicular or luteal phases in sedentary non-hormonal contraceptives users, but not among hormonal contraceptives users.[40] It was suggested that differences in the responses between groups may be a result of less variability in hormones across the hormonal contraceptive cycle.[40] Because RPE may be difficult to differentiate from pain, another explanation could be that RPE values at the end of the MLSS trial may have been affected by a decreased perception of pain in the mid-luteal phase compared with the late-follicular phase (known as the "luteal analgesia" effect).[41] Although it is unclear why perception of effort was different when all physiological responses remained the same, it would be interesting to know how this elevated RPE in the mid-follicular phase would have affected performance during

a longer ride. For example, if the MLSS ride was prolonged until volitional exhaustion, it is possible that these women not using oral contraceptives may disengage from the exercise trial earlier due to the greater perception of effort. Future studies should further examine these responses to exercise.

4.2 | Maximal responses to exercise

4.2.1 | Exercise performance during the ramp-incremental test

$\dot{V}O_{2max}$, PO_{peak}, maximal HR, and end-exercise RPE were unchanged by the menstrual and oral contraceptive cycle phases. This finding is comparable to the majority of literature examining the effects of the menstrual or oral contraceptive cycle phases on these variables.[12-14,42] Even in a study conducted by Lebrun et al[16] that found absolute $\dot{V}O_{2max}$ was greater in the early-follicular phase compared with the mid-luteal phase of the menstrual cycle, this difference was no longer present when $\dot{V}O_{2max}$ was expressed relative to body mass. Another study examining maximal/peak responses to exercise indicated that high-altitude native women residing at an altitude of 3500 m, who were relatively sedentary, showed a greater maximal power output in the mid-luteal phase compared with the mid-follicular phase.[15] However, no significant difference in $\dot{V}O_{2max}$ were observed in that study.[15] Contrary to the majority of studies testing women with a large spectrum of activity level, the present study examined maximal exercise performance in active women (mean $\dot{V}O_{2max}$ greater than 45 mL kg^{-1} min^{-1}) to minimize a large variability in the response and increase the sensitivity to detect smaller changes.

The present study also showed that [BLa] following maximal exercise is unaffected by the menstrual cycle phase. Current data on this variable are also controversial, with some studies indicating no significant menstrual cycle phase effect on [BLa],[19,43] and others showing a lower [BLa] following exhaustive exercise in the mid-luteal phase compared with the mid-follicular phase.[18,20] Significant differences in [BLa] between phases of the cycle could be explained by the slower plasma glucose kinetics and lower total carbohydrate oxidation in the luteal phase compared with the follicular phase of the menstrual cycle.[2] The results of this investigation, however, show that potential changes in substrate metabolism due to the fluctuations in estrogen concentrations have no impact on [BLa] following the ramp-incremental test to exhaustion.

From a submaximal perspective, ventilatory thresholds corresponding to GET and RCP, which are important markers of exercise intensities,[44] were also unaffected by the menstrual and oral contraceptive cycle phases. Previous literature examining these thresholds are limited in number when investigating within the menstrual and oral contraceptive cycles, yet the present study is in agreement with the few studies showing no menstrual cycle phase effects on GET and RCP.[13,14]

Thus, data from the present investigation demonstrate that physiological responses, as well as perception of effort, to maximal and submaximal performance derived from a ramp-incremental test are not affected by the phase of the menstrual and oral contraceptive cycles.

4.2.2 | Time-to-exhaustion

The findings from this investigation show that $\dot{V}O_2$ and duration of TTE following the $MLSS_p$ trial were not different between the menstrual and oral contraceptive cycle phases. Given the short duration and high intensity of the trials, it is unlikely that potential changes in substrate metabolism observed with high estrogen or ethinyl estradiol levels played a prominent role in energy provision. Rather, non-oxidative sources likely played a major role in substrate utilization. Interestingly, a study that has examined TTE trials at a high intensity and found that TTE at 90% PO_{peak} was doubled in the mid-luteal phase compared with the mid-follicular phase.[18] A recent study agrees with the findings from Jurkowski et al,[21] demonstrating that intermittent isometric TTE with the knee extensors was longer in the mid-luteal phase, compared with the early- and late-follicular phases. The minimal literature examining longer duration time-to exhaustion trials have shown no significant difference across the different phases.[16,17] While the majority of the variables measured during the TTE remained unchanged by the menstrual and oral contraceptive cycles, the [BLa] at the end of the TTE was greater in the mid-follicular phase compared with the mid-luteal phase of the menstrual cycle. Future research is needed to explain this observation.

4.3 | Experimental considerations

Similar to all studies examining the menstrual cycle effects, confidence regarding the timing of testing is a potential limitation. Based on the participants tracking their normal menstrual cycles for at least two months and confirmation of ovulation using a urine analysis ovulation kit which detects the LH surge with more than 99% accuracy, we are confident that the testing was done in the follicular and luteal phases of the menstrual cycle, despite not having blood hormonal levels for each phase. However, lacking hormone measurements does limit the ability to distinguish ovulatory cycles from luteal phase deficient cycles.[38] Given the average menstrual cycle length and day of ovulation, testing days were conducted in the mid-follicular phase, where estrogen levels are increasing but have not peaked. It could be argued that if testing was conducted in the late-follicular phase, when estrogen concentration is

highest, the theoretical advantages of high estrogen may have been more impactful on the exercise variables of interest in the present study. While this result is plausible, a study examining the hormonal fluctuations throughout the monophasic oral contraceptive cycle has shown that while the consumption of exogenous hormones is absent during the inactive-pill phase, endogenous estrogen rises to a peak and endogenous progesterone concentrations stay low, mirroring the follicular phase of the menstrual cycle.[45] Moreover, the active-pill phase contains high levels of both ethinyl estradiol and a progestin, mirroring the luteal phase of the menstrual cycle.[45] Therefore, it is likely that the potential theoretical advantages of high estrogen concentrations during the late-follicular phase of menstrual cycle would have been reflected in the oral contraceptive cycle, but our data indicate that this was not the case. In addition, a learning effect between the cycle phases may have impacted the maximal performance outcomes during the ramp-incremental test to exhaustion as a familiarization visit prior to the testing sessions was not performed and, specifically for the non-oral contraceptive group, the visits could not be randomized. However, submaximal physiological responses would not have been impacted by an order effect.

5 | CONCLUSION AND PERSPECTIVE

This investigation provides strong evidence that any endogenous or exogenous hormonal fluctuations between the mid-follicular and mid-luteal phases of the menstrual cycle or the inactive-pill and active-pill phases of the oral contraceptive cycle do not influence a wide range of submaximal and maximal responses to exercise in young, healthy, active women. The present data indicate that: (a) active eumenorrheic women or women using the monophasic oral contraceptive pill should not focus on the timing within their respective cycle with regards to optimizing acute submaximal or maximal aerobic exercise performance, at least when the duration of exercise does not exceed ~30 minutes; and (b) depending on the research question, studies evaluating exercise performance should not avoid testing women solely on the idea that the phase of the cycle might act as a confounding factor for the outcome measures.

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CONFLICT OF INTERESTS

No conflicts of interest, financial or otherwise, are declared by the authors. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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