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ORIGINAL ARTICLE

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Impact of menstrual cycle phase on the exercise status of young, sedentary women

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Abstract The purpose of the present study was to compare exercise status during the follicular (FP) and luteal (LP) phases of the menstrual cycle of a single group of young, sedentary women, where the marked differential in the blood concentrations of 17β-oestradiol ([E_2]) and progesterone ([P_4]) has the potential to alter the metabolic response to exercise. Fourteen females [21.8 (4.0) years, peak oxygen uptake ($\dot{V}O_{2peak}$) <45 ml·kg $^{-1}$·min^{-1}] performed both incremental exercise to exhaustion and steady-state submaximal cycle ergometer exercise while measurements were made of several metabolic and hormonal variables. With the incremental exercise test, time to exhaustion, maximal power output and total work done were not different between the two phases, nor were the absolute values for $\dot{V}O_{2peak}$ or the corresponding values for ventilation ($\dot{V}E$), respiratory frequency (f_R) and heart rate (HR). Resting, end-exercise and peak (post-exercise) plasma lactate concentrations and the lactate threshold were not different between the two phases either. However, as the workloads increased during the incremental protocol, plasma lactate concentration, carbon dioxide output ($\dot{V}CO_2$) and the respiratory exchange ratio (RER) all were lower during LP, while oxygen uptake ($\dot{V}O_2$) was higher. With steady-state submaximal exercise, at workloads corresponding to 25% and 75% of menstrual cycle phase-specific $\dot{V}O_{2peak}$, $\dot{V}O_2$ and the oxygen pulse ($\dot{V}O_2$/HR) were higher and RER and plasma lactate concentration lower during LP. Regardless of phase, [E_2] increased with both incremental and steady-state submaximal exercise, while [P_4] was unchanged. It is concluded that while exercise capacity, as defined by $\dot{V}O_{2peak}$ and the lactate threshold, is unaffected by cycle phase in young, sedentary women, the metabolic responses in the LP during both incremental and steady-state submaximal exercise suggest a greater dependence on fat as an energy source.

Keywords Lactate threshold · Oestrogen · Oxygen uptake · Progesterone · Substrate utilisation

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Introduction

The menstrual cycle is a natural monthly event in young women, coordinated by the hypothalamic-pituitary-ovarian axis and influenced by physiological and pathological changes that occur throughout the lifetime of the woman (Norman and Phillipson 1998). It is characterised by fluctuations in the blood concentrations of several hormones, including 17β-oestradiol ([E_2]), progesterone ([P_4]), luteinising hormone ([LH]) and follicle-stimulating hormone ([FSH]), but can be divided into two distinct hormone environments or phases separated by ovulation. Throughout the pre-ovulation or follicular phase (FP), plasma [E_2] and [P_4], the two principal menstrual cycle hormones, are low, while during the post-ovulation or luteal phase (LP), both [E_2] and [P_4] are increased several-fold. Both [E_2] and [P_4] can affect energy substrate metabolism (Hackney 1999; Hackney et al. 1994), thermoregulation (Kolka and Stephenson 1997) and body water and electrolyte homeostasis (De Souza et al. 1989), all of which are crucial to exercise status. Therefore, the different hormone environments in the follicular and luteal phases of the menstrual cycle have the potential to impact on both exercise capacity and exercise performance.

While there have been some studies of the effect of menstrual cycle phase on exercise status in women with normal menstrual cycles (Jurkowski et al. 1978; Hall Jurkowski et al. 1981; Bonen et al. 1983), they have failed to expose a consistent interaction with the changing [E_2] and [P_4] profile. In terms of exercise performance, an increased time to exhaustion has been reported in the LP of the menstrual cycle (Hall Jurkowski et al. 1981; Nicklas et al. 1989; Lebrun et al. 1995). This may reflect a greater reliance on lipid metabolism as an energy source in LP, a contention supported by reports of a lower respiratory exchange ratio (RER) (Hackney et al. 1993, 1994; Hackney 1999), coupled with lower carbohydrate oxidation and utilisation rates (Kanaley et al. 1992; Hackney et al. 1994). There is, however, no consensus with regard to the relationship between the changing hormone profiles during the menstrual cycle and exercise capacity (Jurkowski et al. 1978; Hall Jurkowski et al. 1981; Bonen et al. 1983). Maximal or peak oxygen uptake ($\dot{V}O_{2max}$or $\dot{V}O_{2peak}$), a critical determinant of exercise capacity, appears to be independent of menstrual cycle phase (De Souza et al. 1989; Nicklas et al. 1989; Bemben et al. 1995; Beidleman et al. 1999) although higher values have been reported in the FP (Schoene et al. 1981; Lebrun et al. 1995). There have been no studies of the effect of menstrual cycle phase on the lactate profile during exercise or the lactate threshold, which is the other major determinant of exercise capacity (Coyle et al. 1988; Robergs and Roberts 2000). There is some degree of consensus that the ventilatory threshold, an indirect index of the onset of lactic acidosis during exercise, is unaffected by phase of the menstrual cycle (Schoene et al. 1981; Stephenson et al. 1982; Dombovy et al. 1987), although one study found a lower relative ventilatory threshold during the early FP (Bemben et al. 1995).

The inconsistent findings regarding the interaction between menstrual cycle phase and exercise status may be a consequence of differences in the methods used to define normal menstrual function and to identify the phase of the menstrual cycle (body temperature versus hormone assay), and the choice of phase studied (menstruation, FP, ovulation, LP) (Ashley et al. 2000). Furthermore, the cross-sectional nature of the studies and variations in subject selection criteria with regard to age, menstrual cycle history, fitness status and menstrual cycle length make the interpretation of previous studies difficult.

The present study was an attempt to address previous shortcomings in this field by employing a more tightly controlled protocol design. To this end our subject cohort comprised a single group of young, sedentary females who were studied at hormone-defined time points in the FP and LP of consecutive menstrual cycles. We explored exercise status in both cycle phases and, given the general literature consensus that lipids are the preferred fuel in the luteal phase cycle, we hypothesised a corresponding enhancement in exercise status.

Methods

Subject selection

Twenty-seven healthy sedentary females with normal menstrual cycles were recruited by advertisement and were required to complete a questionnaire assessing their general and gynaecological health. Of the 27 subjects, 16 matched the inclusion criteria for this study (the subjects had normal menstrual function, were sedentary, non-smokers and neither pregnant nor using oral contraceptives for at least 6 months prior to the study). One member of this subject group withdrew from the study due to illness. The results of one other individual were not included in the final data analysis as her $\dot{V}O_{2peak}$ was greater than 60 ml $\cdot$ kg^{-1} $\cdot$ min^{-1}, suggesting she was not sedentary (Bruce et al. 1973) and therefore did not meet the inclusion criteria for the study. All data therefore are for 14 subjects [age, 21.4 (3.7) years; height, 1.7 (0.5) m; body mass, 63.7 (11.8) kg; BMI 22.5 (4.0) kg $\cdot$m^{-2}; mean (SD)].

The definition of sedentary for this study was that the subjects' occupation did not require physical labour and the subjects did not perform structured physical activity more than once a week (Sargent and Scroop 2002). All subjects had demonstrated normal menstrual function during the 6 months prior to the study, as defined by a consistent menstrual cycle length [29.9 (2.9) days] with a bleeding interval no longer than 6 days [4 (1) days]. The study was approved by The University of Adelaide Human Research Ethics Committee and subjects gave their written consent following provision of a written and oral explanation of the procedures, protocols and risks entailed.

Menstrual cycle monitoring

Subjects were required to record their menstrual cycle histories for the 6 months prior to the study and complete a menses calendar (ReproMed, Australia) throughout the study. Duration of menstrual bleeding was recorded and the timing of ovulation detected by using ClearPlan Home Ovulation Kits (Unipath, Bedford, England) to identify a surge in urinary [LH]. The timing of ovulation was confirmed for each subject by the post hoc measurement of a rise in [P_4] concentration (ADVIA Centaur System, Bayer Diagnostics, USA).

Experimental design

Subjects were required to attend the laboratory on five separate occasions. An initial familiarisation session was followed by incremental exercise to exhaustion (incremental exercise test) and steady-state submaximal exercise tests which were completed during the follicular (days 5–7) and luteal (days 21–23) phases of two consecutive menstrual cycles. Incremental exercise testing (first cycle) preceded submaximal testing (second cycle) and in each case the order of testing (follicular versus luteal) was randomised such that eight subjects commenced in the FP and six in the LP. Subjects recorded their dietary intakes in the 24 h prior to the first exercise session and this diet was replicated in the 24 h preceding each subsequent visit. On the day of testing, subjects reported to the laboratory following a 4-h fast having abstained from alcohol, caffeine and strenuous physical activity for the previous 24 h. On arrival in the laboratory, body mass was measured and chest electrodes applied for ECG (Nihon Koden Lifescope 6 Portable Patient Monitor) and heart rate monitoring (Polar Vantage NV heart rate monitor). Following 10 min of supine rest, a Teflon catheter (Jelco 18G, 1.2 mm x 44 mm length) was inserted under local anaesthesia (lignocaine hydrochloride, 2%) into the deep muscle branch of an ante-cubital vein with its tip directed distally. A 30-cm polyethylene extension (Braun Minimum Volume Extension Tubing, dead space 0.3 ml), filled with normal saline and sealed with a three-way stop cock (Discofix), was attached to the

catheter and taped to the skin. To prevent forearm exercise and catheter displacement a simple elbow splint (Lemmco Elbow Immobilisers, Velcropedic Ace Surgical Supply, Massachusetts, USA) supported the arm. The subject was then seated on a pre-calibrated Monark cycle ergometer (Model 818E, Varburg, Sweden) and a low resistance respiratory valve (Hans Rudolph R2700, Kansas City, Mo., USA) was fitted to enable gas exchange measurements. The valve was held in place by a head support (Hans Rudolph Head-Support for Rudolph Valves, Model No. 2766).

Exercise protocols

Incremental exercise to exhaustion

Following a 5-min rest period, exercise began with 2 min of unloaded cycling (0 W) at 50 rev · min^{-1} and thereafter the power output was incremented by 25 W every 2 min until, despite strong vocal exhortation, the subject could not maintain the target pedal cadence. Venous blood samples were drawn at rest, in the last 30 s of each 2-min workload during exercise and every min for 10 min post-exercise and assayed for plasma lactate concentration. Plasma [E_2] and [P_4] were measured before and 10 min after exercise.

Steady-state submaximal exercise at 25% and 75% $\dot{V}O_{2peak}$

The workloads corresponding to exercise intensities of 25% and 75% $\dot{V}O_{2peak}$ (Table 1) were determined from individual regression analyses of workload against relative exercise intensity (% $\dot{V}O_{2peak}$) for each cycle phase. Following a 5-min rest period, exercise began at a workload corresponding to a relative exercise intensity of 25% of each subject's phase-specific $\dot{V}O_{2peak}$ and continued for 20 min, at which time point the workload was increased to 75% $\dot{V}O_{2peak}$ and exercise continued for a further 20 min. Exercise then ceased and the subject sat resting on the ergometer seat or seated in a chair for a further 10 min. Venous blood samples for plasma lactate analysis were taken at rest, in the last 30 s of each 5-min interval throughout the consecutive exercise periods, and at 1, 5 and 10 min post-exercise. Blood samples for hormone analysis were taken at rest, in the last 30 s of each 20-min exercise period, and at 10 min post-exercise.

Cardiorespiratory variables

Minute ventilation ($\dot{V}E$), oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were calculated from 30-s sampling epochs throughout each test using an open-circuit indirect spirometry system, similar to that described previously (Gore et al. 1992). In brief, subjects breathed through a low resistance respiratory valve with a pre-calibrated, high-flow, turbine transducer (P.K. Morgan, London) attached to the inspiratory port. Expired air was directed to a 2.6-l mixing chamber (Sportech, Canberra, Australia) from which dried gas was sampled continuously (~500 ml · min^{-1}) and passed to oxygen (O_2) and carbon dioxide (CO_2) analysers (ServoMax, UK). The analysers were calibrated prior to each exercise test with two commercially produced gas mixtures of known O_2 and CO_2percentages (BOC Gases, Australia) covering the physiological range of measurement. The ventilometer and analysers were interfaced with an IBM-compatible computer that performed all of the necessary calculations using standard algorithms and Labview-based software (Metabolic Analyser) developed by ICON technologies for the Western Australian Institute of Sport. $\dot{V}E$, $\dot{V}O_2$, $\dot{V}CO_2$ and RER were calculated by averaging the values obtained from the two consecutive 30-s sampling epochs within each minute. Tidal volume (litres) and breathing frequency (f_R, breaths · min^{-1}) were displayed as 30 s averages and heart rate (HR, beats · min^{-1}) was recorded continuously throughout each test (consecutive five beat averages) with the highest value designated as the maximal heart rate (HR_{max}). $\dot{V}O_{2peak}$ was designated as the mean $\dot{V}O_2$ during the minute in which the highest 30 s epoch value was recorded and was expressed in l · min^{-1}.

Table 1 Actual workloads corresponding to 25% and 75% peak oxygen consumption ($\dot{V}O_{2peak}$) applied during the submaximal exercise tests. Values are mean (SE), $n = 14$

Parameter	25% $\dot{V}O_{2peak}$		75% $\dot{V}O_{2peak}$	
	FP	LP	FP	LP
Power output (W)	30 (2.2)	31 (1.8)	131 (11.3)	133 (5.9)
$\dot{V}O_2$ (l · min^{-1})	0.7 (0.0)	0.7 (0.0)	2.0 (0.1)	2.0 (0.1)
HR (beats · min^{-1})	104 (4)	101 (2)	162 (3)	160 (2)

Data analysis

The changes in $\dot{V}O_2$ (l · min^{-1}) with increasing exercise intensity (expressed as a percentage of the workload at exhaustion, % WL_{peak}) and the changes in workload, HR, $\dot{V}CO_2$ and RER with increasing exercise intensity (expressed % $\dot{V}O_{2peak}$) were modelled as linear regression equations. For each subject, the values for workload, $\dot{V}O_2$, HR, $\dot{V}CO_2$, and RER, which corresponded, respectively, to consecutive 10% increments in WL_{peak} or $\dot{V}O_{2peak}$, were predicted from the slope and intercept of each linear regression equation. For each comparison, the coefficient of determination (r^2) and the mean squared error (MSE) were calculated to examine the goodness of fit between the observed data and the data predicted by the linear regression model.

The changes in $\dot{V}E$ and plasma lactate concentration with increasing exercise intensity (% $\dot{V}O_{2peak}$) were modelled for each subject as single exponential functions given by the equation:

$$y = a + b.e^{c.x}$$

where, at a given percentage of $\dot{V}O_{2peak}(x)$, y is the predicted value for $\dot{V}E$, or plasma lactate concentration, and a,b and c are mathematical parameters estimated by minimising the residual sum of squares between the values for $\dot{V}E$ and plasma lactate concentration and the curve fit (Hughson et al. 1987). For each comparison, the r^2 and the MSE were calculated to examine the goodness of fit between the observed data and the data predicted by the exponential model.

Lactate threshold and ventilation threshold determination

This was determined according to the method of Beaver et al. (1985) using a purpose-designed computer program in Basic (Microsoft Version 3.2) to transform the plasma lactate concentration and $\dot{V}O_2$ and the $\dot{V}E$ and $\dot{V}O_2$ data into logarithms. The log-log relationships were plotted and regression lines fitted through the upper and lower segments of the resultant plots whilst minimising the residual sum of squares. The lactate threshold and ventilation thresholds were designated as the $\dot{V}O_2$ corresponding with the point of intersection of the two regression lines. For each subject, the r^2 and the MSE were calculated to examine the goodness of fit of the observed data to the logarithmic model. The power output and HR corresponding to the $\dot{V}O_2$ at the lactate threshold and the ventilation threshold were also determined from linear regression equations of $\dot{V}O_2$ versus power output and HR, respectively.

Blood analyses

Hormones

Hormone concentrations were determined from 5 ml of whole blood collected into glass syringes rinsed with sodium heparin

(500 lU · ml^{-1}), immediately dispensed into a tube containing lithium heparin gel. Blood samples were kept on ice until centrifuged (Model TJ-6R Refrigerated Centrifuge, Beckman, USA) at 4000 *g* for 15 min at 4°C, and the plasma separated and frozen at –20°C for subsequent assay. Female sex hormone concentrations ([E_2], [P_4], [LH] and [FSH]) were analysed in duplicate in the one assay run using an automated chemi-luminescent assay system (ADVIA Centaur System, Bayer Diagnostics, USA).

Plasma lactate

Whole blood (2 ml) was collected into a glass syringe rinsed with sodium heparin (500 lU · ml^{-1}) and kept on ice before analysis of the plasma lactate concentration using an ABL 620 (Radiometer Medical, Copenhagen, Denmark) which was regularly serviced and maintained by local company representatives. The analyser was calibrated at hourly intervals throughout the day, and checked for accuracy, precision and reproducibility before each experiment using quality control solutions with known plasma lactate concentrations.

Statistical analysis

Statistical analysis revealed that a subject cohort of 14 was required to demonstrate a 10% change in hormone, cardio-respiratory and metabolic variables between the two phases of the normal menstrual cycle. To determine differences between the follicular and luteal phases of the menstrual cycle and pre and/ or post exercise data a Student's paired *t*-test and analysis of variance (ANOVA) were used where appropriate. To compare the cardiorespiratory and plasma lactate responses during incremental exercise to exhaustion between the phases of the menstrual cycle a two-factor repeated measures ANOVA (RMANOVA), incorporating a Greenhouse-Geisser adjustment for multi-sample sphericity, was used. The factors tested in the RMANOVA were cycle phase, level of exercise intensity (10 time points throughout exercise: rest, 20% to 100% $\dot{V}O_{2peak}$ or WL_{peak}) and interaction between cycle phase and level of exercise intensity for the incremental exercise test and cycle phase and exercise time (5-min intervals during the low- and high-intensity test) for the submaximal test. Where the two-way RMANOVA showed a significant interaction effect between cycle phase and level of exercise intensity, planned comparisons were performed between phases using Tukey's HSD post hoc analysis incorporating Bonferroni's correction to allow for multiple comparisons. The relationships between basal hormone concentrations and peak cardiorespiratory and metabolic variables were investigated using Pearson product-moment correlation coefficient. Unless otherwise stated, data are reported as mean (SE) and the level of significance for all statistical tests was set at$P \leq 0.05$.

Results

Incremental exercise to exhaustion

Hormone profiles

Rest Basal [E_2] and [P_4] were higher during the LP when compared with the FP {[E_2]: FP, 131.7 (34.9); LP, 348.0 (61.6) pmol · l^{-1}; [P_4]: FP, 1.7 (0.2); LP, 26.8 (5.6) nmol · l^{-1},$P < 0.001$}. Plasma [FSH] was lower during the LP [FP: 4.1 (0.2), LP: 1.6 (0.2) IU · l^{-1}, $P < 0.001$] but there was no observed difference in plasma [LH] between cycle the phases [FP: 4.5 (0.5), LP: 3.6 (0.9) IU · l^{-1}].

Exercise While [E_2] increased with exercise in both the FP [169.5 (40.4) pmol · l^{-1},$P < 0.01$] and LP [448.7 (81.4) pmol · l^{-1},$P < 0.001$] there was no effect of exercise on [P_4] in either phase [FP: 2.0 (0.2); LP: 28.4 (5.0) nmol · l^{-1}].

Work parameters

There was no effect of menstrual cycle phase on peak power output [WL_{peak}, FP: 175 (7.9); LP: 175 (8.9) W], time to exhaustion [FP: 15.9 (0.6); LP: 15.9 (0.7) min] or total work done [FP: 96.6 (12.9), LP: 89.7 (8.6) kJ].

Cardiorespiratory variables

Oxygen uptake There was no effect of cycle phase on the absolute $\dot{V}O_2$ at rest or at the end of exercise ($\dot{V}O_{2peak}$, Table 2) and no correlation between $\dot{V}O_{2peak}$ and either basal [E_2] or [P_4]. However, when the relationship between cycle phase and exercise intensity, expressed as $\%WL_{peak}$, was examined throughout exercise, the RMANOVA and post hoc analysis detected a significant interaction effect, such that at exercise intensities from 80 to 100% WL_{peak}, $\dot{V}O_2$ was higher in the LP (Fig. 1).

Table 2 Effect of menstrual cycle phase on cardio-respiratory variables at rest and following incremental exercise to exhaustion in sedentary women. Values are mean (SE), $n = 14$. (*FP* Follicular phase, f_R breathing frequency, *HR* heart rate, *LP* luteal phase, *RER* respiratory exchange ratio $\dot{V}CO_2$:$\dot{V}O_2$, $\dot{V}CO_2$ carbon dioxide output, $\dot{V}E$ ventilation, $\dot{V}O_2$ oxygen uptake)

Parameter	Rest		$\dot{V}O_{2peak}$	
	FP	LP	FP	LP
$\dot{V}O_2$ (l · min^{-1})	0.20 (0.02)	0.21 (0.01)	2.64 (0.15)	2.73 (0.15)
HR (beats · min^{-1})	86 (3)	81 (2)	184 (2)	182 (2)
$\dot{V}E$ (l · min^{-1})	5.90 (0.65)	6.52 (0.56)	77.07 (4.40)	77.93 (3.27)
$\dot{V}CO_2$ (l · min^{-1})	0.19 (0.02)	0.19 (0.02)	3.04 (0.16)	2.93 (0.14)*
RER	0.90 (0.03)	0.88 (0.03)	1.16 (0.04)	1.05 (0.03)*
f_R (breaths · min^{-1})	7.5 (1.0)	9.9 (1.2)	46.1 (2.0)	44.9 (1.7)
Tidal volume (l)	0.9 (0.1)	0.7 (0.)	1.7 (0.1)	1.8 (0.0)*

*Significantly different from follicular phase, $P < 0.05$

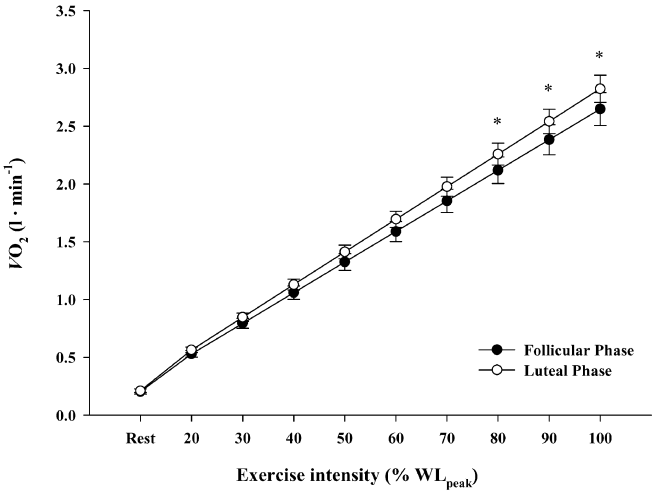

Fig. 1 Effect of menstrual cycle phase on oxygen uptake ($\dot{V}O_2$) during incremental exercise to exhaustion in sedentary females (*Closed circles* Follicular phase, *open circles* luteal phase.) *Significantly different from follicular phase, $P < 0.05$. Values are mean (SE)

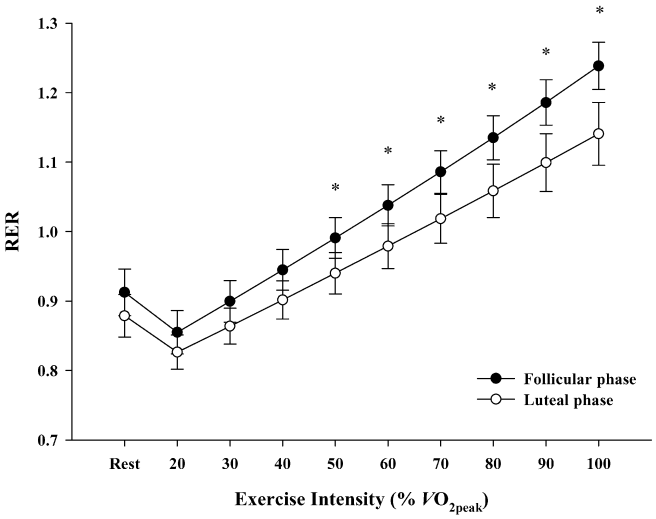

Fig. 2 Effect of menstrual cycle phase on respiratory exchange ratio (*RER*) during incremental exercise to exhaustion in sedentary women. (*Closed circles* Follicular phase, *open circles* luteal phase.) Values are mean (SE). *Significantly different from follicular phase, $P < 0.05$

Carbon dioxide output There was no effect of cycle phase on $\dot{V}CO_2$ at rest but $\dot{V}CO_2$ was lower [4.1 (2.7)%] in the LP at $\dot{V}O_{2peak}$ (Table 2). There was a significant interaction between the cycle phase and exercise intensity with $\dot{V}CO_2$ being lower in the LP at 100% $\dot{V}O_{2peak}$.

Respiratory exchange ratio While cycle phase had no effect on RER at rest (Table 2), RER was lower [10.1 (4.2)%, $P = 0.01$] in the LP at $\dot{V}O_{2peak}$. There was a significant interaction between cycle phase and exercise intensity (Fig. 2) such that RER was lower in the LP from 50% to 100% $\dot{V}O_{2peak}$. Furthermore there was a positive correlation ($y = 0.0006x + 1.1552$; $r = 0.65$; $P < 0.05$) between basal [E_2] and peak RER in the FP, but not in the LP of the menstrual cycle.

Ventilation The phase of the menstrual cycle had no effect on $\dot{V}E$, either at rest or $\dot{V}O_{2peak}$(Table 2) and there was no interaction between menstrual cycle phase and $\dot{V}E$ with increasing exercise intensity.

Breathing frequency and tidal volume Cycle phase had no effect on f_R or tidal volume at rest (Table 2). At $\dot{V}O_{2peak}$, tidal volume was higher in the LP [5.8 (2.1)%, $P < 0.05$] whilst f_R was not affected by phase of the menstrual cycle.

Heart rate There was no effect of menstrual cycle phase on either resting or maximal HR (HR_{max}) (Table 2) or the HR responses with increasing exercise intensity. Subjects attained 98.7 (1.1)% and 98.8 (1.2)% of their age-predicted HR_{max} in the FP and LP respectively.

Plasma lactate concentration and lactate threshold

Plasma lactate concentrations in the FP and LP were not different at rest [FP: 1.0 (0.1); LP: 0.8 (0.1) mmol · l^{-1}] or at the end of exercise [FP: 5.8 (0.3); LP: 5.5 (0.4) mmol · l^{-1}]. Furthermore, there were no differences between the peak values post-exercise [FP: 8.3 (0.6); LP: 7.7 (0.5) mmol · l^{-1}] or the time to reach the peak concentration [FP: 5.2 (0.5); LP: 5.4 (0.7) min]. However, when the changes in plasma lactate concentration with increasing exercise intensity were modelled as single exponential functions in both cycle phases (Fig. 3), a RMANOVA detected a significant main effect for cycle phase, with plasma lactate concentration being *lower* in the LP at all exercise intensities ($P < 0.04$). The lactate threshold, when expressed in terms of $\dot{V}O_2$ (l · min^{-1} or % $\dot{V}O_{2peak}$), was not affected by the menstrual cycle phase. Furthermore, there was no difference in the HR, plasma lactate concentration or power output at the lactate threshold between the phases of the menstrual cycle (Table 3).

Ventilatory threshold

There was no effect of the menstrual cycle phase on the ventilatory threshold [$\dot{V}E$: FP, 24.86 (2.21); LP, 21.76 (1.97) l · min^{-1}. % $\dot{V}O_{2peak}$: FP, 50.6 (3.6); LP, 45.7 (3.8)%] or HR and power output at the threshold.

Submaximal exercise

Workloads corresponding to 25% and 75% $\dot{V}O_{2peak}$

Table 1 lists the workloads corresponding to 25% and 75% of each subject's phase-specific $\dot{V}O_{2peak}$ which were

applied during the submaximal exercise tests. There were no differences in these workloads between the menstrual cycle phases.

Hormone profiles

Rest Basal [E_2] and [P_4] were higher in the LP, whereas [FSH] was lower and no phase difference was observed in [LH] (Table 4).

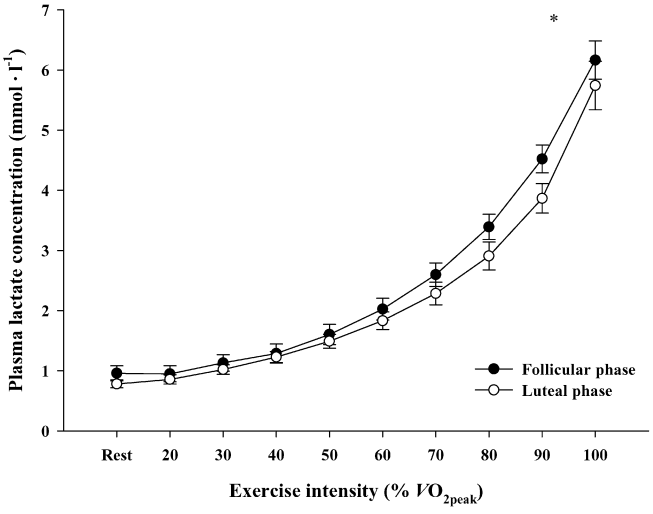

Fig. 3 Effect of menstrual cycle phase on plasma lactate concentrations during incremental exercise to exhaustion in sedentary women. (*Closed circles* Follicular phase, *open circles* luteal phase.) Values are mean (SE). *Significant main effect of menstrual cycle phase, follicular phase (FP) > luteal phase (LP) at all time points, $P < 0.05$

Table 3 Effect of menstrual cycle phase on physiological responses at the lactate threshold. Values are means (SE), $n = 14$

Parameter	FP	LP
$\dot{V}O_2$ ($l \cdot min^{-1}$)	1.22 (0.11)	1.32 (0.13)
% $\dot{V}O_{2peak}$	46.1 (3.2)	47.8 (3.9)
HR ($beats \cdot min^{-1}$)	130 (6)	123 (7)
% HR_{max}	67.9 (3)	66.9 (4)
Power output (W)	74.5 (8)	80.1 (11)
Plasma lactate concentration ($mmol \cdot l^{-1}$)	1.2 (0.2)	1.2 (0.1)

At 25% $\dot{V}O_{2peak}$ Exercise had no effect on [E_2] or [P_4] in either menstrual cycle phase (Table 4).

At 75% $\dot{V}O_{2peak}$ Exercise in the FP had no effect on [E_2] or [P_4], but during the LP [E_2] was significantly higher than both the pre-exercise value and that following low-intensity exercise (Table 4). At 10 min following the completion of both low- and high-intensity exercise, [E_2], [P_4], [FSH] and [LH] were not different from resting values in either the FP or LP. At this time point, however, both [E_2] and [P_4] were higher in the LP (Table 4).

Cardio-respiratory variables

Rest There was no effect of cycle phase on resting values of $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}E$ and HR. RER, however, was lower at rest during the LP [FP: 0.92 (0.04); LP: 0.82 (0.02), $P < 0.05$].

At 25% $\dot{V}O_{2peak}$ Absolute $\dot{V}O_2$ was higher during the LP compared with the FP [FP: 0.66 (0.04); LP: 0.72 (0.03) $l \cdot min^{-1}$, $P < 0.05$], but there was no phase difference in $\dot{V}O_2$ when expressed relative to body mass [FP: 10.36 (0.56); LP: 11.30 (0.40) $ml \cdot kg^{-1} \cdot min^{-1}$, $P = 0.06$]. RER throughout exercise (Fig. 4) was higher during the FP ($P < 0.05$) but there were no phase differences in $\dot{V}CO_2$, $\dot{V}E$ and HR.

At 75% $\dot{V}O_{2peak}$ Absolute and relative $\dot{V}O_2$ ($P < 0.01$) and the oxygen pulse ($\dot{V}O_2$/ HR) were higher ($P < 0.01$) and the RER lower ($P < 0.05$) during the LP compared to the FP (Fig. 4) but there were no phase differences in $\dot{V}CO_2$, $\dot{V}E$ and HR.

Plasma lactate concentration

Rest Cycle phase had no effect on resting plasma lactate concentrations.

Table 4 [E_2], [P_4], [FSH] and [LH] at rest (pre-exercise), in the last 30 s of exercise (25% and 75% $\dot{V}O_{2peak}$) and 10 min following all exercise (post-exercise). (*[E_2]* 17β-Oestradiol, *FP* follicular phase, [*FSH*] follicle-stimulating hormone, [*LH*] luteinising hormone, *LP* luteal phase, *[P_4]* progesterone). Values are mean (SE)

Hormones	Phase	Pre-exercise	25% $\dot{V}O_{2peak}$	75% $\dot{V}O_{2peak}$	Post-exercise
[E_2] ($pmol \cdot l^{-1}$)	FP	86.3 (33.6)	121.5 (35.8)	159.0 (34.5)	161.5 (35.3)
	LP	372.3 (48.3)[a]	367.2 (57.1)[a]	488.6 (77.7) [a,b]	476.0 (68.5)[a]
[P_4] ($nmol \cdot l^{-1}$)	FP	2.5 (0.4)	2.7 (0.4)	3.2 (0.3)	3.1 (0.3)
	LP	22.4 (5.4)[a]	23.8 (5.7)[a]	25.9 (6.4)[a]	22.3 (5.2)[a]
[FSH] ($IU \cdot l^{-1}$)	FP	3.4 (0.3)	–	–	4.0 (0.3)
	LP	2.3 (0.3)[a]	–	–	1.9 (0.3)
[LH] ($IU \cdot l^{-1}$)	FP	3.6 (0.7)	–	–	3.6 (0.6)
	LP	3.7 (0.7)	–	–	3.1 (0.6)

[a]Significantly different from follicular phase
[b]Significantly different from rest, $P < 0.05$

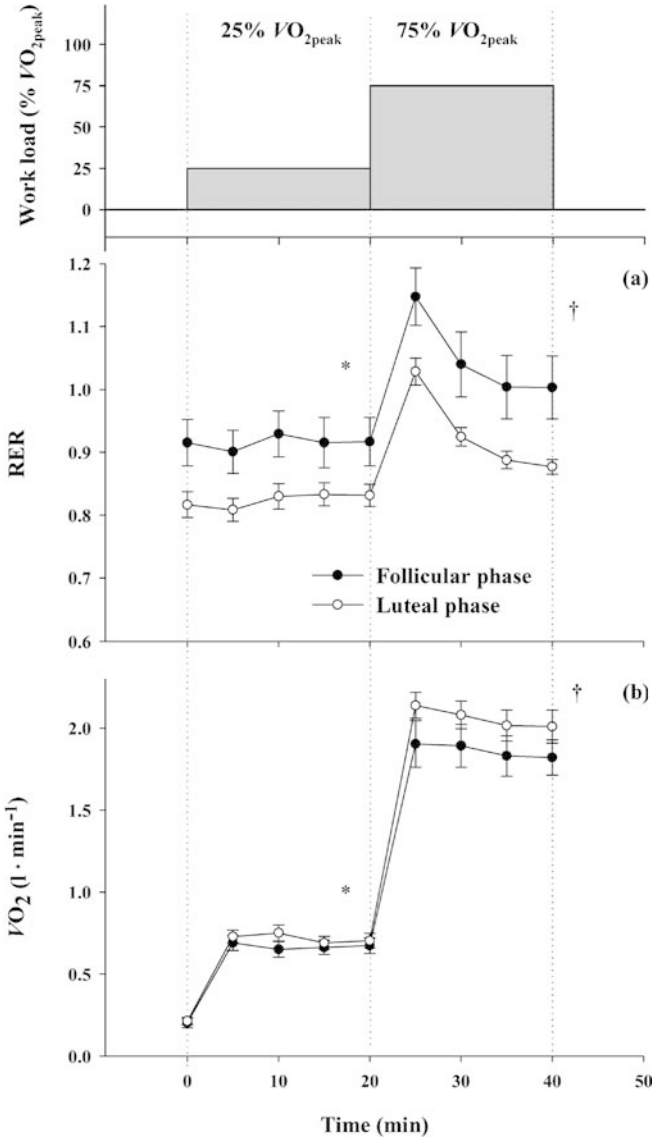

Fig. 4a, b Effect of menstrual cycle phase on the **a** RER and **b** $\dot{V}O_2$ responses during submaximal exercise at 25% and 75% $\dot{V}O_{2peak}$. (*Closed circles* Follicular phase, *open circles* luteal phase.) Values are mean (SE). *Main effect of menstrual cycle phase at 25% $\dot{V}O_{2peak}$, FP < LP at all time points, $P < 0.05$. †Main effect of menstrual cycle phase at 75% $\dot{V}O_{2peak}$, FP < LP at all time points, $P < 0.05$. (*RER* Respiratory exchange ratio, $\dot{V}CO_2$:$\dot{V}O_2$)

At 25% $\dot{V}O_{2peak}$ Plasma lactate concentration remained at or near resting levels throughout low-intensity steady-state exercise in both phases.

At 75% $\dot{V}O_{2peak}$ During high-intensity steady-state exercise there was an interaction between the effects of the phase of the menstrual cycle and the duration of exercise. Specifically, plasma lactate concentration was higher during the FP at min 15 [FP: 5.6 (0.3); LP: 5.2 (0.4) mmol · l^{-1}] and min 20 [FP: 5.7 (0.3); LP: 5.1 (0.4) mmol · l^{-1}, $P < 0.02$] of exercise. End-exercise plasma lactate concentration was also higher during this phase [FP: 6.0 (0.3); LP: 5.5 (0.3) mmol · l^{-1}, $P < 0.03$].

Discussion

The important features of the present study are the careful definition of cycle phase and its longitudinal design, ensuring a well-controlled study of the interaction between exercise status and the menstrual cycle. This was achieved by hormonal identification of the FP and LP study time points in consecutive menstrual cycles in the same individual and by defining both the hormone environment at the time of each study and the hormone responses to both incremental and steady-state submaximal exercise. Within these design constraints, the main findings were that exercise capacity, as defined by $\dot{V}O_{2peak}$ and the lactate threshold, was not affected by menstrual cycle phase in young sedentary women. Furthermore, at submaximal workloads during the incremental test, the observed metabolic responses suggested an increased utilisation of lipids as an energy source in the LP. This latter observation was supported by results from steady-state exercise at both low and high intensities, where $\dot{V}O_2$ was higher throughout exercise in the LP, despite the same absolute workloads in both cycle phases.

These results support several earlier studies of exhaustive exercise in young eumenorrheic women, which detected no cycle phase difference in the physiological end-points in terms of $\dot{V}O_{2peak}$ (Beidleman et al. 1999), $\dot{V}O_{2max}$ (Dombovy et al. 1987; De Souza et al. 1990; Bemben et al. 1995), exercise time to exhaustion (Dombovy et al. 1987; De Souza et al. 1990; Bemben et al. 1995; Lebrun et al. 1995), HR_{max} and peak power output (Jurkowski et al. 1978; Hall Jurkowski et al. 1981). Two studies, however, have reported an increased $\dot{V}O_{2max}$ in the FP during cycle (Schoene et al. 1981) and treadmill (Lebrun et al. 1995) ergometry in untrained and trained subjects respectively. Most previous studies have focused solely on the physiological end-points of incremental exercise and have neglected to explore the metabolic profiles throughout exercise, which are of equal importance. The expression of metabolic responses in terms of relative exercise intensity (% $\dot{V}O_{2peak}$ or % WL_{peak}) is essential to obtaining a more comprehensive understanding of physiological responses to continuous exercise in women across the entire exercise intensity spectrum. The importance of this approach was exemplified in the present study where, although $\dot{V}O_{2peak}$ and the lactate threshold were independent of cycle phase, the metabolic profiles throughout both forms of exercise suggested that the underlying metabolic fuel used in support of this work was influenced by phase of the menstrual cycle. In particular, while the measured metabolic responses during incremental exercise to exhaustion were similar in both cycle phases up to ~50% WL_{peak}, beyond this intensity $\dot{V}O_2$ was higher, and RER, $\dot{V}CO_2$ and plasma lactate concentration were lower, during the LP. As the exercise intensified the cycle phase differences in $\dot{V}O_2$, $\dot{V}CO_2$, RER and plasma lactate concentration became more pronounced. The

contrast between the novel finding of an unchanged lactate threshold yet lower plasma lactate concentrations during both incremental and submaximal exercise in the LP at intensities beyond the lactate threshold ($\sim$50% WL_{peak}) reinforces the value of the present analysis of responses to both submaximal and incremental exercise.

The data obtained from steady-state submaximal exercise at both intensities were in agreement with those during the incremental exercise test at submaximal workloads greater than 50% $\dot{V}O_{2peak}$. In particular, $\dot{V}O_2$ was higher while RER, plasma lactate concentration and $\dot{V}CO_2$ were in general lower during steady-state exercise in the LP. These findings support the notion of an increased metabolism of fat during exercise in this cycle phase. While there are no previous reports of submaximal exercise responses during a continuous incremental protocol, several groups have explored the effects of menstrual cycle phase on steady-state exercise at varying submaximal intensities and duration. However, in agreement with our study, previous metabolic data during steady-state submaximal exercise have suggested a greater reliance on fat as an energy source during the LP, this contention being supported by findings of a lower RER (Dombovy et al. 1987; Hackney et al. 1993; Berend et al. 1994; Hackney et al. 1994; Wenz et al. 1997), lactate concentration (Hall Jurkowski et al. 1981; Lavoie et al. 1987; Berend et al. 1994; McCracken et al. 1994; Wenz et al. 1997) and decreased carbohydrate utilisation rates (Hackney et al. 1994; Wenz et al. 1997; Hackney 1999; Campbell et al. 2001; Zderic et al. 2001). In contrast to our findings, most found no cycle phase differences in $\dot{V}O_2$ (Jurkowski et al. 1978; Hall Jurkowski et al. 1981; Stephenson et al. 1982; De Souza et al. 1989; Beidleman et al. 1999), $\dot{V}CO_2$, (Jurkowski et al. 1978; Hall Jurkowski et al. 1981; Stephenson et al. 1982), HR (Dombovy et al. 1987; De Souza et al. 1990; Beidleman et al. 1999), or $\dot{V}E$ (Stephenson et al. 1982; Dombovy et al. 1987; De Souza et al. 1990; Beidleman et al. 1999). Given the vast array of protocols adopted in previous studies it is not surprising that the results are far from unanimous. That, in the present study, there is close agreement between the submaximal data during both steady-state and incremental exercise testing provides justification for the adoption of a well-controlled longitudinal study in sedentary individuals.

An important aspect of the present study was the hormonal confirmation of ovulation in all menstrual cycles and that all subjects were eumenorrheic, given the normal cycle lengths and [E_2], [P_4], [FSH] and [LH] profiles in both phases. Such a careful assessment of menstrual function is critical to any evaluation of the physiological response to exercise in women. Incremental exercise to exhaustion and steady-state exercise at both submaximal intensities were effective stimuli for increasing circulating [E_2] in the absence of [P_4], [FSH] and [LH]. Given this it appears unlikely that the increase in [E_2] with acute exercise is due to an increase in gonadal steroid production, but maybe due to increased steroid synthesis from androgens in the extragonadal tissues, such as adipose tissue (Simpson 2000) and skeletal muscle (Matsumine et al. 1986). Alternatively, the half-life of [E_2] is 1.7 h (Faigle and Schenkel 1998), which also suggests that the increase in [E_2] with acute exercise could be due to a decrease in clearance of this hormone from the circulation.

In conclusion this study highlights the importance of further research in this area where menstrual cycle lengths, prediction and timing of exercise testing days and basal hormone concentrations are better controlled. The large inter- and intra-subject variability in basal hormone concentrations within and between menstrual cycles in young women creates a problem in interpreting the results in any investigation especially when small subject cohorts are used. Hormone manipulation of menstrual cycle via the oral contraceptive pill may provide one solution. Under these circumstances the ratio between synthetic [E_2] and [P_4] can be pharmacologically manipulated to explore the single and coupled effects of female sex steroids on substrate selection and exercise performance with more precision.

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