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Menstrual cycle phase and sex influence muscle glycogen utilization and glucose turnover during moderate-intensity endurance exercise

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Devries, Michaela C., Mazen J. Hamadeh, Stuart M. Phillips, and Mark A. Tarnopolsky. Menstrual cycle phase and sex influence muscle glycogen utilization and glucose turnover during moderate-intensity endurance exercise. *Am J Physiol Regul Integr Comp Physiol* 291: R1120–R1128, 2006. First published May 11, 2006; doi:10.1152/ajpregu.00700.2005.—Numerous studies from our and other laboratories have shown that women have a lower respiratory exchange ratio (RER) during exercise than equally trained men, indicating a greater reliance on fat oxidation. Differences in estrogen concentration between men and women likely play a role in this sex difference. Differing estrogen and progesterone concentrations during the follicular (FP) and luteal (LP) phases of the female menstrual cycle suggest that fuel use may also vary between phases. The purpose of the current study was to determine the effect of menstrual cycle phase and sex upon glucose turnover and muscle glycogen utilization during endurance exercise. Healthy, recreationally active young women ($n = 13$) and men ($n = 11$) underwent a primed constant infusion of [6,6-²H]glucose with muscle biopsies taken before and after a 90-min cycling bout at 65% peak O₂ consumption. LP women had lower glucose rate of appearance (R_a, $P = 0.03$), rate of disappearance (R_d, $P = 0.03$), and metabolic clearance rate (MCR, $P = 0.04$) at 90 min of exercise and lower proglycogen ($P = 0.04$), macroglycogen ($P = 0.04$), and total glycogen ($P = 0.02$) utilization during exercise compared with FP women. Men had a higher RER ($P = 0.02$), glucose R_a ($P = 0.03$), R_d ($P = 0.03$), and MCR ($P = 0.01$) during exercise compared with FP women, and men had a higher RER at 75 and 90 min of exercise ($P = 0.04$), glucose R_a ($P = 0.01$), R_d ($P = 0.01$), and MCR ($P = 0.001$) and a greater PG utilization ($P = 0.05$) compared with LP women. We conclude that sex, and to a lesser extent menstrual cycle, influence glucose turnover and glycogen utilization during moderate-intensity endurance exercise.

carbohydrate metabolism; menstrual phase; stable isotopes; humans; sex differences; exercise

SOME (9, 16, 20, 33, 39, 47, 48, 52), but not all (11, 37), studies have shown that women, compared with men, utilize less carbohydrate (CHO) to fuel endurance exercise, as evidenced by a lower respiratory exchange ratio (RER). More specifically, women, compared with men, have less muscle glycogen utilization (47), higher glycerol turnover (9), lower plasma lactate concentration (16), higher plasma free fatty acid (FFA) concentration (3, 31), lower catecholamine levels (9, 16, 20), lower glucose rate of disappearance (R_d; see Ref. 16), and greater intramyocellular lipid (IMCL) oxidation (37, 44) during endurance exercise. It has been suggested that these sex differences during exercise are due to differences in estrogen concentration/activity (5, 24).

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Studies involving 17 β -estradiol (E₂), the main circulating form of estrogen, supplementation in male or ovariectomized female rats have shown that E₂ administration robustly improved endurance exercise performance (23, 24), spared muscle and liver glycogen during endurance exercise (23, 24, 40), and increased FFA (19, 24, 40) and IMCL concentrations (14). Similar studies have been conducted in men and amenorrheic women and have shown that E₂ decreases glucose rate of appearance (R_a), R_d, and metabolic clearance rate (MCR; see Refs. 7 and 41) with no effect on RER (7, 41, 49) or muscle glycogen utilization (49). A recent study completed in our laboratory found that 8 days of E₂ supplementation in men lowered RER, glucose R_a and R_d, leucine oxidation, and muscle glycogen stores [18% proglycogen (PG), 24% macroglycogen (MG), 20% total glycogen (G_{tot})]; however, there was no effect on net muscle glycogen utilization (13, 18). These findings support a role for estrogen in mediating sex differences during endurance exercise.

Because estrogen and progesterone levels change over the course of the menstrual cycle, there may be differences in substrate utilization during exercise when performed in the follicular phase (FP) as opposed to the luteal phase (LP) of the menstrual cycle (4, 6, 17, 45, 54). Women in the LP, compared with women in the FP, have a lower RER (17, 54), deplete less muscle glycogen (17, 54), have a lower glucose R_d (6), and oxidize less CHO (6, 54) and more fat (6). Collectively, these observations indicate a reduction in muscle glycogen use in women during the LP of the menstrual cycle and suggest that sex differences in CHO utilization during exercise may be more apparent during the LP. In contrast, several studies have not found differences in substrate utilization during exercise across the menstrual cycle (10, 32, 45, 46).

There are reports that oral contraceptive (OC) use lowers blood glucose concentration (4), increases plasma FFA (4), decreases glucose R_a and R_d (45), and increases glycerol R_a (10) during exercise, further indicating that the estrogens can modify fuel selection during exercise. OC use has been shown to increase cortisol concentrations, which is known to increase lipolysis and decrease peripheral glucose uptake and utilization, suggesting a potential mechanism for the observed shift in fuel selection with OC (10, 50).

To date, none of the studies investigating the effect of menstrual cycle phase or OC use have looked at the effects of these factors on a broader spectrum of CHO metabolism, specifically by simultaneously conducting muscle biopsies to study glycogen utilization, by using the stable isotope [6,6-²H]glucose tracer to measure whole body glucose kinetics, and

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by calculating the RER to investigate the change in substrate oxidation during moderate-intensity endurance exercise. The purpose of the current study was to understand the physiological changes in fuel use during moderate-intensity endurance exercise as influenced by hormonal changes during the menstrual cycle in women and whether these differences persist when compared with men. To do that, we used both stable isotope methodology to measure glucose R_a , R_d , and MCR and muscle biopsies to directly measure muscle glycogen utilization, together with breath measurements to measure the ratio of CHO to fat oxidation. Women were compared during both phases of the menstrual cycle, and men were compared with women in the FP and LP of the menstrual cycle.

MATERIALS AND METHODS

Participants. Thirteen healthy (6 on OC), recreationally active women participated in this study. Eleven healthy, recreationally active men were recruited for the sex comparison and were matched with the women based on peak O_2 consumption ($\dot{V}O_{2\text{ peak}}$) expressed as milliliters per kilogram fat-free mass (FFM) per minute. Subject characteristics are presented in Table 1. One woman (not on OC) withdrew for reasons unrelated to the study after completing one-half of the study; hence, only the data from her first test day will be presented and compared with men. Informed consent was obtained before commencing the study after a description of the study and advisement of the risks and benefits of participation. This study was approved by the Research Ethics Committee of McMaster University and was conducted in accordance with the guidelines of the Declaration of Helsinki.

Study protocol. At least 1 wk before the first trial date, subjects performed a progressive exercise test on an electronically braked cycle ergometer (Excalibur Sport; Lode, Groningen, The Netherlands) to determine their $\dot{V}O_{2\text{ peak}}$, as previously described (18). The $\dot{V}O_{2\text{ peak}}$ was used to determine the work intensity needed to elicit 65% of the subject's $\dot{V}O_{2\text{ peak}}$ for subsequent testing and was confirmed by measuring $\dot{V}O_2$ at the calculated workload intensity for each subject ~30 min after the test. A relative power output of 65% $\dot{V}O_{2\text{ peak}}$ was used to investigate the outcome variables during endurance exercise at moderate intensity to allow for comparisons between this and previously published papers (7, 9, 29, 36, 47, 49). Additionally, exercise performed at 65% $\dot{V}O_{2\text{ peak}}$ results in a significant contribution from both CHO and fat from muscle stores and plasma (38).

Table 1. Subject characteristics and dietary composition for 13 women and 11 men

Measurement	Men	Women
Age, yr	21 ± 1	22 ± 2
Weight, kg	80 ± 3 ^a	63 ± 2
Height, cm	178 ± 1 ^a	165 ± 1
BMI, kg/m ²	25 ± 1	23 ± 1
FFM, kg (% body wt)	59 ± 1 (81) ^a	52 ± 1 (71)
$\dot{V}O_{2\text{ peak}}$		
ml·kg body wt ⁻¹ ·min ⁻¹	45 ± 1 ^a	39 ± 2
ml·kg FFM ⁻¹ ·min ⁻¹	56 ± 1	54 ± 3
Total energy intake		
kcal	2,416 ± 331 ^b	1,884 ± 393
kJ	10,099 ± 1,384 ^b	7875 ± 1,643
CHO, g (%)	298 ± 69 (49)	277 ± 54 (53)
Protein, g (%)	94 ± 28 (16) ^b	64 ± 12 (12)
Fat, (g) (%)	87 ± 18 (33) ^b	61 ± 22 (26)

Data are means ± SD; n = 13 women and 11 men. BMI, body mass index; FFM, fat-free mass; $\dot{V}O_{2\text{ peak}}$, peak O_2 consumption; CHO, carbohydrate. Unpaired t-tests: ^aHigher than women, P < 0.05; ^bhigher than women, P < 0.02.

Women were tested during the FP (day 9 ± 1) and LP (day 20 ± 1) of the menstrual cycle in a single-blind, randomized, crossover design. Subjects provided a history of their menstrual cycle for at least the last three phases. In addition, ovulation was determined in women not using OC to confirm the time of ovulation and/or status of the LP (ClearPlan Easy Ovulation test kit; Novartis Consumer Health Care, Mississauga, ON, Canada). The sex hormone concentrations (Table 2) ascertain their menstrual phase. On the morning of the test day, subjects reported to the laboratory and performed 90 min of exercise on a cycle ergometer at 65% $\dot{V}O_{2\text{ peak}}$. Subjects returned to the laboratory and completed the second arm of the study during the opposite phase of the menstrual cycle. Male subjects reported to the laboratory for testing on one occasion.

During the course of the study, subjects were asked to maintain and record their normal activity level. Food intake was recorded for at least 3 days (minimum of 2 weekdays and 1 weekend day) and was used to control food intake during the second arm of the study. Because this was part of a larger study where men were tested on two occasions, diet records were obtained from three men and two women on both arms of the study to ensure that habitual diet did not change between trials. Diet records were analyzed using commercially available analysis software (Nutritionist Pro, version 2.2; First DataBank, San Bruno, CA). Subjects were not tested on or around major holidays or during times when their diets deviated from normal.

Subjects consumed the same meal on the evening before both test days. On the morning of the test day, subjects reported to the laboratory 10–12 h postabsorptive. Body weight was recorded, and body composition was determined using bioelectric impedance analysis (BIA-101A; RJL Systems, Mt. Clemens, MI). A 20-gauge catheter (BD Insite Autoguard; Becton-Dickinson Canada, ON, Canada) was placed in the antecubital vein of the right arm to allow for blood sampling for the duration of the testing. To “arterialize” the blood, the arm was placed in a heating pad (65 ± 5°C) 15 min before the withdrawal of each blood sample when the subjects were not exercising. Another catheter (BD Insite Autoguard; Becton-Dickinson Canada) was placed in the antecubital vein of the left arm to allow for infusion of stable isotopes with a constant infusion pump (model 74900; Cole-Palmer). Subjects then underwent a primed constant infusion of [6,6-²H]glucose (99% enriched; Cambridge Isotope Laboratories) for 2.5 h. Glucose was mixed with 0.9% saline and filtered through a 0.2- μ m sterile, nonpyrogenic filter (Acrodisc; Pall Gelman, Ann Arbor, MI) in single-use vials at the McMaster University Medical Center pharmacy. Before initiating the infusion protocol, a baseline blood sample was taken to allow for determination of the natural background enrichment of glucose. A priming dose of [6,6-²H]glucose (17 μ mol/kg) was given followed immediately by a constant infusion at a rate of 0.22 μ mol·kg⁻¹·min⁻¹ for 60 min to reach steady state. At the onset of exercise, the infusion rate was increased in a stepwise fashion at time (t) = 0, 5, and 10 min of exercise to 0.33, 0.44, and 0.55 μ mol·kg⁻¹·min⁻¹, respectively. The infusion rate remained at 0.55 μ mol·kg⁻¹·min⁻¹ for the remainder of the 90-min exercise session.

Before and after exercise, a muscle biopsy was taken from the vastus lateralis muscle ~10 cm proximal to the knee joint using a custom suction-modified Bergström needle (5 mm diameter). Biopsies were taken from the same leg before and after exercise and from the contralateral leg on the subsequent test day. Approximately 30 mg of muscle tissue was immediately snap-frozen and stored in liquid nitrogen until transferred to -86°C for subsequent PG/MG analyses.

Blood samples were taken at baseline and 15 min before commencing exercise and at t = 60, 75, and 90 min of exercise. For glucose and lactate analysis, blood samples were collected in heparinized tubes, placed on ice, centrifuged at 1,750 g at 4°C for 10 min, and stored at -50°C for subsequent analysis. For determination of hormone levels (E₂, testosterone, progesterone), blood samples were collected and allowed to stand for 30 min in untreated tubes, centrifuged at 1,200 g

Table 2. Serum hormone concentrations for 13 women during the FP and LP of the menstrual cycle using oral contraceptives or not using oral contraceptives and 11 men

Treatment	FP	FOC	FNOC	LP	LOC	LNOC	Men
Total testosterone, nmol/l	0.9±0.1 ^g	0.7±0.1 ^{b,g}	1.1±0.1 ^g	0.8±0.0 ^g	0.7±0.0 ^g	1.0±0.2 ^g	21.0±1.0
17β-Estradiol, pmol/l	125±45	42±18 ^d	184±71 ^c	203±75	14±5 ^{a,d}	361±107 ^e	128±13
Progesterone, nmol/l	2.0±0.1 ^d	2.0±0.3 ^f	2.0±0.2 ^d	6.0±2.0	2.0±0.3 ^{a,f}	10.0±3.0 ^e	3.0±0.3

Data are means ± SE; *n* = 13 women and 11 men. FP, follicular phase; FOC, follicular phase and using oral contraceptives; FNOC, follicular phase and no oral contraceptives; LP, luteal phase; LOC, luteal phase and using oral contraceptives; LNOC, luteal phase and no oral contraceptives. Repeated-measures ANOVA: ^alower than LNOC, *P* ≤ 0.03; ^blower than FNOC, *P* < 0.01; ^clower than LNOC, *P* < 0.01; ^dlower than men, *P* < 0.01; ^ehigher than men, *P* < 0.001; ^flower than men, *P* < 0.05; and ^glower than men, *P* < 0.001.

at room temperature for 30 min, and the serum stored at -50°C for subsequent analysis.

Respiratory measures ($\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}E$, and RER) were taken at *t* = 120 and 20 min before exercise and at *t* = 0, 5, 30, 60, 75, and 90 min during exercise using a computerized open-circuit gas collection system (Moxus Modulator $\dot{V}O_2$ system with O₂ analyzer S-3A/I and CO₂ analyzer CD-3A; AEI Technologies, Pittsburgh, PA).

Blood glucose, lactate, and hormones. Plasma glucose and lactate concentrations were analyzed with an automated lactate and glucose analyzer (2300 STAT plus; YSI). Serum E₂, testosterone, and progesterone were analyzed using a single incubation RIA (Coat-a-count: Kit no. TKE21, Kit no. TKTE1, and Kit no. TKIN5; Diagnostics Products, Los Angeles, CA).

Isotopic enrichment, and glucose R_a, R_d, and MCR. Isotopic enrichment of glucose was determined using gas chromatography-mass spectrometry (GCMS; GC model 6890 and MS model 5973; Agilent Technologies, Palo Alto, CA) of the pentaacetate derivative, as described previously (7). To isolate the pentaacetate derivative, the samples were first deproteinized using barium hydroxide and zinc sulfate. The supernatant was passed through an anion-cation (AG 1X8-400 and AG 50X8-400; Sigma Chemicals, St. Louis, MO) exchange column, and the eluted extract was evaporated (SpeedVac Plus SC210A; ThermoSavant, Holbrook, NY). The derivative was prepared for GCMS analysis by adding 100 μl of 2:1 acetic anhydride and pyridine solution to each sample.

A 15-m fused silica capillary column with 0.2 mm diameter and 0.2 μm film thickness (Supelco, Bellefonte, PA) was used in the GC oven. To monitor selected ions, a mass-to-charge ratio of 200 and 202 atomic mass units was used with electron impact ionization mode.

Glucose R_a and R_d were calculated using the Steele equation (43) as modified (22, 38) for use with stable isotopes, since the amount of tracer infused is no longer considered negligible. The modified Steele equation is as follows

$$R_a (\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) = \frac{F - \left(V_d \frac{C_m}{1 + E} \frac{dE}{dt} \right)}{E}$$

$$R_d (\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) = R_a - \left[\frac{V_d \left(\frac{dC_m}{dt} (1 + E) - C_m \frac{dE}{dt} \right)}{(1 + E)^2} \right]$$

where *F* is the infusion rate in μmol/kg/min, *C_m* is the measured glucose concentration in μmol/L, *E* is the enrichment, *V_d* is the volume of distribution, assumed to be 100 ml/kg, and *t* is the time in min.

Glucose R_a and R_d were calculated at rest and at 60, 75, and 90 min during exercise. The average of the exercise time points was combined and reported as the average R_a and R_d over the exercise bout. MCR was determined as follows

$$\text{MCR} (\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) = R_d / [(C_1 + C_2) / 2]$$

where *C₁* and *C₂* are plasma glucose concentrations at sampling times 1 and 2, respectively.

Biochemical glycogen determination. Muscle PG and MG content was analyzed as previously described (1, 27, 28). Briefly, snap-frozen muscle samples were freeze-dried for 24 h, powdered, dissected free of any blood and connective tissue, and weighed. Ice-cooled perchloric acid (200 μl, 1.5 M) was added to 1.5–2.5 mg of tissue and was pressed with a plastic inoculating loop for 20 min on ice. Samples were then centrifuged for 15 min at 1,620 g at 4°C, and 100 μl of the supernatant were removed for MG determination. The remaining supernatant was aspirated off. HCl (1 ml, 1 M) was added to each sample, PG samples were briefly pressed, and MG samples were vortexed for several seconds. Samples were hydrolyzed for 2 h at 100°C and neutralized with 2 M Tris base, vortexed, centrifuged at 1,620 g for 5 min, and stored at -86°C until subsequent determination of glucosyl units. Values for PG and MG were then added to give total muscle glycogen content. Muscle glycogen utilization was calculated as follows

$$\text{Glycogen utilization} = [\text{glycogen}]_{\text{pre}} - [\text{glycogen}]_{\text{post}}$$

where Pre and Post indicate before and after exercise, respectively.

Estimated glycogen utilization and percent contribution of plasma glucose and muscle glycogen to total CHO oxidation. Muscle glycogen utilization was estimated as follows

$$\text{Estimated muscle glycogen utilization} = \text{total CHO oxidation} - \text{glucose } R_d$$

Total CHO oxidation was calculated from the RER as previously described (15). The percent contribution of plasma and muscle CHO oxidation was determined by dividing the glucose R_d or estimated muscle glycogen utilization, respectively, by the total CHO oxidized over the 90-min exercise bout and multiplying by 100.

Statistical analysis. Serum hormone concentrations and rest samples for RER and glucose R_a, R_d, and MCR were analyzed using paired and unpaired *t*-tests for within-group and between-group analyses, respectively. Data were analyzed using two-way, repeated-measures ANOVA with menstrual phase and time being the experimental variables. To compare men with women, two-way, repeated-measures ANOVA was used, with sex (FP vs. men, LP vs. men) and time being the experimental variables. One-tailed tests were used for glucose turnover and muscle glycogen utilization when comparing LP with FP women and men with women (FP and LP) because we hypothesized a priori that higher estrogen concentrations would decrease glycogen utilization and glucose turnover. When significance was attained, Tukey's honest significant difference post hoc test was used to determine the source of the difference. Subject characteristics were analyzed using unpaired *t*-tests.

Statistical analyses were conducted on all data to compare women taking OC (OC users) with women not taking OC (non-OC users). Because there were no significant differences in any of the measures between OC vs. non-OC users, data for all the women were pooled. Statistical analyses were performed on glucose kinetic data (R_a, R_d, and MCR) corrected for body weight. When corrected for kilogram FFM, results are maintained. Analyses were performed using a com-

puterized statistics program (STATISTICA for Windows, version 5.1; StatSoft, Tulsa, OK). All data were normal based on the Kolmogorov-Smirnov test. Statistical significance was set at $P \leq 0.05$. Data are presented as means \pm SE unless otherwise indicated.

RESULTS

Subject characteristics. Body weight in women did not change between trials. Women were lighter, shorter, less lean (lower FFM), and had a lower $\dot{V}O_{2\text{ peak}}$ per kilogram body weight ($P < 0.05$) compared with men (Table 1). However, when $\dot{V}O_{2\text{ peak}}$ was expressed relative to kilogram FFM, there was no difference between men and women.

Diet. Total energy intake and CHO, protein, and fat gram consumption for men and women are presented in Table 1. There were no differences in energy intake or dietary composition between women using OC and those who were not. Women ate significantly less kilocalories ($P < 0.001$), protein ($P < 0.001$), and fat ($P = 0.015$) compared with men. The relative contributions of CHO, protein, and fat were not different between men and women. Diet composition of the five subjects who completed diet records on both arms of the study was not different between trials, indicating that habitual diet did not change.

Hormone concentrations. Serum E_2 and progesterone concentrations were higher during the LP compared with the FP; however, this difference was not significant (Table 2). Testosterone levels did not change during the course of the menstrual cycle. Serum testosterone levels were higher in FP women not using OC compared with FP women using OC ($P = 0.006$). Serum E_2 ($P = 0.02$) and progesterone ($P = 0.03$) levels were higher in LP women not using OC compared with LP women using OC. Serum E_2 levels were not different between men and women during either phase of the menstrual cycle (Table 2). However, serum E_2 levels were lower during the FP and LP in women taking OC ($P = 0.003$ and $P < 0.001$, respectively) and higher during the LP in women not using OC ($P < 0.0001$) compared with men. Serum progesterone levels were lower in women during the FP compared with men ($P = 0.002$); however, this difference disappeared during the LP. When comparing men with women not using OC, men had a higher progesterone concentration than women in the FP ($P = 0.01$) and a lower progesterone concentration than in the LP ($P < 0.001$). During both phases of the menstrual cycle, women taking OC had a lower progesterone concentration compared with men ($P = 0.02$ vs. FP and $P = 0.03$ vs. LP). Women had a lower serum testosterone concentration compared with men ($P < 0.001$).

OC vs. non-OC. OC use did not influence the measured variables of CHO metabolism; thus, data presented are pooled for all women (Table 3).

RER. Because of complications during the sample acquisition, data from one man could not be included in the respiratory analyses. There was no difference in RER during exercise between menstrual cycle phases (Fig. 1). FP women had a lower RER during exercise compared with men ($P = 0.02$), and LP women had a lower RER than men at 75 and 90 min of exercise ($P = 0.04$; Fig. 1). RER decreased significantly over time during exercise ($P < 0.001$).

Plasma glucose and lactate concentrations. There were no differences in plasma glucose or lactate concentrations between menstrual cycle phases (Table 4). There were no differ-

ences in plasma glucose or lactate concentrations when comparing FP or LP women with men (Table 4). Plasma lactate concentrations were higher in all subjects during exercise compared with at rest ($P < 0.001$).

Glucose R_a , R_d , and MCR. Because of technical difficulties during the infusion, data from one man could not be used in the glucose turnover analyses. Glucose R_a , R_d , and MCR were lower in the LP (6%, $P = 0.03$, 0.03, and 0.04, respectively) at $t = 90$ min compared with FP of the menstrual cycle (Fig. 2). However, when the average of the last 30 min of exercise was compared, there were no differences between phases. During exercise, FP and LP women had a lower glucose R_a (22 and 20%, $P = 0.03$ and 0.01, respectively), R_d (22 and 20%, $P = 0.03$ and 0.01, respectively), and MCR (25 and 22%, $P = 0.01$ and 0.001, respectively) compared with men (Fig. 2). At rest, LP women had a higher MCR than men ($P = 0.03$). Exercise increased glucose R_a , R_d , and MCR compared with rest ($P < 0.001$). When glucose R_a , R_d , and MCR were corrected for kilogram FFM, as opposed to kilogram body weight, significance was maintained.

Muscle glycogen utilization. Data from one woman subject are not presented since there was not an adequate amount of sample to perform the assay; hence, data presented are for 12 women. LP women, compared with FP women, utilized less PG (30%, $P = 0.04$), MG (16%, $P = 0.04$), and G_{tot} (24%, $P = 0.02$; Fig. 3). LP women utilized less PG (25%, $P = 0.05$), with a trend toward a lower G_{tot} (18%, $P = 0.1$) compared with men (Fig. 3). Exercise decreased all muscle glycogen fractions in both men and women ($P < 0.001$).

Estimated glycogen utilization and percent contribution of plasma glucose and muscle glycogen to total CHO oxidation. There was no difference in the estimated muscle glycogen utilization during the 90-min exercise bout between FP and LP women or between men and FP or LP women (FP, $1,540 \pm 187$; LP, $1,537 \pm 194$; men, $1,624 \pm 185$ mg/kg). Plasma glucose contributed 29, 32, and 38% of total CHO oxidation during exercise in FP, LP, and men, respectively. Muscle glycogen contributed 71, 68, and 62% of total CHO oxidation during exercise in the FP, LP, and men, respectively. There was no difference in the proportion of plasma and muscle glycogen used during exercise between LP and FP women. FP women, compared with men, used a greater proportion of muscle glycogen and a lesser proportion of plasma glucose over the exercise bout ($P = 0.03$).

DISCUSSION

The main findings of this study were that LP women had a lower glucose R_a , R_d , and MCR at 90 min of exercise and a lower PG, MG, and G_{tot} utilization during exercise compared with FP women. In addition, FP women had a lower RER and glucose R_a , R_d , and MCR during exercise, and LP women have a lower RER at 75 and 90 min of exercise, glucose R_a , R_d , and MCR during exercise, lower MCR at rest, and a greater PG utilization compared with equally fit men.

To conduct a meaningful comparative analysis between the sexes, we matched men and women based on their aerobic capacity ($\dot{V}O_{2\text{ peak}} \cdot \text{kg FFM}^{-1} \cdot \text{min}^{-1}$). Endurance training is known to alter substrate utilization during exercise, resulting in a lesser reliance on CHO sources (2, 9, 30, 34) and a greater reliance on lipid sources (21, 29, 34). If men and women have

Table 3. RER, muscle proglycogen, macroglycogen, and total glycogen concentration and glucose R_a , R_d , and MCR in 13 women (6 OC, 7 non-OC) in the FP and LP of the menstrual cycle

	Follicular Phase		Luteal Phase	
	OC	Non-OC	OC	Non-OC
RER				
Pre	0.88±0.06	0.87±0.04	0.90±0.07	0.91±0.10
30	0.93±0.06	0.92±0.03	0.93±0.04	0.94±0.02
60	0.91±0.05	0.89±0.03	0.90±0.03	0.91±0.03
75	0.91±0.05	0.88±0.03	0.90±0.03	0.90±0.02
90	0.90±0.04	0.87±0.03	0.90±0.03	0.90±0.03
Proglycogen, mmol/kg dry wt				
Pre	323±114	266±47	322±125	251±57
Post	132±65	117±50	141±71	123±69
Macroglycogen, mmol/kg dry wt				
Pre	222±113	204±71	200±88	170±44
Post	90±56	45±11	121±74	61±12
Total glycogen, mmol/kg dry wt				
Pre	545±218	471±75	522±207	385±95
Post	222±117	150±64	263±140	149±89
Glucose R_a , ($\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)				
Pre	22±2	22±11	24±6	29±8
60	43±11	47±14	53±20	49±9
75	47±14	50±17	49±10	49±10
90	52±18	54±20	49±7	51±13
Glucose R_d , ($\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)				
Pre	22±2	22±11	24±6	29±8
60	43±11	47±14	53±20	49±9
75	46±13	50±17	49±10	49±10
90	52±18	54±20	49±7	51±13
MCR, ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)				
Pre	4±1	4±2	5±2	6±2
60	8±2	10±3	11±5	10±2
75	9±3	11±4	10±3	10±3
90	9±3	12±5	10±2	11±3

Values are means \pm SE. RER, respiratory exchange ratio; Pre, before exercise; 30, 60, 75, and 90 = 30, 60, 75, and 90 min of exercise, respectively; Post, after exercise; R_a , rate of appearance; R_d , rate of disappearance; MCR, metabolic clearance rate.

different aerobic capacities and/or training profiles, sex-based comparisons would be unreliable. We (8, 9, 18, 36, 48) and others (9, 16, 20, 33, 39, 47, 48) have matched male and female subjects using $\dot{V}O_{2\text{ peak}}$ per kilogram FFM per minute in previous research.

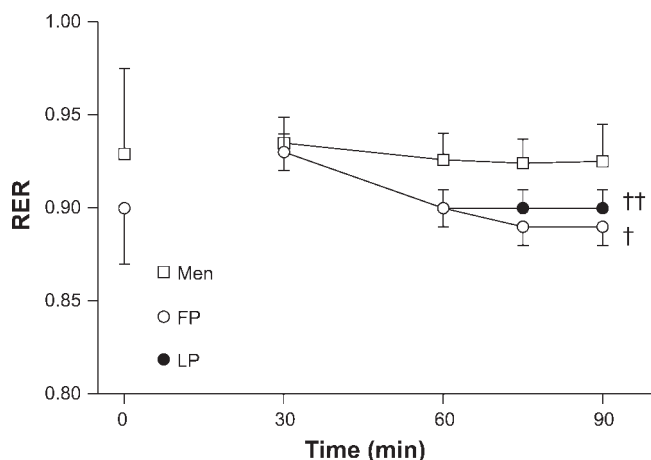


Fig. 1. Respiratory exchange ratio (RER) for 13 women during the follicular phase (FP, \circ) and luteal phase (LP, \bullet) of the menstrual cycle and 10 men (\square) at rest and during 90 min of cycling at an intensity of 65% peak O_2 consumption ($\dot{V}O_{2\text{ peak}}$). Data are means \pm SE. †FP lower than men, $P < 0.05$. ††LP lower than men at 75 and 90 min exercise, $P < 0.05$. RER decreased over time during exercise, $P < 0.05$.

Results from studies supplementing men and women with E_2 have shown that E_2 modifies substrate selection, as evidenced by a decrease in glucose R_a , R_d , and MCR (7, 41). However, E_2 supplementation trials did not find an impact of E_2 on muscle glycogen utilization during endurance exercise (49). In one E_2 supplementation study involving women, a modest change in estrogen concentration was shown to decrease glucose R_a , R_d , and MCR (41), suggesting that minor changes in estrogen concentration, similar to those that occur during the course of the menstrual cycle, can elicit a change in substrate metabolism during exercise in women. Indeed, in the current study, we

Table 4. Plasma lactate and glucose concentrations for 13 women during the FP and LP of the menstrual cycle and 11 men

Treatment	Rest	60 min Exercise	75 min Exercise	90 min Exercise
Glucose, mmol/l				
FP	5.1±0.1	4.8±0.2	4.7±0.1	4.7±0.2
LP	4.8±0.1	4.8±0.1	4.8±0.1	4.9±0.1
Men	5.1±0.1	4.8±0.2	4.7±0.1	4.7±0.2
Lactate, mmol/l				
FP	0.9±0.5	2.2±0.2*	2.2±0.2*	2.0±0.1*
LP	0.9±0.6	2.0±0.2*	2.0±0.2*	2.0±0.2*
Men	1.0±0.1	2.5±0.3*	2.4±0.2*	2.6±0.3*

Data are means \pm SE; $n = 13$ women and 11 men. Repeated-measures ANOVA: *significantly higher than rest, $P < 0.001$.

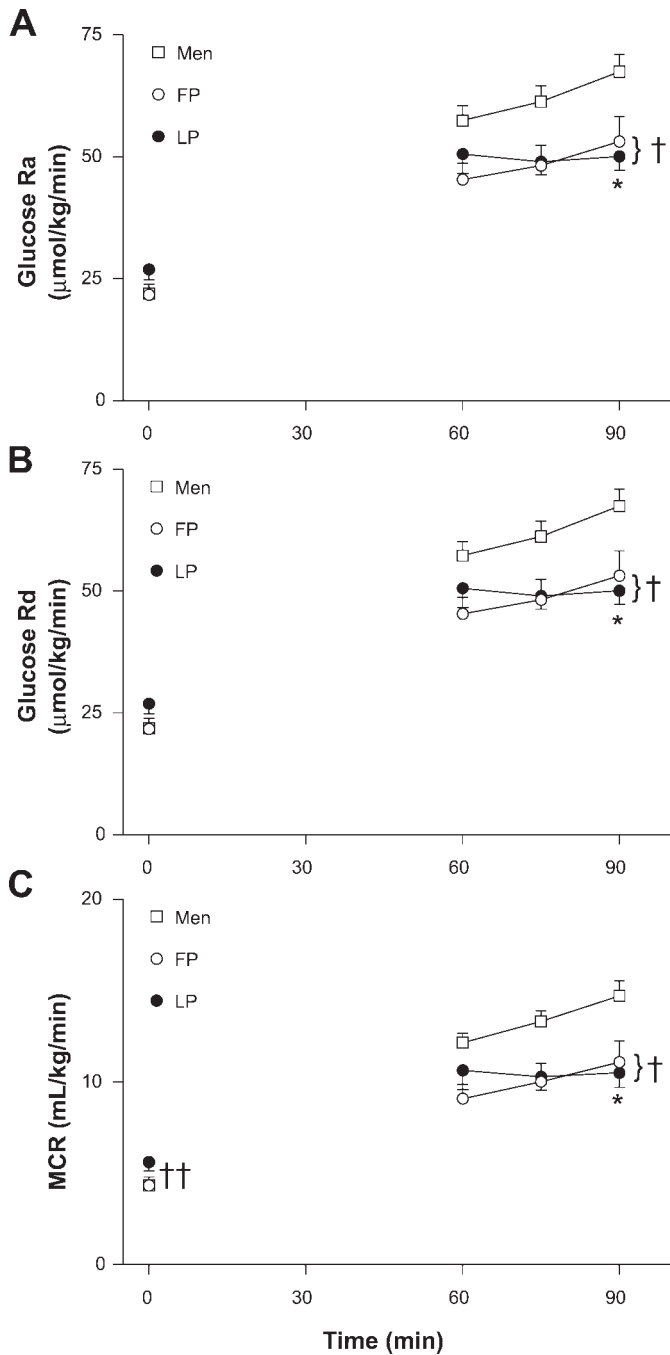


Fig. 2. Glucose rate of appearance (R_a , A), rate of disappearance (R_d , B), and metabolic clearance rate (MCR, C) for 13 women during the FP (○) and LP (●) of the menstrual cycle and 10 men (□) at rest and during 90 min of cycling at an intensity of 65% $\dot{V}O_{2\text{ peak}}$. Data are means \pm SE. *LP lower than FP at 90 min, $P \leq 0.04$. †FP and LP lower than men ($P \leq 0.03$). ‡LP higher than men at rest ($P = 0.03$).

found that women in the LP of the menstrual cycle used less PG (30%), MG (16%), and G_{tot} (24%) during endurance exercise compared with women in the FP. Interestingly, no difference in muscle glycogen utilization was seen when comparing women during the FP of the menstrual cycle with men. However, differences in muscle PG utilization became apparent when comparing women in the LP of the menstrual cycle with men (LP < men, 25%), suggesting that perhaps a greater

difference in estrogen concentration is needed before changes in muscle glycogen utilization are detected. Alternatively, because comparing women with men involved a mixed design, we may have lacked the statistical power needed to identify differences in muscle glycogen utilization between women in the FP and men. However, this is only the second time that an effect of sex on muscle glycogen utilization during exercise has been found. Of the studies conducted previously, women were in the FP of the menstrual cycle when tested in two studies (37, 48), and no effect of sex was found on muscle glycogen utilization during exercise. To the best of our knowledge, the only other study to have found an effect of sex on muscle glycogen utilization did not control for the menstrual cycle phase (47); thus, it is possible that the majority of women were tested in the LP of the menstrual cycle and that is why an effect of sex on muscle glycogen utilization was found. Therefore, it is likely that an effect of sex on muscle glycogen utilization

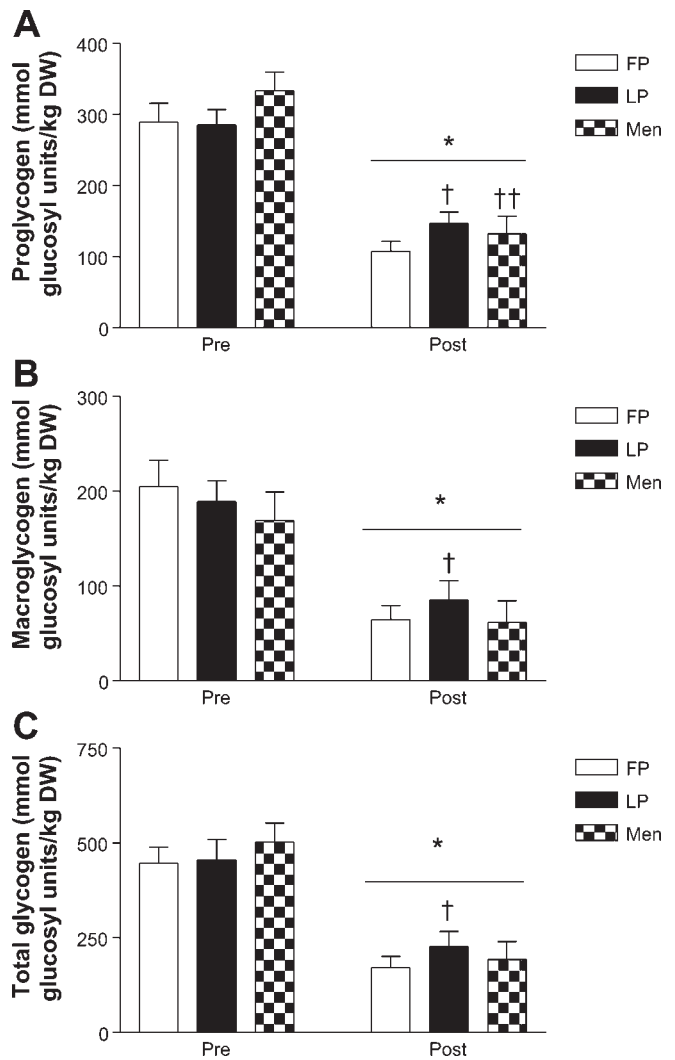


Fig. 3. Progycogen (A), macroglucogen (B), and total glucogen (C) concentrations for 12 women in the FP (open bars) and LP (filled bars) of the menstrual cycle and 11 men (cross-hatched bars) at rest (Pre) and after 90 min of cycling (Post) at an intensity of 65% $\dot{V}O_{2\text{ peak}}$. Data are means \pm SE. DW, dry wt. *Exercise decreased glycogen concentration ($P < 0.001$). †LP used less than FP ($P < 0.05$). ‡LP used less than men, $P < 0.05$.

during exercise is only apparent when comparing men with women during the LP of the menstrual cycle.

This was the first study to investigate the effect of menstrual cycle phase and gender on muscle PG and MG utilization during endurance exercise. We found that both men and FP women used more PG during exercise (25% vs. men, 30% vs. FP) compared with LP women, but only FP women utilized more MG (16%) compared with LP women. One would expect metabolic changes to be evident first in the PG fraction followed by changes in the MG fraction, since the PG fraction is believed to be the more dynamic form of glycogen (1, 27). The results of this study support the theory that PG is the more dynamic form of glycogen, since both the menstrual cycle phase and gender influenced its utilization, whereas only menstrual cycle phase influenced MG utilization.

The role of estrogen in regulating fuel selection during exercise was first hypothesized when women began to overtake men during longer endurance events (42). Women can complete a 90-km run in less time than men, which has been attributed to women being better able to sustain a higher percentage of their $\dot{V}O_{2\text{ peak}}$ compared with men (42). When the distance is reduced to 42.2 km, women perform similarly to men, but men outperform women in 10-km runs (42). These findings suggest that, from a performance standpoint, differences in estrogen concentration between men and women during longer-endurance exercise result in improved performance in women after men have depleted their glycogen stores. In that study, the menstrual phase was not indicated. This does not detract from our observation that changes in substrate utilization still occur even with smaller changes in estrogen concentration between different phases of the menstrual cycle, as opposed to differences between men and women. This is confirmed in the current study with glucose R_a and R_d being significantly different during the last 15 min of the 90-min endurance exercise bout when comparing FP vs. LP.

In the current study, one-half of the woman subjects were using triphasic OC. No differences in CHO storage or utilization during endurance exercise were found when comparing women using OC with women not using OC, despite differences in the hormonal milieu. Women in the LP not using OC had a higher estrogen concentration compared with women in the LP using OC. However, we measured serum E_2 levels, which are suppressed in women taking OC because of their intake of ethinyl estradiol (25); however, total estrogen (E_2 + ethinyl estradiol) in all women was likely similar. Ethinyl estradiol has been shown to be more potent than E_2 in its ability to bind to the estrogen receptor; thus, it can exert its metabolic effects more readily (51). In fact, a previous crossover design study showed that OC decreased glucose R_a and R_d beyond what is seen in women when they were not using OC (45). Because of the relatively small number of subjects within each group ($n = 6$ OC, $n = 7$ non-OC), we could have masked a smaller effect resulting from a type II error.

Previous studies examining the effect of the menstrual cycle on substrate utilization during exercise have collectively shown that, during endurance exercise performed in the LP, women attenuate muscle glycogen utilization (6, 17, 54). We found an effect of menstrual cycle phase on glucose R_a , R_d , MCR and muscle glycogen utilization. This is the first study to measure both pro- and MG measurements in men and women in both

phases of the menstrual cycle, and we found that women in the LP utilize less of both the PG (30%) and MG (16%) glycogen fractions compared with FP women. Whether the decreased muscle glycogen utilization seen in LP women is the result of phase differences in estrogen or progesterone concentration cannot be directly determined from the current results. However, a recent E_2 supplementation trial in men completed in our laboratory found no effect of E_2 on PG, MG, or G_{tot} utilization (13). It is possible that estrogen may not be the primary mediator of the decrease in glycogen utilization observed during the LP; it is possible that it is either progesterone, a synergistic effect of the two hormones, or that the absolute E_2 concentration is higher in the LP. Progesterone has been found to act in an anti-estrogenic fashion (12, 19, 35). In studies with rats involving administration of either E_2 alone or E_2 plus progesterone, the E_2 and progesterone combination prevented the increase in lipid availability and oxidation (19) and the decrease in CHO utilization seen in the E_2 trial (35). Interestingly, only progesterone in combination with E_2 , and not progesterone alone, influenced CHO metabolism (35), suggesting that the basal hormonal milieu favors an environment where the anti-estrogenic actions of progesterone predominate. One study in humans has found that administration of E_2 and progesterone in combination inhibited the decrease in muscle glycogen utilization but not the decrease in glucose R_d observed with E_2 administration (12). However, the latter study (12) used estimated values, not direct measures, to determine muscle glycogen utilization. Also, studies have consistently shown (6, 17, 54), and this study confirms, that women in the LP use less CHO during endurance exercise compared with FP women. In addition, studies in rats have found that E_2 administration alone decreases muscle glycogen utilization during exercise (23, 24, 40). Thus it is likely that E_2 is mediating the decrease in glycogen utilization during exercise performed in the LP, and the reason no difference in muscle glycogen utilization was previously seen in E_2 -supplemented men (13, 49) could be because of a relatively shorter dosage period, that the E_2 dosage was insufficient to counteract the actions of physiological testosterone concentrations, or that E_2 receptors were lower in men.

Although the sex differences in the current study were robust and consistent with numerous other studies (9, 16, 20, 33, 39, 47, 48), the difference in E_2 concentration between women during the FP of the menstrual cycle and men was comparatively small. Part of the explanation is that one-half of the women were taking OC, which suppressed endogenous E_2 concentration, whereas synthetic estrogens (i.e., ethinyl estradiol) would have been high in the women (not measured in the current study). Another explanation is likely to be the number and activity of estrogen receptors in skeletal muscle and liver of women compared with men. It is known that estrogen receptor- β mRNA and protein are present in male skeletal muscle (26, 53); however, receptor density and protein content are not necessarily indicative of protein activity. To date, studies that have been conducted looking at estrogen receptor mRNA and protein content in men and women have involved a small number of subjects (3 men, 3 women); thus, no sex comparisons have been conducted (53). However, when closely examining the data, women had consistently higher estrogen receptor mRNA content and percent estrogen-positive nuclei (53) compared with men. As such, it is likely that sex

differences in substrate selection exist between men and women in the FP because estrogen can exert its metabolic effects more readily in FP women. Finally, it is possible that the cyclical nature of the menstrual cycle may influence the observed sex differences, since the continual fluctuations in E₂ concentration across the menstrual cycle may prevent the downregulation of estrogen receptors during the LP when estrogen is high.

Previous studies examining sex-based differences in substrate utilization during endurance exercise have typically compared men with women in the FP of the menstrual cycle (9, 16, 20, 33, 37, 48) or have neglected to control for menstrual cycle phase (11, 39, 47). It is reasonable to suggest that sex-comparative studies should include comparisons of men with women in the LP as well as the FP. To the best of our knowledge, we are the first to conduct such a study. In the current study, we found differences in glucose turnover and glycogen utilization during endurance exercise between different phases of the menstrual cycle and between men and women in the different phases of the menstrual cycle. Because of the high variance in estrogen concentrations in women during the FP of the menstrual cycle, sex differences in substrate utilization during endurance exercise may not be as prominent in the FP as when comparing men with women during the LP. Taking the aforementioned points into consideration, men should be compared with women in both phases of the menstrual cycle when making sex comparisons.

In the current study, we used two methodologies to determine muscle glycogen utilization during exercise. There was little agreement between the measured (analyzing muscle biopsies for glycogen content before and after exercise) and estimated (calculated from total CHO oxidation and glucose R_d) muscle glycogen utilization ($r = 0.1$). This finding likely indicates the numerous assumptions and sources of error in each method but cannot provide any information on which method should be considered as the "gold standard." The data clearly indicate that researchers using one method cannot validly compare data between studies that use different methods to estimate glycogen utilization.

In summary, we have demonstrated that LP women have a lesser reliance on CHO sources to fuel endurance exercise compared with FP women, as evidenced by a lower glucose R_a, R_d, and MCR at 90 min of exercise, a higher PG concentration, and a lower PG, MG, and G_{tot} utilization during exercise. In addition, both LP and FP women differ from equally trained men with respect to substrate selection during exercise, with FP women having a lower RER and glucose R_a, R_d, and MCR during exercise and LP women having a lower RER at 75 and 90 min of exercise, lower glucose R_a, R_d, and MCR during exercise, and a lesser PG utilization compared with men. Future sex-comparative studies should consider comparing men with women in both phases of the menstrual cycle to fully elucidate the effect of sex and estrogen on CHO metabolism during endurance exercise.

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