No effect of menstrual cycle phase on glycerol or palmitate kinetics during 90 min of moderate exercise

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Horton, Tracy J., Emily K. Miller, and Kristen Bourret. No effect of menstrual cycle phase on glycerol or palmitate kinetics during 90 min of moderate exercise. J Appl Physiol 100: 917–925, 2006; doi:10.1152/japplphysiol.00491.2005.—The systemic flux of glycerol and palmitate [a representative nonesterified free fatty acid (NEFA)] was assessed in three different phases of the menstrual cycle at rest and during moderate-intensity exercise. It was hypothesized that circulating glycerol and NEFA turnover would be greatest in the midfollicular (MF) phase of the menstrual cycle, when estrogen is elevated but progesterone low, followed by the midluteal phase (ML; high estrogen and progesterone), and lowest in the early follicular (EF) phase of the menstrual cycle (low estrogen and progesterone). Subjects included moderately active, eumenorrheic, healthy women. Testing occurred after 3 days of diet control and after an overnight fast (12–13 h). Resting and exercise (50% maximal oxygen uptake, 90 min) measurements of tracer-determined glycerol and palmitate kinetics were made. There was a significant increase in both glycerol and palmitate turnover from rest to exercise in all phases of the menstrual cycle (P < 0.0001). No significant differences, however, were observed between cycle phases in the systemic flux of glycerol or palmitate, at rest or during exercise. Maximal peripheral lipolysis during exercise, as represented by glycerol rate of appearance at 90 min, equaled 8.45 ± 0.96, 8.35 ± 1.12, and 7.71 ± 0.96 mol·kg⁻¹·min⁻¹ in the EF, MF, and ML phases, respectively. Circulating free fatty acid utilization also peaked at 90 min of exercise, as indicated by the palmitate rate of disappearance (3.51 ± 0.35, 3.17 ± 0.39, and 3.47 ± 0.26 mol·kg⁻¹·min⁻¹) in the EF, MF, and ML phases, respectively. In conclusion, systemic rates of glycerol and NEFA turnover (as represented by palmitate flux) were not significantly affected by the cyclic fluctuations in estrogen and progesterone that occur throughout the normal menstrual cycle, either at rest or during 90 min of moderate exercise.

nonesterified free fatty acid kinetics; lipolysis

Recently, there have been a number of studies that have addressed the effect of the normal menstrual cycle on whole body substrate oxidation during exercise. This has been of interest both from the perspective of establishing any potential role of the normal variation in estrogen and progesterone on substrate metabolism as well as establishing the necessity to control for sex steroid hormone status in metabolic studies including women. In general, the majority of studies have shown that the normal cyclic fluctuations in estrogen and progesterone observed throughout the menstrual cycle do not affect whole body lipid or carbohydrate oxidation, at rest or during moderate exercise (5, 28, 29, 57); nevertheless, menstrual cycle phase may affect substrate metabolism under certain conditions. For example, lower rates of glucose flux and carbohydrate oxidation have been observed in the midluteal (MF) vs. midfollicular (MF) phase when the demand for glucose production and utilization during exercise begins to exceed a certain level (potentially > 25 μmol·kg⁻¹·min⁻¹) and/or if hepatic glycogen is limited due to fasting overnight (7, 28, 62). In addition, an acute elevation in estrogen via synthetic hormone administration, on a background of low or deficient endogenous estrogen, can decrease glucose flux (9, 11, 52), although whether this also drives a decrease in whole body carbohydrate oxidation is equivocal.

With respect to lipid metabolism, there can be differences in the sources of lipid utilized, and patterns of lipolysis, even if differences in whole body lipid oxidation are not observed. These former variables can be assessed using tracer measures of systemic nonesterified free fatty acid (NEFA) turnover and systemic glycerol turnover. Using these techniques, it has been shown that, during moderate exercise, both circulating NEFA and triglyceride (TG)-derived free fatty acids (FFA) are utilized as lipid fuel sources (1, 40, 54). Although it has been assumed that the predominant source of the TG-derived FFA is intramyocellular TG (1, 45), a contribution from circulating (VLDL) TG cannot be ruled out (40). Similar methodology has been employed to address the effect of oral contraceptives (OC) on lipid kinetics during exercise in eumenorrheic women. Results showed that 4 mo of OC use significantly increased systemic glycerol turnover (8) but had no effect on NEFA turnover, suggesting increased intracellular reesterification of NEFA (25). In addition, there was a significant decrease in the proportion of NEFA uptake that was oxidized (25), and because whole body lipid oxidation did not change, estimated TG-derived FFA oxidation was significantly increased. In a novel study that used a gonadotropin-releasing hormone (GnRH) antagonist to acutely suppress endogenous hormone production, although exercise lipid kinetics were not measured directly, data showed a significant increase in circulating NEFA concentrations, suggesting increased lipolysis, after estrogen replacement (9). Very high levels of progesterone, however, antagonized the effects of estrogen on NEFA levels (9). In contrast, the administration of exogenous estrogen to amenorrheic women or men has been reported to have no effect on exercise glycerol turnover (7, 47). These latter studies suggest a chronic lack, or absence, of endogenous female sex steroids might diminish the lipolytic response to exogenous estrogen.

Studies of the effects of exogenous female sex steroids on exercise lipid kinetics (8), along with animal and human studies that show significant effects of estrogen and progester-
one on various aspects of lipid metabolism (7, 9, 11, 18, 19, 33, 34, 36, 37, 39, 43, 46), suggest the potential for an effect of menstrual cycle phase on lipolysis and the sources of lipid utilized. Even though resting glycerol (8) and NEFA turnover (21) have been reported not to differ across the menstrual cycle, the greatly increased fuel utilization that occurs with exercise, and the concurrent hormonal changes, may accentuate any potential effects of normal cyclic fluctuations in estrogen and progesterone on lipid metabolism. Only very recently have data been published from one study on the effect of menstrual cycle phase on exercise lipid kinetics. Results showed that in five eumenorrheic women, there were no differences in systemic glycerol (8) and NEFA (25) turnover between the follicular and ML phases of the menstrual cycle. The later study, however, assessed lipid kinetics in the follicular phase when estrogen was very low (<30 pg/ml), as was progesterone, and compared this with the luteal phase when both estrogen and progesterone were elevated. Hence, an effect of the endogenous elevation in estrogen alone, such as occurs in the MF phase of the menstrual cycle, was not evaluated. Furthermore, subjects in this study were tested 3 h after eating a high-carbohydrate (75% energy) snack. Even though this snack had a low glycemic index, it would still have resulted in an insulin response sufficient to significantly suppress lipolysis and lipid utilization in the preexercise period. Furthermore, because insulin is a potent antilipolytic hormone, some carryover effect to the measures of lipid kinetics during exercise were likely, thus confounding any potential influence of the sex steroids (2, 25). Hence the effect of normal cyclic fluctuations in the female sex steroids, on exercise lipid kinetics, warrants further investigation.

The aim of the present study, therefore, was to determine systemic lipid mobilization and utilization across three phases of the menstrual cycle characterized by very different estrogen and progesterone levels. The phases included the early follicular (EF; low estrogen and progesterone), MF (elevated estrogen and low progesterone), and ML (elevated estrogen and progesterone) phases. Glycerol kinetics and palmitate kinetics (representative of plasma NEFA turnover) were measured during 90 min of moderate-intensity exercise, after an overnight fast (12–13 h) in moderately active women. Measure-ments were made in the overnight-fasted state because this is more likely to eliciting an effect of cycle phase vs. measures in the fed state (25). It was hypothesized that systemic NEFA turnover, and systemic glycerol turnover, would be greatest in the MF phase of the menstrual cycle, when estrogen is elevated but progesterone low, followed by the ML phase (high estrogen and progesterone), and lowest in the EF phase of the menstrual cycle (low estrogen and progesterone). It was further hypothesized that there would be reciprocal cycle phase differences in the estimate of TG-derived NEFA contribution to total lipid oxidation because our laboratory has previously observed that the latter is unaffected by cycle phase (23).

METHODS

Subjects

Lean, healthy women were recruited for the study. Participants were required to have a regular menstrual cycle (>11 cycles over the past year) and to be habitually active (>90 min aerobic exercise/wk) but not competitive athletes. A health and physical examination was performed on subjects to confirm there was no medical reason for their exclusion from the study.

Medical exclusions included past or present history of cardiovascular disease, high blood pressure, diabetes, any hormonal imbalance or metabolic abnormality, or use of OCs or other hormones. A total of 13 subjects took part in the study. One subject was excluded on the basis of her study day hormone concentrations, which suggested she was anovulatory. Subject characteristics are given in Table 1. The study protocol was approved by the University of Colorado Committee Institutional Review Board for the Protection of Human Subjects. All subjects read and signed an informed consent form before admission into the study.

Preliminary assessments. Measurement of maximal O2 uptake (V\textsubscript{O2 max}), body composition, and resting metabolic rate (RMR) were performed in the follicular phase of the menstrual cycle as previously described (23). Briefly, V\textsubscript{O2 max} was determined by a graded exercise test to exhaustion using a cycle ergometer (Monark, Varberg, Sweden). Body composition was determined by underwater weighing with percent body fat estimated from body density (5). RMR was measured in the overnight-fasted state using indirect calorimetry (Sensormedics 2900, Sensormedics, Yorba Linda, CA). V\textsubscript{O2 max} is not affected by menstrual cycle phase (10, 30), and body composition, assessed by densitometry, would not be detectably affected by cycle phase. The RMR was used to estimate energy requirements during the period of pretest diet control. Any increase in energy requirements during the luteal phase (35, 44) was accommodated by provision of optional food modules (23).

Menstrual cycle monitoring. Subjects provided details of prior menstrual history as far back as possible and monitored basal body temperature for at least 3–4 mo before their first study day. Determination of cycle length and time of ovulation were obtained from these records. Day 1 of the menstrual cycle was designated as the first day of menses. Ovulation was indicated by a significant and sustained rise in basal body temperature (0.3°C or more) midcycle. The last day of the menstrual cycle was the day before the next menses. Subjects also used an ovulation prediction kit (First Response Ovulation Predictors Test, Tambrands Lake Success, NY) to help identify the time of ovulation. Using a typical cycle length of 28 days, with ovulation at day 14, the EF studies were performed between days 1 and 4 (low estrogen and progesterone), the MF studies were performed between days 8 and 11 (elevated estrogen, low progesterone), and the ML studies were performed between days 19 and 23 (high estrogen and high progesterone). Adjustments in the study days were made if cycle length and/or day of ovulation differed from the typical 28-day

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age, yr</th>
<th>Height, m</th>
<th>Body Weight, kg</th>
<th>Body Fat, %</th>
<th>V\textsubscript{O2 max}, ml kg \textsuperscript{-1} min \textsuperscript{-1}</th>
<th>EF Phase</th>
<th>MF Phase</th>
<th>ML Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>E\textsubscript{2}, pg/ml</td>
<td>Prog, ng/ml</td>
<td>E\textsubscript{2}, pg/ml</td>
<td>Prog, ng/ml</td>
</tr>
<tr>
<td>Mean</td>
<td>29</td>
<td>1.67</td>
<td>59.6</td>
<td>25.4</td>
<td>39.9</td>
<td>24\dagger</td>
<td>0.41</td>
<td>72</td>
</tr>
<tr>
<td>± SD</td>
<td>± 5</td>
<td>± 0.06</td>
<td>± 7.1</td>
<td>± 1.5</td>
<td>± 5.8</td>
<td>± 0.3†</td>
<td>± 0.04</td>
<td>± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SD. V\textsubscript{O2 max}, maximal O2 uptake; EF, early follicular; MF, midfollicular; and ML, midluteal; E\textsubscript{2}, estradiol concentration; Prog, progesterone concentration. \*Significantly different from MF and EF phases, P < 0.0001. †Significantly different from MF and ML phases, P < 0.0001.
pattern. Measurements were made in the same or in consecutive cycles with at least 2 wk separating study days.

Prestudy diet and exercise control. Subjects were fed a controlled diet, prepared by the General Clinical Research Center (GCRC) diet kitchen, for 3 days before each study day (23). The diet had the same composition for all subjects (25% fat, 15% protein, and 60% carbohydrate) and was designed to maintain energy balance (1.6–1.8 × RMR based on subjects’ habitual activity level). Subjects were required to refrain from planned exercise the day before each study.

Study Days

Subjects spent the evening before each study day in the GCRC. The evening meal was consumed between 1900 and 2000. The study began at 0800 the next day, with samples being drawn for resting measurements at ~0930–1000 (12–13 h fasted). The aim was for all subjects to complete 3 test days (EF, MF, and ML) and to test order was random (23). Confirmation of cycle phase was always based on serum estrogen and progesterone levels measured on the day of the study. In three different subjects, the data from 1 day had to be excluded, because they were obtained in an incorrect cycle phase. Two other subjects were only able to complete 2 study days.

Study protocol. On the morning of the study, subjects were awakened at ~0545. An intravenous catheter was placed in an antecubital vein for infusion of stable isotopes. In the contralateral arm, a sampling catheter was placed retrograde fashion into a dorsal hand vein or, if necessary, in a wrist vein. The heated hand technique (38) was used to obtain arterialized blood samples. Initial blood samples were drawn for determination of background enrichment followed by a primed (2 μmol/kg), constant (0.09 μmol·kg⁻¹·min⁻¹) infusion of [1,2,3,3-²H₄]glycerol (Cambridge Isotopes, Andover, MA) starting from the minute rate of nitrogen excretion calculated from a 24-h urine collection. Previously, our laboratory calculated a difference of less than ±0.5% in the estimation of carbohydrate or fat grams oxidized when using the study period urine collection vs. the rate of nitrogen excretion estimated from the 24-h urine collection (22, 24).

Determination of glycerol isotope enrichment. This was measured via gas chromatography-mass spectrometry (GC-MS; GC models 5992 and 5985B, Hewlett-Packard, Palo Alto, CA) using the triacetate derivative of glycerol. Before GC-MS analysis, plasma samples were deproteinized with iced ethanol, and the supernatant was lyophilized. Samples were then derivatized using 200 μl of acetic anhydride-pyridine solution (1:1) and heating for 10 min at 60°C. Samples were transferred to GC-MS vials for analysis. Injector temperature of the GC-MS was set at 250°C, and initial oven temperature was set at 195°C. Oven temperature was increased 10°C/min until a final temperature of 265°C was achieved. Helium was used as the carrier gas with a 35:1 ml/min splitless injection ratio; transfer line temperature was set at 250°C, source temperature at 200°C, and quadrupole temperature at 116°C, and electron impact ionization was used to monitor selective ions with a mass-to-charge ratio (m/z) of 145 and 148 atomic mass units. Standard curves containing known amounts of natural and labeled glycerol were run with the biological samples.

Determination of palmitate isotope enrichment and concentration. NEFAs were extracted from plasma (0.5 ml) using Dole’s mixture. Heptadecanoic acid and 7,7,8,8-²H₄ (D₄)-palmitic acid were added to samples as internal standards to a concentration of 50 nmol. All standard solutions were prepared in n-heptane to a concentration of 0.05 mg/ml. A mixture of standard NEFA (palmitic, palmitoleic, linoleic, oleic, and stearic acid) was also prepared to the same concentration. Trimethylsilyl (TMS) esters of the NEFAs were obtained by derivatization with bis(trimethylsilyl)trifluoroacetamide (BTFSA) containing 1% chlorotrimethylsilane (TMCS) and acetonitrile (BTFSA/1% TMCS-acetonitrile, 1.5 vol/vol). Samples were heated for 1 h at 60–70°C and transferred to autosampler vials for analysis. FFA isotope enrichments were measured using GC-MS (GC model 5890, MS model 5970, Hewlett-Packard). Injector temperature was set at 275°C, and a DB-1 capillary column (30 m × 1 μm) was operated at 200°C isothermal for 4 min past injection and then programmed to 250 at 4°C/min. The transfer line was set at 280°C. Helium was used as a carrier gas with ~1.5 ml/min splitless injection ratio at the upper temperature limit program. The m/z values were monitored for the TMS esters of natural, unenriched, palmitate at m/z 313 (M-15) and enriched [1-¹³C]palmitate at m/z 314. D₄-palmitic acid was monitored at m/z 317 (M-15). Heptadecanoic acid, m/z 327, was only monitored as a marker. The m/z of the following NEFAs were also monitored: palmitoleic at m/z 311 and 312; linoleic at m/z 337 and 338; oleic at m/z 339 and 340; and stearic at m/z 341 and 342. Palmitate concentration was determined by measuring the ratio (313/317) of palmitate to the D4 internal standard. The concentrations of palmitoleic, linoleic, oleic, and stearic acid in plasma were determined using the D4 internal standard because these comprise the majority (~96.5%) of the circulating NEFAs (25), and this enabled the calculation of the palmitate contribution to the total plasma NEFA pool.

Calculations. Glycerol and palmitate enrichment and concentration data were spline fitted to remove noise introduced by analytical and sampling errors (55). Glycerol and palmitate rate of appearance (Rₐ) and rate of disappearance (Rₚ) were then calculated using the Steele equation as modified for use with stable isotopes (45, 55).

\[
R_a = -\frac{F - V_a([C_2 + C_4]/2)(E_2 - E_1)/t_2 - t_1)}{E_2 + E_1/2}
\]

\[
R_p = R_a - V_4([C_2 - C_1]/t_2 - t_1)
\]

where Rₐ is rate of appearance of trace (μmol·kg⁻¹·min⁻¹), F is infusion rate of tracer (μmol·ml⁻¹·min⁻¹), E is plasma enrichment, Vₐ is effective volume of tracee distribution [230 ml/kg body wt for glycerol and 40 ml/kg body wt for palmitate (45)], t₁ is time 1 of sampling, t₂ is time 2 of sampling, Cₐ is trace concentration at t₁, Cₐ
is tracee concentration at \( t_2 \), \( E_1 \) is plasma enrichment at \( t_1 \), and \( E_2 \) is plasma enrichment at \( t_2 \).

**Determination of circulating hormone and substrate levels.** Approximately 7 ml of whole blood were allowed to clot, and the serum was separated off after spinning. This was used for determination of hormone and substrate concentrations. All plasma, serum, and supernatant samples were stored at \(-70°C\) until analysis. For each subject, samples from all study days were run simultaneously for all assays.

**Data Analysis**

For each dependent variable, a repeated-measures ANOVA was used to determine the effect of menstrual cycle phase, time, and their interaction. If a significant menstrual cycle \( \times \) time interaction was observed, an unpaired \( t \)-test was used to determine whether there were specific time points where cycle phase differences occurred. Analyses were performed using JMP statistical software (SAS Statistical Software, Cary, NC).

**RESULTS**

Energy expenditure, \( O_2 \) uptake and heart rate during the 90-min cycle exercise were not different between cycle phases, as previously reported (23). On average, subjects exercised at 51.1, 50.1, and 50.6% of \( V_{\text{O}_2}\)max during the EF, MF, and ML phases, respectively. Similarly, whole body, protein, fat, and carbohydrate oxidation were not different between phases of the menstrual cycle (23).

**Circulating Substrate and Hormone Concentrations**

As expected, resting concentrations of progesterone were significantly greater in the ML phase \((P < 0.0001)\) vs. the EF and MF phases (Table 1). Estradiol concentration was significantly lower in the EF phase vs. both the MF and ML \((P < 0.0001)\) phases. There was no significant difference in the estradiol concentration between the MF and ML phases.

Figure 1 shows the change in circulating lipids and lipid-derived substrates from rest to exercise during each phase of the menstrual cycle. Values were adjusted for changes in plasma volume. There was a significant effect of time, but not cycle phase, on all parameters. Circulating glycerol increased significantly with the onset of exercise, whereas significant increases in NEFA concentrations were not observed until 45 min of exercise. TG levels remained relatively constant until 75 and 90 min of exercise when they significantly increased compared with rest. \( \beta\)-HBA concentrations gradually increased such that they were significantly elevated by 90 min of exercise. Our laboratory has previously reported no significant effect of cycle phase on rest and exercise changes in insulin and counterregulatory hormones (23).

**Glycerol Kinetics**

Plasma isotope enrichments for glycerol are shown in Fig. 2A. Glycerol \( R_a \) and \( R_d \) at rest and during exercise, are
shown in Fig. 3. At rest, average glycerol turnover did not differ between the EF, MF, or ML phases (Ra = 2.64 ± 0.58, 2.89 ± 0.63, and 2.57 ± 0.44 µmol·kg⁻¹·min⁻¹, respectively). Exercise resulted in a significant increase in glycerol Ra compared with rest, in all phases of the cycle (P < 0.0001). The maximum increase in glycerol Ra was observed at 90 min of exercise when values were approximately threefold above resting. There was no significant effect of menstrual cycle phase on exercise glycerol kinetics, including the maximum glycerol Ra (8.46 ± 0.95, 8.36 ± 1.12, and 7.71 ± 0.96 µmol·kg⁻¹·min⁻¹ in the EF, MF, and ML phases, respectively) or glycerol Rd (7.91 ± 0.89, 8.00 ± 1.17, and 7.18 ± 0.97 µmol·kg⁻¹·min⁻¹, respectively).

**Palmitate Kinetics and Total NEFA Utilization**

Plasma isotope enrichments for palmitate are shown in Fig. 2B. Figure 4 shows the palmitate Ra and Rd at rest and throughout exercise. At rest, palmitate turnover did not differ between the EF, MF, or ML phases (Ra = 1.52 ± 0.13, 1.53 ± 0.10, and 1.79 ± 0.17 µmol·kg⁻¹·min⁻¹, respectively). Again, there was no significant effect of cycle phase on palmitate kinetics, whereas there was a significant effect of time (P < 0.0001). Maximum palmitate Rd was observed at 90 min of exercise and equaled 3.31 ± 0.35, 3.17 ± 0.39, and 3.47 ± 0.26 µmol·kg⁻¹·min⁻¹ in the EF, MF, and ML phases, respectively.

Palmitate’s contribution to total plasma NEFA was not significantly different between cycle phases at rest (26.5 ± 0.02% EF, 28.0 ± 0.03% MF, and 28.8 ± 0.04% ML) or during exercise (27.6 ± 0.02, 28.0 ± 0.03, and 28.7 ± 0.04%, respectively) with no difference between rest and exercise values. Consequently, when total NEFA turnover was estimated from the contribution of palmitate to total NEFAs, no effect of menstrual cycle phase was also observed on resting NEFA Ra (5.66 ± 0.25, 5.46 ± 0.19, and 6.16 ± 0.25 µmol·kg⁻¹·min⁻¹ in the EF, MF, and ML phases, respectively) or exercise Ra (maximal Ra at 90 min equaled 12.15 ± 0.38, 11.59 ± 0.44, and 12.26 ± 0.26 µmol·kg⁻¹·min⁻¹, respectively). The same was true for NEFA Rd at rest (EF 5.73 ± 0.57, MF 5.46 ± 0.40, and ML 6.13 ± 0.61 µmol·kg⁻¹·min⁻¹) and during exercise (maximal Rd at 90 min equaled 11.84 ± 1.26, 11.34 ± 1.40, and 11.95 ± 0.88 µmol·kg⁻¹·min⁻¹, respectively). The total NEFA Ra was used to estimate the minimum and maximum potential contribution
endogenous lipid oxidation.

Table 2. Estimate of the contribution of circulating NEFAs to total lipid oxidation during exercise

<table>
<thead>
<tr>
<th>Cycle Phase</th>
<th>Total Lipid Oxidation (kcal/90 min)</th>
<th>60% NEFA Rd Oxidized</th>
<th>100% NEFA Rd Oxidized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kcal/90 min</td>
<td>% Total lipid oxidation</td>
<td>kcal/90 min</td>
</tr>
<tr>
<td>Early follicular</td>
<td>10 277±29</td>
<td>87±6 34.3±4</td>
<td>145±11 57.1±7</td>
</tr>
<tr>
<td>Midfollicular</td>
<td>11 255±19</td>
<td>91±15 35.7±3</td>
<td>151±16 59.5±5</td>
</tr>
<tr>
<td>Midluteal</td>
<td>10 256±23</td>
<td>87±6 35.0±2</td>
<td>145±9 58.3±4</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of subjects. Minimum and maximum potential contribution of nonesterified free fatty acids (NEFAs) to total fat oxidation assumes that 60–100% of NEFA uptake [rate of disappearance (Rd)] is oxidized; kcal, is kilocalories and assumes a caloric equivalent of 9.44 kcal/g for endogenous lipid oxidation.

DISCUSSION

This study determined the effect of normal menstrual cycle fluctuations in estrogen and progesterone on resting and exercise lipid kinetics. It was unique in that it compared the response to moderate-intensity, long-duration exercise, across three phases of the menstrual cycle [EF (low estrogen and progesterone), MF (elevated estrogen, low progesterone), and ML (elevated estrogen and progesterone)] in the overnight-fasted state. Contrary to what was hypothesized, we observed no difference in systemic glycerol and/or palmitate kinetics between the different phases of the menstrual cycle, either at rest or during exercise. This suggests no effect of cyclic changes in estrogen and progesterone on systemic lipolysis and NEFA utilization.

The present data agree with previous studies that show no difference in resting glycerol (8) or NEFA (21) kinetics when comparing the follicular with the ML phase of the menstrual cycle. Only one other study has investigated the effect of menstrual cycle phase on glycerol (8) and plasma NEFA (25) kinetics during moderate exercise (45 and 65% of peak O2 uptake, 60-min duration). This study differed from the present investigation in that it investigated only two phases of the menstrual cycle (follicular vs ML), in a small number of subjects (n = 5), with measurements made 3 h after a light meal. The present data were collected in three phases of the menstrual cycle (EF, MF, and ML), on 10–11 subjects, with testing performed in overnight-fasted state. Despite these differences, both studies are consistent in the observation of no significant effect of menstrual cycle phase on systemic glycerol and NEFA kinetics during exercise. Given the fact that estrogen and progesterone have been shown to significantly affect lipolysis and lipid metabolism in both animals and humans (7, 9, 11, 18, 19, 33, 34, 36, 37, 39, 43, 46), present and previous data (8, 25) suggest that the fluctuations (2, 4, 15, 31, 32, 56) in estrogen and progesterone that cover the majority of the days in the normal menstrual cycle are of insufficient magnitude, and/or duration, to significantly affect resting or exercise lipid kinetics and whole body lipid oxidation. This does not rule out subtle differences that the current techniques are not sensitive enough to detect. Indeed, in our laboratory’s previous report on glucose kinetics across the menstrual cycle, there was greater correspondence between glucose Rd or Rq measured within the same phase of the cycle vs. glucose kinetics compared across all phases of the menstrual cycle, despite no significant effect of cycle phase per se (23). These previous data show that
studying women within the same phase of the cycle can reduce the variability in the metabolic parameter(s) of interest. Thus it may be prudent to control for menstrual cycle phase when conducting metabolic studies in women, to decrease within- or between-subject variation and enhance the statistical power to detect a significant effect of group and/or treatment.

It is probable that the degree and/or duration of exposure to elevated estrogen and/or progesterone may be important in determining whether or not there are significant effects on lipid metabolism. Indeed, a reduction in endogenous estrogen and progesterone (to ~50 pg/ml and 1.36 ng/ml, respectively), via short-term suppression of GnRH, has been observed to result in a significant reduction in resting and exercise NEFA concentration compared with when estrogen was replaced for 3 days (9). Coincident with this elevation in NEFA levels was a significant increase in resting and exercise lipid oxidation and a decrease in circulating glucose utilization (9). Compared with the present study, sex steroid hormone levels in the GnRH-suppressed condition were similar to those observed in the EF progesterone-replaced condition (24 pg/ml estrogen and 0.41 ng/ml progesterone), whereas the elevation in endogenous estrogen observed during the MF (72 pg/ml) and ML (107 pg/ml) phases was much lower than those achieved in the GnRH-suppressed + estrogen-replaced condition (~260 pg/ml). This may explain why no menstrual cycle differences in lipid metabolism were observed in the present study between low- and high-estrogen states. In the GnRH suppression study, the effects of estrogen were negated by the addition of progesterone, although the circulating progesterin level achieved (~47 ng/ml) (9) was approximately three- to fourfold higher than the progesterone level we observed during the ML phase of the menstrual cycle (11.5 pg/dl). More chronic exposure to elevated sex steroid levels, via 4-mo treatment with a combined OC, resulted in a significant increase in glycerol Ra during exercise but not rest, with no effect on whole body lipid oxidation or NEFA kinetics (8, 25). It was estimated that the exogenous estrogen concentration achieved during the 3 wk of active combined OC use was ~400 pg/ml (8, 25), but no estimate was made of the exogenous progesterin level. Studies of exogenous hormone administration, therefore, suggest that only in the 2–3 days of the late-follicular phase of the menstrual cycle, when estrogen is greatly elevated but progesterone is still low, may there be measurable increases in lipid mobilization and differences in the pattern of lipid utilization. Any small effects of elevated estrogen in the ML phase of the cycle may be decreased by the presence of progesterone, and the level of progesterone may determine the extent of this antagonism.

The results from the present and previous studies on menstrual cycle effects on lipid metabolism apply to eumenorrheic women who are habitually moderately active. If the degree of exposure to circulating estrogen and/or progesterone is important in determining whether or not effects on lipid metabolism are observed, then similar results are likely in trained, eumenorrheic women because such individuals generally demonstrate a reduction in the magnitude of the sex steroid fluctuations across the menstrual cycle (3). It is debatable, however, as to whether individuals characterized by greater cyclic changes in estrogen and/or progesterone, such as sedentary individuals, might demonstrate more pronounced effects of the menstrual cycle on lipid metabolism. Because an effect of combined OC use was observed on exercise glycerol kinetics, it raises the question of whether or not there are effects of single or combined sex steroid administration on lipid metabolism in other groups, such as postmenopausal women or women with polycystic-ovarian syndrome. Although estrogen replacement therapy has been observed to decrease resting systemic NEFA kinetics in postmenopausal women (28), observations during exercise have not been made.

In the present investigation, we estimated the potential contribution of TG-derived FFA (circulating TG and muscle TG) to total lipid oxidation across the three phases of the menstrual cycle. This was calculated on the basis of the difference between whole body lipid oxidation and systemic NEFA Ra. The unknown fate of the NEFA taken up within tissues, however, is a limitation to this estimate because the NEFA taken up by tissues can be partitioned between reesterification (to stored TG) and oxidation, with small amounts directed toward structural or functional components. At rest, the majority of NEFA Ra undergoes reesterification (13, 25, 54), whereas during exercise more is directed toward oxidation. The exact proportion of NEFA Ra that is oxidized during exercise appears to vary considerably (~60–100%), and it is likely due to factors such as exercise intensity and duration, subject training status, sex, and whether measurements are made systemically vs. across the active limb (13, 14, 20, 25, 48–50, 53, 54). Using this range for the percentage of NEFA Ra oxidized, we estimated that the potential minimum and maximum contribution of plasma NEFA to total lipid oxidation was 34 and 56%, respectively. Importantly, this was true irrespective of menstrual cycle phase. Consequently, the estimated contribution of TG-derived FFA to total lipid oxidation was significant, potentially ranging from 44 to 66%. This agrees well with other studies that have directly measured plasma NEFA oxidation, via tracers, to estimate the contribution of circulating plasma NEFA vs. TG-derived NEFA to total lipid oxidation during exercise (16, 25, 40, 45, 54). Although the present study suggests that there was no effect of menstrual cycle phase on the proportion of whole body lipid oxidation derived from the different lipid sources, it has to be recognized that this assumes no effect of menstrual cycle phase on the proportion of NEFA Ra that is directed toward reesterification vs. oxidation. Nevertheless, data from the study of Jacobs et al. (25) suggest this is the case.

It is worth considering the interpretation of the data obtained from the measurement of systemic glycerol and NEFA (palmitate) turnover. Both of these isotopic measures of tracer Ra predominantly represent peripheral lipolysis. Peripheral sources of systemic glycerol and NEFA release include subcutaneous adipose tissue, muscle TG, and circulating TG. Under resting or overnight-fasted conditions, subcutaneous adipose tissue is by far the greatest contributor to systemic glycerol and NEFA Ra (27, 42), whereas with exercise the contribution from muscle TG, and possibly circulating TG, increases (16, 25, 40, 45, 54). Whether glycerol or NEFA Ra is a better measure of lipolysis is debatable, and both measures have their limitations. During exercise, NEFA Ra may underestimate peripheral lipolysis due to a portion of the NEFA that are released from TG hydrolysis not entering the circulation, for example, due to oxidation within muscle, and/or some intracellular reesterification (55). NEFA Ra, therefore, may be more representative of net NEFA export from subcutaneous
adipose tissue. With respect to the systemic measure of glycerol Ra, this may better represent peripheral lipolysis during exercise (26). There is no reutilization of glycerol within subcutaneous adipose tissue because this tissue lacks glycerol kinase (12) and potentially just a small amount of reutilization within muscle (17). By using both glycerol and NEFA tracers to measure lipid mobilization in the present investigation, limitations of each tracer were somewhat offset, thus strengthening the observation of no effect of menstrual cycle phase on total peripheral lipolysis and net NEFA export.

Because circulating TG and β-HBA are other potential sources of lipid-derived fuels, we measured changes in the circulating levels of these substrates at rest and with exercise throughout the menstrual cycle. If circulating TG-derived FFA were a significant fuel source during exercise, it may be predicted that circulating TG concentrations would fall because the production rate may not be able to increase sufficiently to match utilization over the relatively short time frame of the exercise. This is because VLDL-TG has a slow turnover rate (41). However, no decrease in circulating TG levels was observed, nor was there an effect of menstrual cycle phase. Although this could be interpreted to suggest that the source of TG-derived FFA during exercise was predominantly muscle TG, this is a tenuous conclusion without more direct measures of circulating and/or muscle TG utilization. Nevertheless, the lack of a difference in circulating TG levels during exercise throughout the menstrual cycle does correspond with the lack of a difference in the estimated utilization of non-plasma-derived FFA (muscle and circulating TG). Although we did not measure the kinetics of β-HBA production and utilization, the lack of difference in concentration between cycle phases would also suggest no effect on either of these parameters. This is reinforced by the observation that the precursor for β-HBA production, plasma NEFA, differed neither in concentration nor in rate of production and clearance throughout the menstrual cycle. The pattern of change in β-HBA did, however, correspond with the change in circulating NEFA levels.

In summary, menstrual cycle variations in the level of estrogen to progesterone, ranging from low to moderately high, had no significant effect on lipolysis and circulating NEFA utilization during prolonged, moderate-intensity exercise in habitually active women. Data also suggest that there is a significant contribution of TG-derived NEFA, derived from circulating and/or intramuscular TG, to total lipid oxidation during exercise in all phases of the menstrual cycle.

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