Menstrual cycle phase and oral contraceptive effects on triglyceride mobilization during exercise

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Casazza, Gretchen A., Kevin A. Jacobs, Sang-Hoon Suh, Benjamin F. Miller, Michael A. Horning, and George A. Brooks. Menstrual cycle phase and oral contraceptive effects on triglyceride mobilization during exercise. J Appl Physiol 97: 302–309, 2004—We examined the effects of menstrual cycle phase and oral contraceptive (OC) use on triglyceride mobilization during 90 min of rest and 60 min of leg ergometry exercise at 45 and 65% peak O2 uptake (V̇O2peak) in eight moderately physically active, eumenorrheic women (24.8 ± 2.1 yr). Subjects were tested during the follicular phase (FP) and the luteal phase (LP) before OC use and during the inactive phase (IP) and high-dose phase (HP) after 4 complete mo of OC use. Glycerol rate of appearance (Ra), a measure of triglyceride mobilization, was determined in a 3-h postabsorptive state using a primed constant infusion of [1,1,2,3,3-2H]glycerol. Before OC use (BOC), there were no significant differences between LP and LP in any of the variables studied. Dietary composition, exercise patterns, plasma glycerol concentrations, growth hormone concentrations, and exercise respiratory exchange ratio did not change with OC use. However, 4 mo of OC use significantly (P < 0.05) increased glycerol Ra in HP during exercise at 45% V̇O2peak (6.2 ± 0.2, 6.5 ± 0.4, and 7.7 ± 1.1 μmol·kg−1·min−1 for BOC, IP, and HP, respectively) and in IP and HP at 65% V̇O2peak (6.6 ± 0.1, 8.2 ± 0.6, and 8.1 ± 0.7 μmol·kg−1·min−1 for BOC, LP, and HP, respectively). Plasma cortisol concentrations were significantly higher with OC use at rest and during exercise at 45 and 65% V̇O2peak. In summary, although fluctuations of endogenous ovarian steroids have little effect on triglyceride mobilization, the synthetic ovarian steroids found in OCs significantly increase triglyceride mobilization and plasma cortisol concentrations in exercising women. We conclude that the hierarchy of effects of ovarian steroids and their analogs on triglyceride mobilization in exercising women is as follows: energy flux > OC use > recent carbohydrate nutrition, menstrual cycle effects, substrate utilization; glycerol kinetics; lipolysis; synthetic steroids; exertion.

Despite the extensive use of oral contraceptives (OCs) by both sedentary and athletic women, few investigators have evaluated the effects of these exogenous ovarian steroids on the metabolic responses to physical exercise. Although there are some gender differences (19, 20, 25, 35, 40) in substrate metabolism during prolonged exercise, there appear to be only small differences due to the endogenous ovarian hormone fluctuations across the normal menstrual cycle (3, 6, 11, 17, 21, 22, 29, 31, 34, 42), with many investigations reporting no difference in blood glucose kinetics, whole body respiratory exchange ratio (RER), or blood glucose or lactate concentrations due to menstrual cycle variations (7, 16, 24, 27, 30, 38).

OCs are used for birth control in normally menstruating young women, and OCs have been used to prevent bone loss in amenorrheic athletes (33), although this use of OCs is controversial. However, there is concern among athletes that these exogenous ovarian hormones may have cardiovascular and metabolic effects. Few studies have examined the influences of OCs on metabolism in exercising women (2, 5, 39). Typically, investigators used cross-sectional study designs to compare OC users with nonusers. For example, Bonen et al. (5) examined women who were not taking OCs and who were taking several types of OCs and observed a significant increase in free fatty acid (FFA) concentration in the women taking OCs. Bemben et al. (2) found a significant decrease in RER during exercise in OC users vs. nonusers, suggesting a shift toward lipid metabolism with OC use. Only a few studies have used stable isotope tracers to examine the effects of exogenous ovarian hormones on metabolism. Ruby et al. (36) found decreased glucose appearance rate (Ra) and disappearance rate (Rd) in amenorrheic athletes exercising at 65% of peak O2 uptake (V̇O2peak) for 90 min after 72 h of transdermal estradiol administration. Also, when men were given short-term doses of estradiol, glucose Ra and Rd decreased (13). Our laboratory previously reported that OCs decreased glucose Ra, Rd, and metabolic clearance rate, but OC use did not affect whole body RER during 60 min of exercise at 65% V̇O2peak (39).

To our knowledge, there are no published reports that have used a longitudinal experimental design and stable isotope tracers to determine the influences of menstrual cycle phase and OC use on triglyceride mobilization (lipolysis) during rest and exercise in humans. Therefore, to evaluate the hypothesis that endogenous ovarian steroids as well as their synthetic analogs as found in OCs would stimulate lipolysis, we undertook a study on eight young women to investigate the metabolic effects of the phases of normal menstrual cycle and OC use on triglyceride mobilization.

MATERIALS AND METHODS

Subjects. Eight healthy, nonsmoking, female subjects were recruited from the University of California, Berkeley, campus to participate in a series of experiments to examine the effects of ovarian hormones on cardiorespiratory function and substrate utilization during peak and prolonged submaximal exercises. Results from the peak exercise trials (14) and submaximal exercise trials to assess menstrual cycle effects on glucose kinetics (38, 39) on normally menstruating women are reported separately. Subjects habitually exercised 2–6 h/wk (3.7 ± 0.7 h/wk), but they were not competitive athletes. The women were nulliparous; had been diet, weight, and exercise stable; the costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
and had not taken OCs for ≥6 mo. All subjects reported regular menstrual cycles (24–32 days) and were injury and disease free as determined by health history questionnaire and physical examination. Informed, written consent was provided, and the University of California Committee for the Protection of Human Subjects approved the study protocol (no. 2001-8-132).

Experimental design. VO₂ peak tests were conducted, in a randomized order, during the early follicular phase (FP), i.e., 4–8 days after the start of menses, and during the midluteal phase (LP), i.e., 17–25 days after the start of menses and 6–9 days after ovulation. After the maximal exercise test, four stable isotope tracer infusion trials were randomly conducted within two sequential menstrual cycles, with each trial consisting of a 90-min rest period, followed by a 60-min exercise protocol. Before OC use, trials were conducted, in a randomized order, during FP, 3–9 (6.3 ± 0.8) days after the start of menses, and during LP, 17–25 (21.6 ± 0.5) days after the start of menses and 4–10 (7.2 ± 0.6) days after ovulation. Exercise tasks involved leg ergometer cycling at 45 or 65% VO₂ peak, separated by ≥3 days to complete the four trials within one to two menstrual cycles. Ovulation was predicted by using urine ovulation predictor kits (First Response, Carter Products). FP and LP were confirmed by plasma estradiol and progesterone concentrations from blood samples taken before exercise. All subjects had luteinizing hormone surges as detected from urinalysis of every menstrual cycle studied. However, trials were reassessed during the week of the inactive pills [inactive phase (IP), 32 days] and were injury and disease free as determined by health history questionnaire and physical examination.

Subjects were instructed to refrain from exercise, caffeine, and medications 24 h before testing. The isotope trials were conducted in a postabsorptive state in the morning, and dietary intake was controlled for the 24 h immediately preceding each of the eight isotope trials (2,183 kcal, 63% carbohydrate, 22% fat, and 15% protein). In addition, subjects consumed a standardized, low-glycemic index breakfast (308 kcal, 75% carbohydrate, 9% fat, and 16% protein) in the laboratory 3 h before the start of exercise. We chose to test our subjects in a rested and recently fed, postabsorptive state to control for the effects of meal size, composition, and timing and to mimic conditions in a nonlaboratory environment.

To estimate the rate of lipolysis, we measured the glycerol Rₐ. On the morning of a trial, a catheter was placed in a hand or wrist vein to obtain “arterialized” blood samples by using the heated hand vein technique, and a forearm venous catheter was placed in the contralateral arm before isotope tracer infusion. In previous studies in our laboratory (23), radial artery and heated hand vein blood samples were drawn simultaneously for determination of glucose, glycerol, and palmitate isotopic enrichments (IEs) and were not found to be significantly different. After collection of background blood samples, a 15-ml priming bolus of [1,1,2,3,3-²H]glycerol (D₅-glycerol; 225 × the resting minute infusion rate or 5.5 mg/ml) was given, and subjects rested supine or semisupine for 90 min while D₅-glycerol was continuously infused (0.06 µmol·kg⁻¹·min⁻¹ or 0.37 mg/min; Baxter Travensol 6300 infusion pump). On the basis of prior experience with the tracers to be infused and exercise tasks to be performed (7, 19, 20), to achieve constant IEs in the measurement range during exercise, tracer infusion rates were increased to three times the resting rate during exercise at 45% VO₂ peak and four times the resting rate during exercise at 65% VO₂ peak. Isotope tracers were obtained from Cambridge Isotope Laboratories (Nashua, NH), diluted in 0.9% sterile saline, and chromatographically tested for sterility and pyrogenicity (School of Pharmacy, University of California, San Francisco, CA), and on the day of the experiment they were passed through a 0.2-µm Millipore filter (Nalgene, Rochester, NY) before infusion.

At each of the blood sampling points, respiratory gas exchange was determined, heart rate was recorded by using an electrocardiograph (model Q750, Quinton, Seattle, WA), and blood pressure measured by auscultation.

Blood sampling and analysis. Blood samples were taken at 0, 60, 75, 90 min of rest and at 15, 30, 45, and 60 min of exercise; thoroughly mixed and spun in a refrigerated centrifuge at 2,800 g for 13 min; decanted; and stored at −20 or −80°C until analysis. Blood samples for plasma FFA and glycerol determination were collected in 3-ml sterile Vacutainers, containing EDTA, mixed before centrifugation, and measured by using WAKO (Richmond, VA) and Sigma (Sigma Chemical, St. Louis, MO) kits, respectively. Blood samples for determination of estradiol, progesterone, cortisol, and growth hormone levels were collected and transferred to sterile Vacutainers containing EDTA. Plasma hormone concentrations were determined by ¹²⁵I radioimmunooassay (Coat-A-Count kits, Diagnostic Products, Los Angeles, CA). Samples obtained from each subject in all trials were analyzed together. The intraassay coefficient of variation was 2–5%, and the sensitivities of the assays were 8.0 pg/ml for estradiol, 0.02 ng/ml for progesterone, 0.2 µg/dl for cortisol, and 0.9 ng/ml for growth hormone. The estradiol and progesterone kits can measure only endogenous ovarian hormones and do not reflect the amount of exogenous ovarian hormones found in OCs. Hematocrit was measured at each sampling point using circular microcapillary tube reader (no. 2201, International Equipment) to assess any changes in plasma volume due to exercise. Subjects drank tap water ad libitum during each trial to maintain hydration status.

IE analysis. Whole blood samples for glycerol IEs were collected in an 8% perchloric acid solution. Glycerol IEs were determined by using gas chromatography-mass spectrometry [GC-MS; model 5890 series II (GS) and model 5989A (MS), Hewlett-Packard] of the trimethylsilyl derivative. In preparation for GC-MS analysis, samples were neutralized with 2 N KOH and transferred to cation (AG 5-X8, X-ADH 50–100 mesh formate resin)- and anion (AG 1-X8, 100–200 mesh formate resin)-exchange columns, and the glycerol was eluted with distilled deionized water. Samples were then lyophilized, resuspended in methanol, and transferred to a 1-ml GC-MS vial. One
hundred microliters of 2:1 acetic anhydride-pyridine solution were added to each vial, and each vial was heated at 65°C for 10 min. Samples were subsequently dried under nitrogen, resuspended in 30 ml of ethyl acetate, and transferred to GC-MS vials for analysis. For GC-MS analysis, the injector temperature was set at 200°C, and initial oven temperature was set at 110°C. Oven temperature was gradually increased by 35°C/min until it reached a final temperature of 255°C. Helium was used as the carrier gas for all analyses with a 3:1 ml/min splitless injection ratio. The transfer line temperature was set at 250°C, the source temperature was set at 200°C, and the quadrupole temperature was set at 116°C. Chemical ionization was performed by using methane gas, and selected ion monitoring was used to monitor ions mass-to-charge ratios of 159 and 164 for [1H]glycerol and [3H]glycerol, respectively.

Calculations. Glycerol Rₐ and Rₐ and metabolic clearance rate (MCR) were calculated using the model described by Steele and modified for use with stable isotope tracers (41).

\[ R_a (\mu mol \cdot kg^{-1} \cdot min^{-1}) = \frac{F \cdot V [(C_1 + C_2)/2] / [(IE_2 - IE_1)/(t_2 - t_1)]}{(IE_2 + IE_1)/2} \]

\[ R_d (\mu mol \cdot kg^{-1} \cdot min^{-1}) = R_a - V [(C_1 - C_2)/(t_2 - t_1)] \]

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

where F represents the isotopic infusion rate; V is the estimated volume distribution of glycerol (270 ml/kg); C₁ and C₂ are concentrations at sampling times t₁ and t₂, respectively; and IE₁ and IE₂ are IEs of D₃-glycerol at sampling times t₁ and t₂, respectively. Values for IE were corrected for baseline enrichments from background blood samples taken before infusion of the isotopes.

Statistics. Values are means ± SE. Representative values for metabolite concentrations and glycerol kinetics were averaged from the final 15 min (75 and 90 min) of rest and 15 min (45 and 60 min) of exercise. Because there were no significant differences between resting values for the two trials in each phase before OC use and with OC use, the resting values were pooled to obtain one FP and one LP rest value before OC use and one IP and one HP rest value with OC use. Despite concerted efforts to test subjects during the intended menstrual cycle phase, the ovarian profiles of some of the women on their test day did not meet the criteria for FP (estradiol levels >50 pg/ml despite low progesterone) or LP (progesterone <3.0 ng/ml). For parameters that have not been previously reported, menstrual cycle phase values are included when menstrual cycle phase values are presented in our laboratory’s previous report (39), but they are repeated for convenience.

Physical characteristics and ergometric parameters of subjects before and during OC use were reported previously and are shown in Table 1 (14, 38, 39). There were no significant changes in total energy intake (1.920 ± 191 and 1.819 ± 260 kcal/day before and after OC, respectively), percentage of the energy intake as carbohydrate (58 ± 2.2 and 54 ± 2.9% before and after OC, respectively), percentage of the energy intake as fat (27 ± 2.9 and 31 ± 3.1% before and after OC, respectively) and percentage of the energy intake as protein (15 ± 1.6 and 14 ± 1.3% before and after OC, respectively) due to OC use (14).

There were no significant differences in RER due to ovarian steroids at 45% VO₂peak (0.90 ± 0.01, 0.90 ± 0.01, 0.90 ± 0.01, and 0.89 ± 0.01 for FP, LP, HP, and HP, respectively) and 65% VO₂peak (0.93 ± 0.01, 0.93 ± 0.02, 0.94 ± 0.01, and 0.93 ± 0.01 for FP, LP, HP, and HP, respectively). Whole body lipid utilization (based on indirect calorimetry) was also similar between phases at 45% VO₂peak (2.0 ± 0.1, 2.0 ± 0.4, 2.0 ± 0.2, and 2.2 ± 0.3 kcal/min for FP, LP, HP, and HP, respectively) and 65% VO₂peak (2.2 ± 0.2, 1.8 ± 0.5, 1.6 ± 0.3, and 1.9 ± 0.3 kcal/min for FP, LP, HP, and HP, respectively) (39).

Ovarian hormone concentrations. Resting and exercise estradiol and progesterone levels were presented in our laboratory’s previous report (39), but they are repeated for convenience. Plasma estradiol concentrations in resting women were 27.1 ± 3.7, 84.7 ± 5.8, 23.5 ± 4.4, and 13.4 ± 2.0 pg/ml for FP, LP, HP, and HP, respectively. Corresponding plasma progesterone concentrations were 0.4 ± 0.1, 10.9 ± 1.5, 0.3 ± 0.0, and 0.3 ± 0.0 ng/ml for FP, LP, HP, and HP, respectively. Five of the eight subjects met the phase criteria, and thus only data from those five are included when menstrual cycle phase effects are shown. Both endogenous ovarian hormones were lower during rest and exercise after OC use (39), validating the suppression of endogenous hormone production by synthetic ovarian steroids (9).

Blood metabolite concentrations. Blood FFA concentrations were not significantly different between menstrual cycle phases before OC use: 0.17 ± 0.05 and 0.18 ± 0.06 mM for FP and LP, respectively, at rest; 0.60 ± 0.07 and 0.69 ± 0.08 mM for FP and LP, respectively, at 45% VO₂peak; and 0.67 ± 0.08 and 0.60 ± 0.13 mmol·kg⁻¹·min⁻¹ for FP and LP, respectively, at 65% VO₂peak. Thus they were combined to form one value before OC use on each subject for making statistical comparisons with data obtained on the same subject after OC use. FFA concentrations increased three- to fivefold in response to exercise, but they were not significantly different between 45 and 65% VO₂peak only.

Blood glycerol concentrations were also not significantly different between menstrual cycle phases before OC use: 0.10 ± 0.01 and 0.10 ± 0.01 mM for FP and LP, respectively,

### Table 1. Physical characteristics of young women before OC and with 4 mo of OC

<table>
<thead>
<tr>
<th>Variable</th>
<th>BOC</th>
<th>IP</th>
<th>HP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>24.5 ± 1.3</td>
<td>25.1 ± 1.2</td>
<td>25.3 ± 1.3</td>
</tr>
<tr>
<td>Height, cm</td>
<td>164.6 ± 1.7</td>
<td>164.7 ± 1.8</td>
<td>164.7 ± 1.9</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>60.3 ± 1.7</td>
<td>60.4 ± 1.7</td>
<td>60.5 ± 1.8</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>23.2 ± 1.4</td>
<td>23.3 ± 1.4</td>
<td>23.4 ± 1.5</td>
</tr>
<tr>
<td>Fat-free mass, kg</td>
<td>46.2 ± 1.2</td>
<td>46.3 ± 1.3</td>
<td>46.4 ± 1.4</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>14.1 ± 1.1</td>
<td>14.2 ± 1.2</td>
<td>14.3 ± 1.3</td>
</tr>
<tr>
<td>VO₂peak, l/min</td>
<td>2.54 ± 0.15</td>
<td>2.57 ± 0.16</td>
<td>2.59 ± 0.17</td>
</tr>
</tbody>
</table>

Values are means ± SE for 8 women. OC, oral contraceptives; BOC, before OC; IP, inactive phase; HP, high-dose phase; VO₂peak, peak O₂ uptake. Data are from Refs. 14, 38, and 39. *Significantly different from BOC, P < 0.05.
at rest; 0.24 ± 0.02 and 0.26 ± 0.02 mM for FP and LP, respectively, at 45% \( \dot{V}O_2 \) peak; and 0.29 ± 0.03 and 0.31 ± 0.06 mmol·kg\(^{-1}\)·min\(^{-1}\) for FP and LP, respectively, at 65% \( \dot{V}O_2 \) peak. Thus they were combined to form one value before OC use on each subject for making statistical comparisons with data obtained on the same subject after OC use. Glycerol concentrations were not significantly different among phases, but increased with exercise intensity (rest < 45% \( \dot{V}O_2 \) peak < 65% \( \dot{V}O_2 \) peak) (Fig. 1B).

Blood glucose concentration fell slightly (5–12%) during exercise, but it was unaffected by menstrual cycle phase or OC use during rest or exercise (39). Blood lactate concentration rose significantly during exercise: lactate concentration was significantly elevated over rest during exercise at 45% \( \dot{V}O_2 \) peak and elevated still more during exercise at 65% \( \dot{V}O_2 \) peak (39). However, as with the blood glucose response, the blood lactate response was unaffected by menstrual cycle phase or OC use during rest or exercise (38, 39).

**Blood glycerol kinetics.** As prestudy estimates of tracer infusion rate anticipated metabolic responses, glycerol IEs did not change significantly from rest to exercise or between phases, indicating isotopic steady state (Fig. 2A). Also, background glycerol IEs remained at 0% for all trials (Fig. 2A). Glycerol \( R_a \) was not significantly \((P > 0.05)\) different between the menstrual phases before OC use: 1.8 ± 0.2 and 1.9 ± 0.2 \( \mu \)mol·kg\(^{-1}\)·min\(^{-1}\) for FP and LP, respectively, at rest; 6.2 ± 0.3 and 6.1 ± 0.5 \( \mu \)mol·kg\(^{-1}\)·min\(^{-1}\) for FP and LP, respectively, at 45% \( \dot{V}O_2 \) peak; and 6.2 ± 0.3 and 6.9 ± 0.3 \( \mu \)mol·kg\(^{-1}\)·min\(^{-1}\) for FP and LP, respectively, at 65% \( \dot{V}O_2 \) peak. Glycerol \( R_a \) was not different after 4 mo of OC use at rest and increased significantly during both exercise intensities compared with rest for all phases (Fig. 2B). Four months of OC use significantly increased glycerol \( R_a \) by 24% during exercise.
at 45% \( \dot{V}O_2 \) peak (7.7 ± 1.1) during HP compared with BOC (6.2 ± 0.2 \( \mu \)mol·kg\(^{-1} \)·min\(^{-1} \)) and 18% compared with that in IP (6.5 ± 0.4 \( \mu \)mol·kg\(^{-1} \)·min\(^{-1} \)). Four months of OC use significantly increased glycerol \( R_a \) by >20% during exercise at 65% \( \dot{V}O_2 \) peak in both IP and HP compared with BOC.

Glycerol \( R_a \) was not significantly (\( P > 0.05 \)) different between the menstrual phases before OC use 1.7 ± 0.2 and 1.7 ± 0.2 \( \mu \)mol·kg\(^{-1} \)·min\(^{-1} \) for FP and LP, respectively, at rest; 5.7 ± 0.5 and 5.6 ± 0.4 \( \mu \)mol·kg\(^{-1} \)·min\(^{-1} \) for FP and LP, respectively, at 45% \( \dot{V}O_2 \) peak; and 5.4 ± 0.3 and 6.0 ± 0.4 \( \mu \)mol·kg\(^{-1} \)·min\(^{-1} \) for FP and LP, respectively, at 65% \( \dot{V}O_2 \) peak. Glycerol \( R_a \) was not different after 4 mo of OC use at rest, and increased significantly during both exercise intensities compared with rest (\( P < 0.05 \)) for all phases (Fig. 2C). Four months of OC use significantly increased glycerol \( R_a \) during exercise (~20%) in HP at 45% \( \dot{V}O_2 \) peak and IP and HP at 65% \( \dot{V}O_2 \) peak. Glycerol \( R_a \) was also 19% higher in HP than IP during exercise at 45% \( \dot{V}O_2 \) peak. Metabolic clearance rate did not change significantly as a result of phase, but it did increase from rest to exercise after 4 mo of OC use (Fig. 2D).

**Hormone concentrations.** There were no significant differences in plasma cortisol concentrations between FP and LP before OC use: 13.9 ± 2.0 and 11.9 ± 1.8 \( \mu \)g/dl for FP and LP, respectively, at rest; 15.1 ± 3.2 and 16.8 ± 3.2 \( \mu \)g/dl for FP and LP, respectively, at 45% \( \dot{V}O_2 \) peak; and 21.7 ± 2.9 and 19.7 ± 4.4 \( \mu \)g/dl for FP and LP, respectively, at 65% \( \dot{V}O_2 \) peak. Cortisol concentrations did not change significantly from rest to exercise at 45% \( \dot{V}O_2 \) peak with any phase, but there was a significant increase from rest to exercise at 65% \( \dot{V}O_2 \) peak before OC use and in IP and during the transition from 45 to 65% \( \dot{V}O_2 \) peak in HP only (Fig. 3A). During rest and at both intensities of exercise, there were significant increases in cortisol concentration from before OC use to IP to HP with OC use (\( P < 0.05 \); Fig. 3A).

There were no significant differences in plasma growth hormone concentrations between FP and LP before OC use: 2.8 ± 1.4 and 2.0 ± 0.4 ng/ml for FP and LP, respectively, at rest; 6.0 ± 1.7 and 7.2 ± 2.4 ng/ml for FP and LP, respectively, at 45% \( \dot{V}O_2 \) peak; and 10.1 ± 2.6 and 10.1 ± 2.0 ng/ml for FP and LP, respectively, at 65% \( \dot{V}O_2 \) peak. Growth hormone concentrations increased significantly from rest to 45 and 65% \( \dot{V}O_2 \) peak exercise (Fig. 3B), but it was not significantly different between phases. Although growth hormone tended to be higher in HP than in all other phases at rest and during both intensities of exercise, the difference was not statistically significant.

Insulin values are reported separately (38, 39), but they are reiterated for the convenience of the reader. At rest, insulin levels before OC use were 10.9 ± 1.6 \( \mu \)U/ml and increased by 2–3 \( \mu \)U/ml in individual subjects (\( P < 0.05 \)) with OC use during IP and HP, which were not different from each other (39). Insulin decreased during exercise in all conditions (\( P < 0.05 \)), with a significant exercise intensity effect before OC use (\( P < 0.05 \); rest > 45% \( \dot{V}O_2 \) peak > 65% \( \dot{V}O_2 \) peak), but no significant phase or exercise intensity effect was observed with OC use.

**DISCUSSION**

To our knowledge, this is the first published report using stable isotope glycerol tracers to measure the influences of menstrual cycle phase and OC use on triglyceride mobilization in humans during rest and exercise with a longitudinal study design. The results from our investigation appear to confirm our prediction of no significant effect of endogenous ovarian hormone fluctuations on triglyceride mobilization at rest or during exercise. However, 4 mo of the synthetic steroids used in OCs increase triglyceride mobilization (as estimated from glycerol \( R_a \)) during exercise, with little change in FFA or glycerol concentrations. The increased triglyceride mobilization was associated with increases in the lipolytic hormone cortisol.

Most studies that have examined the effect of menstrual cycle phase on substrate flux have shown no significant difference in blood glucose flux or whole body substrate utilization patterns in resting or exercising postabsorptive women (7, 24, 38). In the present study, we also showed no difference in triglyceride mobilization with the endogenous ovarian hormone fluctuations of normal menstrual cycles. Perhaps the small changes in substrate flux rates due to menstrual phases either were normalized by carbohydrate nutrition being provided 3 h before exercise or possibly the glycerol flux rates due to the endogenous ovarian hormone fluctuations were overwhelmed by changes in flux that occurred due to exercise.

Although there are no known studies that have used stable isotopes to examine the effects of ovarian hormones on fat metabolism in humans, several studies have examined the effects of the exogenous ovarian hormone use on glucose flux.
Ruby et al. (36) determined the effects of transdermal estradiol replacement on substrate turnover during exercise in amenorheic women, and Carter et al. (13) administered oral estradiol to male subjects and measured substrate turnover during 90 min of cycle ergometer exercise. In addition, our laboratory’s previous report (39) determined the effects of OC use on glucose kinetics during exercise. In all these investigations, there was a reduction in glucose flux in response to exogenous estradiol administration. Because total carbohydrate oxidation (measured by indirect calorimetry) during exercise was unaffected by OC use, as our laboratory reported previously (39), OC administration may have increased the use of skeletal muscle glycogen or lactate as a means to compensate for the reduction in glucose availability.

Another means for compensating for reduced glucose mobilization could be an increase in FFA mobilization and utilization. Ellis et al. (18) demonstrated that exogenous administration of estradiol to male rats increased the availability of FFA to exercising muscle, not only from adipose tissue but also from intracellular muscle triacylglycerol. Campbell and Febbraio (12) investigated the effects of estradiol and progesterone administration, separately and in combination, to ovariectomized rats and found increased levels of carnitine palmitoyltransferase I, the enzyme that controls long-chain fatty acid flux into the mitochondria. Estradiol and progesterone administration to rats also increased 3-hydroxyacyl-CoA dehydrogenase, an enzyme important in the β-oxidation pathway for skeletal muscle lipid metabolism. Given the perspectives derived from the work of others on rodents, the increased adipose tissue fatty acid mobilization (glycerol $R_g$) with OC use in our investigation could have been associated with increased fatty acid utilization. However, data from indirect calorimetry do not support such a conclusion. More likely, the elevated fatty acid mobilization could also have been accompanied by increased reesterification of mobilized fatty acids, inasmuch as whole body RER during exercise was unaffected by OC use.

Combined results from our laboratory’s previous (39) and our present reports indicate that OCs have persistent as well as acute effects on metabolism. Triglyceride mobilization was higher in HP, but not in IP, during exercise at 45% $V_{O_2 \text{peak}}$, indicating multiple, transient and long-term effects of exogenous ovarian steroids on fat metabolism. We have no clear explanation for this observation of the different metabolic effects of OC use, but the results may be due to the different hormone receptor numbers and affinities of the different tissues involved in fuel production during exercise.

As noted in our laboratory’s previous report (14), OC use reduced $V_{O_2 \text{peak}}$ by ~13%. This effect of OC on aerobic capacity increased the relative exercise intensity, an effect that usually increases glucose flux and decreases glycerol $R_g$ (8). Therefore, when considering the effects of OC use on women exercising at high relative intensity, the effects of OC use on increasing glycerol $R_g$ are especially notable, because lipid oxidation is suppressed at high relative exercise intensities (8). Furthermore, our subjects consumed a standardized diet 24 h before testing and a standardized breakfast in the laboratory on the morning of testing; hence we report data on rested, glycojen-replete, and 3- to 4-h postabsorptive subjects. The two exercise tasks we studied raised metabolic rate 5- to 8-fold above rest and resulted in a 4- to 11-fold increase in carbohydrate oxidation. In this setting, the overall contributions of carbohydrate and fat to total substrate oxidation, determined by indirect calorimetry, were not different before and after OC use. Nevertheless, glucose production and disposal rates were suppressed (39), and OC use enhanced triglyceride mobilization during exercise without changing glucose (39), glycerol, or FFA concentrations in the blood (Fig. 1).

As has been shown by others (1, 15), in our study OC use was associated with increased cortisol concentrations at rest and during exercise. Cortisol levels are also increased dramatically during rest and exercise with pregnancy, during which estrogen and progesterone concentrations are also elevated (4, 10, 32). Although growth hormone concentrations were unchanged by OC administration in our study, other studies have also shown an increase in growth hormone concentrations with OC administration (2, 5). These hormones are involved in promoting lipolysis and decreasing peripheral (fat and muscle tissue) glucose uptake and utilization (28). Cortisol also stimulates glycogen deposition and gluconeogenesis while inhibiting glycogen mobilization (28). Consequently, it is difficult to know whether the changes in glucose disposal and triglyceride mobilization observed in the present investigation were primarily attributable to the effects of synthetic ovarian steroids or were secondary to increased cortisol levels.

The release of cortisol usually occurs as a means for mobilizing energy to combat stressful situations (fight-or-flight mechanisms). Usually, reductions in blood glucose trigger counterregulatory hormones to maintain glucose homeostasis. In this context, it is difficult to explain why cortisol levels were elevated with OC use, because blood glucose levels did not change (39). The increased cortisol associated with high levels of ovarian hormones (as in pregnancy) may be a protective measure to ensure a rapid metabolic response (26). High levels of cortisol would reduce peripheral glucose uptake (28) (maintaining blood glucose concentrations) and increase adipose tissue triglyceride mobilization (thus providing an alternative fuel source to spare blood glucose) and protein catabolism (providing amino acids for gluconeogenesis).

Summary and conclusions. Results of the present investigation contribute to the growing body of evidence on the relative effects of endogenous and exogenous ovarian hormones on metabolic flux and substrate partitioning in humans. Effects of endogenous ovarian hormones on glucose and glycerol fluxes during exercise are subtle and overridden during exercise by changes in metabolite flux rates and carbohydrate nutrition. Exogenous ovarian hormones, such as the OCs studied in this investigation, exert greater effects on glucose flux and rate of lipolysis than do endogenous hormones, inasmuch as effects of OC use can be observed in glycogen-replete recently fed women. Furthermore, the effects of OC use on glucose flux and lipolysis are persistent with higher intensities of exercise, inasmuch as they can be observed during days of the month when exogenous ovarian hormones are not provided. Finally, exogenous ovarian hormones appear to increase endogenous counterregulatory hormones related to lipolysis (cortisol) at rest and during exercise. Although we conducted a longitudinal study of effects of endogenous and exogenous ovarian hormones on lipid mobilization involving 4 mo of OC use, future investigations will be necessary to provide insight into the long-term metabolic effects of OC use and how long the effects persist after the cessation of “the pill.” In this respect, studies

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examining the cellular effects of exogenous ovarian hormones, such as metabolic regulation and receptor control, are required.

In women fed several hours before study, these results are interpreted to mean that 1) lipolysis is not altered by menstrual cycle phase in women not using OCs, 2) OC use increases lipolysis (glycerol $R_a$) but does not affect overall FFA concentration or whole body RER during moderate-intensity exercise, and 3) the increased triglyceride mobilization seen with OC use is associated with higher cortisol concentrations. On the basis of our present findings, our laboratory’s previous reports (38, 39), and reports of others (11, 24), we conclude that the hierarchy of effects of ovarian steroids and their analogs on triglyceride mobilization in exercising women is as follows:

energy flux > OCs > recent carbohydrate nutrition, menstrual cycle effects.

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DISCLOSURES

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