Fatty acid reesterification but not oxidation is increased by oral contraceptive use in women

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RESULTS OF STUDIES ON LABORATORY rodents indicate that estrogen stimulates adipose lipolysis (31), increases plasma-free fatty acid (FFA) availability (41) and oxidation during exercise (32), and promotes the expression of proteins integral to fatty acid oxidation (11, 12). The influences of ovarian hormones on human lipid metabolism have been studied, but their effects are not fully understood. Studies indicate that women have greater lipolytic responses than men to fasting (45) and exercise (35, 44), but this does not always result in greater whole body fatty acid rates of oxidation (Rox). We (20, 23) and others (38, 57) have found that women derive a greater proportion of energy expenditure (EE) from lipid during exercise than lifestyle-matched men. However, others have not found gender differences in whole body fatty acid Rox (44, 49, 54). Results of gender differences in lipid partitioning during exercise are also equivocal with reports of both greater plasma FFA Rox (44) and greater intramuscular triglyceride (IMTG) use (49, 54) in women compared with men. While gender comparison studies contrast groups with very different ovarian hormone concentrations, their inherent cross-sectional design presents subject-matching issues that have not been fully resolved in the literature.

Longitudinal study designs to assess menstrual cycle and oral contraceptive effects on lipid metabolism avoid the subject-matching problems faced by gender comparison studies. Fluctuations in ovarian hormones between the follicular (FP) and luteal phases (LP) of the menstrual cycle do not influence glycerol turnover (14) or whole body fatty acid Rox at rest or during prolonged exercise (7, 37, 40, 56). Additionally, plasma FFA turnover at rest was similar between the FP and LP (34). Studies reporting subtle increases in whole body fatty acid Rox during exercise in the luteal compared with the FP also demonstrated that these effects were mitigated at lower exercise intensities (63) or by carbohydrate feeding (10). Intersubject variability in ovarian hormone concentrations during the different phases of the menstrual cycle often makes the results of studies on humans difficult to interpret. The only study to tightly control ovarian hormones in eumenorrheic women with pharmacological suppression and replacement of estradiol and progesterone found that conditions of high estradiol-low progesterone resulted in greater whole body fatty acid Rox during prolonged exercise than either low estradiol-low progesterone or high estradiol-high progesterone (17). To date, a detailed examination of the influence of ovarian hormones across the normal menstrual cycle on plasma FFA kinetics at rest and during exercise has not been reported.

The influence of synthetic ovarian hormone supplementation in amounts typical of low-dose oral contraceptives on human lipid metabolism has received little attention. Synthetic estradiol supplementation in men (13) and amenorrheic women (50) did not alter glycerol turnover or whole body fatty acid Rox at rest or during prolonged exercise. Synthetic estradiol and progesterone supplementation through the use of oral contraceptives is common and has been shown to increase plasma cortisol concentration at rest and during exercise (1, 14, 60) and plasma FFA concentration during low-intensity exercise (6). However, 3 mo of oral contraceptive use did not alter plasma olate turnover at rest (26). Cross-sectional investigations have found no difference in whole body fatty acid Rox (63) during 30–90 min of exercise at 45–85% peak oxygen con-
OVARian hormones and plasma FFA turnover

**METHODS**

Detailed descriptions of the methods employed in this study have been previously published (14, 15, 55, 56), but relevant information is reiterated for the convenience of the reader.

**Subjects.** Eight healthy, nonsmoking, weight-stable women were recruited from the University of California, Berkeley community by posted notices and E-mails for participants in a series of experiments to examine the effects of ovarian hormones on maximal aerobic capacity ($V_{\text{O}_2 \text{peak}}$) and substrate use during submaximal exercise. Subjects were nulliparous, reported regular menstrual cycles (24–32 days in duration), and had not taken oral contraceptives for ≥6 mo. Subjects habitually exercised 2–6 h/wk (3.7 ± 0.7 h/wk), but were not trained endurance athletes. Health history questionnaires and physical examination revealed that the women were injury and disease free. The procedures and risks were thoroughly explained to the subjects, and their written, informed consent was obtained. The University of California Committee for the Protection of Human Subjects approved the study protocol (CPHS no. 2001–8–132).

**Experimental design.** After screening, subjects performed eight stable isotope tracer infusion trials: four trials during the menstrual cycle phases and four trials following oral contraceptive use (Fig. 1). Stable isotope tracer infusion trials consisted of 90 min of rest and during moderate-intensity exercise. Assuming that substrate use during exercise is primarily regulated by exercise intensity and its metabolic and endocrine consequences (9), we anticipated that whole body fatty acid $R_{\text{ox}}$ and plasma FFA turnover and $R_{\text{ox}}$ at rest and during moderate-intensity exercise. Assumptions that substrate use during exercise is primarily regulated by exercise intensity and its metabolic and endocrine consequences (9), we anticipated that whole body fatty acid $R_{\text{ox}}$ and plasma FFA turnover and $R_{\text{ox}}$ would be unaffected by either endogenous or synthetic ovarian hormones. Given our expectation that ovarian hormones would promote fatty acid mobilization (14) without affecting oxidation, we tested the hypothesis that fatty acid reesterification would be increased during rest and exercise in the LP and during oral contraceptive use, when ovarian hormone concentrations are high, compared with the early FP.

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and a forearm venous catheter was placed in the contralateral arm for continuous stable isotope tracer infusion. In previous studies in our laboratory (36), radial artery and heated hand vein blood samples were drawn simultaneously for determination of glucose, glyceral, and palmitate isotopic enrichments (IE) and were not found to be significantly different. After collection of background blood and breath samples, subjects rested supine or semisupine for 90 min while [1-13]Cpalmitate was continuously infused (0.61 mg/min; Baxter Travelen 6300 infusion pump). The resting palmitate tracer infusion rate was doubled (1.22 mg/min) during exercise at 45 and 65% VO2 peak to maintain isotopic equilibrium (20, 23). [1-13]Cpalmitate was obtained from Cambridge Isotope Laboratories (Nashua, NH), combined with 100 ml of 25% human albumin, diluted in 400-ml 0.9% sterile saline, and pharmacologically tested for sterility and pyrogenicity (School of Pharmacy, University of California, San Francisco). All palmitate/albumin infusates were used within 5 days of completion of sterility testing. Subjects were simultaneously infused with [6,6-2H2]glycerol and [1,1,2,3,3-2H]glycerol to assess glucose and glycerol kinetics, and these results have been previously published (14, 55, 56).

At each of the blood sampling points, respiratory gas exchange was determined, and an aliquot of expired air was collected in duplicate in 10-ml vacuum Exetainer tubes for the determination of 13CO2 IE. The expired air samples were stored at room temperature and were analyzed by Metabolic Solution (Merrimack, NH) using isotope ratio mass spectrometry (MS). Heart rate was recorded throughout rest and exercise using an electrocardiograph (model Q750, Quinton, Seattle, WA), and blood pressure was measured by auscultation.

**Blood sampling and analysis.** Blood samples were taken at 0, 60, 75, and 90 min of rest and 15, 30, 45, and 60 min of exercise, thoroughly mixed, spun in a refrigerated centrifuge at 2,800 g for 13 min, decanted, and stored at −20 or −80°C until analysis. The sampling and analytic procedures for plasma total FFA (enzymatic assay), glycerol, hormones and blood glucose, and lactate have been previously published (14, 55, 56). Importantly, the estrogen and progesterone kits used detected only endogenous ovarian hormones and not the synthetic ovarian hormones found in the oral contraceptives. Samples for palmitate-stable IE analysis were prepared by thorough mixing exactly 1 ml of plasma with a heptane-isopropanol (30:70) extraction solution containing H2SO4 (0.0033 M) and 100 nmol of pentadecanoic acid (15:0) as an internal standard. Extracted plasma samples were stored at −20°C for later analysis. 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 normal hydration status.

**Plasma catecholamine analysis.** Blood for catecholamine analysis was collected in chilled tubes containing glutathione and EGTA to prevent oxidation. Blood was immediately centrifuged at 3,000 g, and the plasma was transferred to a storage tube and was frozen at −80°C until analysis. Catecholamines were extracted from the plasma using methods adapted from Anton and Sayre (2) using acid-washed WA-4 Alumina (Sigma, St. Louis, MO) and 1.5 M Tris buffer containing 2% EGTA at a pH of 8.6. An internal standard of 500 pg/ml of dihydroxybenzylamine (ESA, Clemsons, MA) was added to each standard and sample before extraction. Standard catecholamine solutions were purchased from ESA (Clemsons, MA). After vigorous shaking for 15 min, samples were rinsed three times with 2 ml deionized water and then transferred to filter tubes and centrifuged at 1,200 rpm in a table-top centrifuge (Microfuge 12, Beckman, Fullerton, CA), and perchloric acid (0.1 M) was used to elute the catecholamines. Finally, 100 µl of the eluent were injected into the HPLC system (ESA Coulochem LC/EC, 5200A, Clemsons, MA). The flow rate was 1.0 ml/min, the mobile phase was Cataphase 2 (ESA, Cambridge, MA), the column was C-18 4.6 × 80 mm, and the electrodes were set at +350, 50, and −350 mV for the 5021 Conditioning cell and 5011 Dual Channel High Sensitivity Analytical cell, respectively. Chromatographs were analyzed using EZChrom Elite data analysis system (ESA).

**IE analysis.** The fatty acids of the extracted plasma samples were isolated by thin-layer chromatography using an oleate [18:1(n-9)] standard. Isolated samples were derivatized to their fatty acid methyl esters to allow easy volatileization by gas chromatography (GC). Individual plasma long-chain fatty acid concentrations and palmitate IE were determined simultaneously by flame ionization detection (FID) and GC-MS, respectively (GC model 6890 and MS model 5973N; Hewlett-Packard). Following separation by GC, a microcontroller was used to split the column and deliver appropriate loads to the FID and MS. For GC-MS analysis, the injector temperature was set at 250°C, and initial oven temperature was set at 50°C. The oven temperature was held at 55°C for 30 s and gradually increased by 25°C/min until it reached a temperature of 195°C and was then ramped at 3°C/min to 205°C and then 8°C/min to 230°C. The column used was a DB225, 30 m × 0.25 µm (Agilent). Helium was used as the carrier gas in constant flow mode for all analyses with an average velocity of 60 cm/s. The source temperature was set at 230°C, and the quadrupole temperature was set at 150°C. Electron impact ionization was performed with selected ion monitoring to monitor ion mass-to-charge ratios of 270 and 271 for [12]Cpalmitate and [13]Cpalmitate, respectively. Glycerol IE was determined as described previously (14).

**Calculations.** Individual plasma long-chain fatty acid concentrations were calculated relative to the abundance of the internal pentadecanoic acid standard and a physiological range of external standards. Palmitate IE values were corrected for baseline enrichments from background blood samples taken before the infusion of the isotopes. Palmitate rate of appearance (Rap) and disappearance (Rd) and metabolic clearance rate were calculated using equations defined by Steele and modified for use with stable isotopes (61), as described previously (21). Palmitate kinetics were converted to FFA kinetics by dividing by the fraction of plasma palmitate to total FFA concentration (~0.30) as determined by FID, assuming that the relative concentration of each individual plasma FFA is directly related to its relative release from adipose tissue. This assumption has been supported by recent work (46), and palmitate and oleate have long been considered optimal for modeling plasma FFA kinetics (25, 28, 29, 33, 46). EE values were derived from respiratory data averaged over the last 5 min of each 10-min collection period. The proportion of EE derived from carbohydrate and lipid and the rates of EE, whole body carbohydrate, and lipid oxidation were calculated using the following stoichiometric equations as described previously (21, 42, 64):

\[
%\text{EE from CHO} = \left(\frac{\text{RER} - 0.71}{0.29}\right) \times 100
\]

\[
%\text{EE from lipid} = 100 - %\text{EE from CHO}
\]

EE (kcal/min) = \left[\left(\%\text{EE from CHO} \times V\hat{O}_2\right) \times 5.05 \text{ kcal/l } O_2\right] + \left[\left(\%\text{EE from lipid} \times V\hat{O}_2\right) \times 4.7 \text{ kcal/l } O_2\right]
\]

Carbohydrate R\text{ox} (g/min) = \left[\left(\%\text{EE from CHO} \times V\hat{O}_2\right) \times 5.05 \text{ kcal/O}_2\right] / 4.2 \text{ kcal/g CHO}

Lipid R\text{ox} (g/min) = \left[\left(\%\text{EE from lipid} \times V\hat{O}_2\right) \times 4.7 \text{ kcal/O}_2\right] / 9 \text{ kcal/g lipid where CHO is carbohydrate; RER is the respiratory exchange ratio; and } V\hat{O}_2 \text{ is oxygen uptake expressed in l/min. Rates of carbohydrate and lipid oxidation were converted to body weight relative units (} \mu\text{mol·kg}^{-1}·\text{min}^{-1} \text{) using the molecular weights of glucose (180 g/mol) and a representative triglyceride (860 g/mol), and lipid } R\text{ox was converted to fatty acid } R\text{ox by multiplying by 3 (3 mol fatty acids/mol triglyceride). Plasma FFA } R\text{ox was calculated as:}
\]

Plasma FFA R\text{ox} (\mu\text{mol·kg}^{-1}·\text{min}^{-1})
\]

\[
= (I_E\text{breath} \times V\hat{CO}_2) / (I_E\text{plasma} \cdot CF \times %\text{palmitate})
\]

where I_E\text{breath} is the IE of 13C in the breath, V\hat{CO}_2 is CO_2 production in \mu\text{mol·kg}^{-1}·\text{min}^{-1}, assuming 22.4 l/mol CO_2, I_E\text{plasma} is the IE of 13C.
[\textsuperscript{13}C]palmitate in the plasma, CF is the bicarbonate correction factor [0.65 for rest and 0.90 for exercise determined previously (43)], and \%palmitate is the fraction of plasma palmitate to total FFA concentration as determined by FID. The \textit{R}_{ox} of other fatty acids (purported to primarily represent IMTG) was calculated by subtracting plasma FFA \textit{R}_o from whole body fatty acid \textit{R}_{ox}. The rate of lipolysis was calculated as three times glycerol \textit{R}_o (assumed that released glycerol enters the plasma pool only as a result of lipolysis, glycerol cannot be reincorporated into triglyceride within adipose tissue or skeletal muscle, and triglyceride molecules are completely hydrolyzed (62). The rates of reesterification (\textit{R}_e) were calculated as:

- Total fatty acid \textit{R}_{e} (\text{\mu mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) = \text{rate of lipolysis} - \text{whole body fatty acid} \textit{R}_{ox}
- Local fatty acid \textit{R}_{e} (\text{\mu mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) = \text{rate of lipolysis} - (\text{plasma FFA} \textit{R}_o + \text{other fatty acid} \textit{R}_{ox})
- Plasma-derived fatty acid \textit{R}_{e} (\text{\mu mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) = \text{Plasma FFA} \textit{R}_e - \text{plasma FFA} \textit{R}_{ox}

Local fatty acid \textit{R}_e (often termed “intracellular \textit{R}_e”) represents the reesterification of fatty acids that presumably never left their site of origin, whereas plasma-derived fatty acid \textit{R}_e (often termed “extracellular \textit{R}_e”) represents the reesterification of fatty acids that exited their site of origin and were reesterified in another tissue (adipose, liver, or skeletal muscle). Local fatty acid \textit{R}_e has previously been calculated by subtracting plasma FFA \textit{R}_o from the rate of lipolysis (59, 62). We feel that this approach may have overestimated local fatty acid \textit{R}_e by ignoring the reesterification of fatty acids within skeletal muscle that originated from the hydrolysis of IMTG and never entered the plasma pool (A. L. Friedlander, personal communication). The equations of the present investigation attempt to correct this oversight by incorporating the oxidation of other fatty acids purported to primarily represent IMTG.

\textbf{Statistics.} Data are represented as means \pm SE. While individual plasma long-chain fatty acid data were analyzed over time, steady-state FFA kinetic data were calculated using the last two (75 and 90 min) and three (30, 45, and 60 min) IE values obtained during rest and exercise, respectively. Results of Shapiro-Wilks tests indicated that the data were normally distributed. The significance of mean differences was assessed by ANOVA with repeated measures followed by post hoc analyses using the least significant difference test. Significance was set a priori at \(P < 0.05\).

\section*{RESULTS}

Despite concerted efforts to test subjects in the intended menstrual cycle phase, three women did not meet the ovarian hormone concentration criteria (one in FP and two in LP). Menstrual cycle phase comparisons (FP vs. LP) were performed in the remaining five women with complete sets of data. No significant differences were noted in any variable measured between FP and LP, and the data from all eight subjects collected BOC were pooled by averaging each subject’s FP and LP values (excluding the individual trials where ovarian hormone criteria were not met). Likewise, data on resting women within each condition (BOC, IP, and HP) were not different and were pooled. Although not statistically different from each other, FP and LP fatty acid turnover and \textit{R}_{ox} data are described where appropriate for the purpose of comparison with previous studies.

\textbf{Subject characteristics.} The physical characteristics of the subjects have been presented previously (14, 15, 55, 56) and are repeated in Table 1 for convenience of the reader. Oral contraceptive use was associated with a small, but significant increase in body weight, body fat percent, and fat mass and an \(-11\%\) decrease in \textit{V}_{O_2\text{peak}} compared with BOC (15)

\textit{Plasma hormone concentrations.} Plasma ovarian hormone, insulin, glucagon, growth hormone, and cortisol concentrations at rest and during exercise have been presented previously (14, 55, 56). Briefly, plasma estradiol concentrations were higher during LP than FP (84.8 \pm 8.5 vs. 30.6 \pm 3.5 pg/ml, \(P < 0.05\)) and were higher during IP than HP (23.5 \pm 4.4 vs. 13.4 \pm 2.0 pg/ml, \(P < 0.05\)). Although it could not be determined with the methods used in the present study, it has previously been shown that triphasic oral contraceptive use results in plasma synthetic estradiol concentrations of \(-400\) pg/ml (58). Plasma progesterone concentration was higher during the LP than FP (10.9 \pm 1.3 vs. 0.4 \pm 0.1 ng/ml, \(P < 0.05\)) and was not different during IP and HP (0.3 \pm 0.1 vs. 0.3 \pm 0.1 ng/ml). Except for higher plasma insulin concentrations at rest in IP and HP than BOC (\(P < 0.05\)), plasma insulin, glucagon, and growth hormone concentrations were not different among BOC, IP, and HP at rest or during exercise. Plasma cortisol concentrations were higher in IP and HP than BOC at rest (25.0 \pm 1.5 and 33.2 \pm 1.9 vs. 12.7 \pm 1.5 \mu g/dl), 45\% \textit{V}_{O_2\text{peak}} (25.6 \pm 3.0 and 29.5 \pm 2.3 vs. 16.0 \pm 2.7 \mu g/dl), and 65\% \textit{V}_{O_2\text{peak}} (29.3 \pm 2.2 and 36.1 \pm 3.1 vs. 21.5 \pm 2.7 \mu g/dl). Plasma norepinephrine increased significantly in a stepwise manner from rest (257.3 \pm 14.4 pg/ml) to 45\% \textit{V}_{O_2\text{peak}} (733.3 \pm 40.3 pg/ml) and 65\% \textit{V}_{O_2\text{peak}} (1,610.8 \pm 62.0 pg/ml), but there were no differences among BOC, IP, and HP. Plasma epinephrine also increased significantly in a stepwise manner from rest (4.4 \pm 2.0 pg/ml) to 45\% \textit{V}_{O_2\text{peak}} (10.9 \pm 2.7 pg/ml) and 65\% \textit{V}_{O_2\text{peak}} (16.9 \pm 5.0 pg/ml), but there were no differences among BOC, IP, and HP.

\textit{Individual plasma long-chain fatty acid concentrations.} Plasma myristate, palmitate, palmitoleate, stearate, oleate, linoleate, and total plasma FFA concentration increased approximately two- to fivefold during exercise compared with rest in all conditions and both exercise intensities (Table 2). Changes in linolenate and arachidonate could not be tested statistically due to missing values when their concentrations were below the level of detection, but collectively they represented <2\% of the total plasma FFA pool. Plasma palmitate concentration was higher in HP than BOC at 45 and 60 min of exercise at 45%
Respiratory gas exchange parameters at rest and during exercise before and after 4 mo of oral contraceptive use

<table>
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<tr>
<th>Variable</th>
<th>Rest BOC</th>
<th>Rest IP</th>
<th>Rest HP</th>
<th>45% BOC</th>
<th>45% IP</th>
<th>45% HP</th>
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<th>65% IP</th>
<th>65% HP</th>
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<tr>
<td>V˙O₂ peak (P &lt; 0.05)</td>
<td>342.9 ± 1.2%</td>
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<tr>
<td>V˙O₂, ml·kg⁻¹·min⁻¹</td>
<td>4.0 ± 0.1</td>
<td>3.7 ± 0.1</td>
<td>3.8 ± 0.1</td>
<td>19.8 ± 0.9*</td>
<td>18.9 ± 0.9*</td>
<td>18.9 ± 1.1*</td>
<td>28.9 ± 1.5*†</td>
<td>26.7 ± 1.6†</td>
<td>26.8 ± 1.4†</td>
</tr>
<tr>
<td>V˙CO₂, ml·kg⁻¹·min⁻¹</td>
<td>3.4 ± 0.1</td>
<td>3.3 ± 0.1</td>
<td>3.5 ± 0.1</td>
<td>17.6 ± 0.8*</td>
<td>16.7 ± 0.7*</td>
<td>16.6 ± 0.8*</td>
<td>26.3 ± 1.4*†</td>
<td>24.5 ± 1.4†</td>
<td>24.3 ± 1.3*†</td>
</tr>
<tr>
<td>RER</td>
<td>0.86 ± 0.01</td>
<td>0.89 ± 0.01</td>
<td>0.87 ± 0.01</td>
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<td>0.92 ± 0.01†</td>
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<tr>
<td>EE, kcal·min⁻¹</td>
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<td>5.9 ± 0.3</td>
<td>5.8 ± 0.3</td>
<td>5.7 ± 0.4</td>
<td>8.6 ± 0.5*†</td>
<td>8.2 ± 0.5*†</td>
<td>8.2 ± 0.5*†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 subjects. V˙O₂, oxygen consumption; V˙CO₂, carbon dioxide production; RER, respiratory exchange ratio (V˙CO₂/V˙O₂); EE, energy expenditure. Significantly different than *rest, †all previous time points, ‡corresponding value at 45% V˙O₂ peak, §BOC; and †IP: P < 0.05.

Table 3. Respiratory gas exchange parameters at rest and during exercise before and after 4 mo of oral contraceptive use
VO_2 peak in all conditions (Table 3). The RER of FP and LP were not statistically different at rest (FP: 0.84 ± 0.01, LP: 0.87 ± 0.02; P = 0.08) or during exercise at 45% VO_2 peak (FP: 0.89 ± 0.01, LP: 0.88 ± 0.01; P = 0.45) or 65% VO_2 peak (FP: 0.92 ± 0.01, LP: 0.91 ± 0.02; P = 0.49). The RER and proportion of energy derived from carbohydrate were significantly higher in IP than BOC at rest (P < 0.05). The RER of BOC was higher than rest during exercise at 45 and 65% VO_2 peak, whereas the RER of IP and HP was only higher than rest at 65% VO_2 peak. These values differ slightly from those previously reported (14, 55, 56) due to the correction of a small number of VCO_2 values that were the result of apparent hyperventilation and resulted in plasma FFA R_{ox} values that were greater than whole body fatty acid R_{ox}.

**Plasma palmitate IE and breath ^{13}C O_2 IE.** Despite the doubling of the palmitate tracer infusion rate in anticipation of a twofold increase in plasma FFA turnover during exercise, plasma palmitate IE decreased during exercise under all conditions and was significantly lower than rest after 15–30 min of exercise (Fig. 2A). Nonetheless, plasma palmitate remained readily detectable (Fig. 2A). Breath ^{13}C O_2 IE decreased from rest during exercise at 65% VO_2 peak but remained unchanged during exercise at 45% VO_2 peak (Fig. 2B).

**Plasma FFA kinetics.** Plasma FFA R_{a} and R_{d} increased approximately threefold from rest to exercise in all conditions and both exercise intensities (Fig. 3, A and B). The plasma FFA R_{a} of FP and LP were not statistically different at rest (FP: 3.17 ± 0.48, LP: 2.94 ± 0.46 µmol·kg\(^{-1}\)·min\(^{-1}\); P = 0.74) or during exercise at 45% VO_2 peak (FP: 12.24 ± 1.16, LP:

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**Fig. 2.** Isotopic enrichment of plasma [\(^{13}\)C]palmitate (A) and breath \(^{13}\)CO_2 (B). MPE, molar percent excess; APE, atom percent excess; BOC, before oral contraceptives. Values are means ± SE; n = 8. Significantly different than *rest at 45% VO_2 peak for all conditions, †rest at 65% VO_2 peak for all conditions, and #corresponding values at 45% VO_2 peak for all conditions except HP at 60 min: P < 0.05.

**Fig. 3.** Effect of exercise intensity and oral contraceptive use on plasma free fatty acid (FFA) rate of appearance (A), plasma FFA rate of disappearance (B), plasma FFA metabolic clearance rate (C), and plasma FFA rate of oxidation (R_{ox}; D). Values are means ± SE of the last 15 and 30 min of rest and exercise, respectively; n = 8. Significantly different than *rest and *corresponding value at 45% VO_2 peak: P < 0.05.

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Fatty acid oxidation. Whole body fatty acid $R_{ao}$ of FP and LP were not statistically different at rest (FP: $3.12 \pm 0.37 \, \text{µmol kg}^{-1} \cdot \text{min}^{-1}$; $P = 0.09$) or during exercise at $45\% \, V\dot{O}_{2\text{peak}}$ (FP: $13.48 \pm 1.24$, LP: $15.44 \pm 1.43 \, \text{µmol kg}^{-1} \cdot \text{min}^{-1}$; $P = 0.53$) or during exercise at $45\% \, V\dot{O}_{2\text{peak}}$ (FP: $8.81 \pm 1.20$, LP: $10.10 \pm 0.78 \, \text{µmol kg}^{-1} \cdot \text{min}^{-1}$; $P = 0.39$) or $65\% \, V\dot{O}_{2\text{peak}}$ (FP: $9.24 \pm 1.09$, LP: $12.19 \pm 1.06 \, \text{µmol kg}^{-1} \cdot \text{min}^{-1}$; $P = 0.09$). No differences were noted in plasma FFA $R_{ao}$ between conditions at rest or during exercise. The percentage of plasma FFA $R_{d}$ oxidized increased from rest to exercise in all conditions and was significantly higher in BOC than IP and HP at rest (36 vs. 26 and 27%), $45\% \, V\dot{O}_{2\text{peak}}$ (69 vs. 55 and 51%), and $65\% \, V\dot{O}_{2\text{peak}}$ (83 vs. 61 and 69%).

Fatty acid reesterification. The extent of fatty acid reesterification is evident as whole body lipolytic rates exceeded whole body fatty acid $R_{ao}$ at rest and during exercise (Fig. 4A). Total and plasma-derived fatty acid $R_{ao}$ increased significantly from rest to exercise in most conditions, whereas local fatty acid $R_{ao}$ did not change from rest to exercise except for IP at $45\% \, V\dot{O}_{2\text{peak}}$ (Table 4, Fig. 4B). A main effect of the treatment was found where total and plasma-derived fatty acid $R_{ao}$ and the percentage of lipolysis reesterified in IP and HP were greater than BOC ($P < 0.02$). Total fatty acid $R_{ao}$ was higher in IP and HP than BOC at 65 and $45\% \, V\dot{O}_{2\text{peak}}$, respectively. Plasma-derived fatty acid $R_{ao}$ was 1.5- to 2-fold higher in IP and HP than BOC at rest and during exercise at 45 and $65\% \, V\dot{O}_{2\text{peak}}$. The increase in plasma FFA $R_{ao}$ from rest to exercise was a function of not only a three- to fourfold increase in whole body lipolytic rate (14) (Fig. 4A) but also a decrease in the proportion of liberated FFA that were reesterified in the transition from rest ($50$–$80\%$) to exercise ($30$–$50\%$). Plasma-derived fatty acid $R_{ao}$ made up a majority of total fatty acid $R_{ao}$ at rest ($63\%$) and during exercise at $45\% \, V\dot{O}_{2\text{peak}}$ ($72\%$), but not $65\% \, V\dot{O}_{2\text{peak}}$ ($46\%$).

### DISCUSSION

As part of our overall effort to describe the effects of exercise intensity and other factors on substrate partitioning, we employed a longitudinal study design to examine the influences of endogenous ovarian hormones and synthetic ovarian hormones contained in oral contraceptives on whole body fatty acid $R_{ao}$ and plasma FFA turnover and $R_{ao}$ at rest and during exercise. The results suggest that neither fluctuations in endogenous ovarian hormones across the normal menstrual cycle nor supplementation with synthetic ovarian hormones through oral contraceptive use exert any measurable influence on whole body fatty acid $R_{ao}$ or plasma FFA turnover or $R_{ao}$ at rest or during moderate-intensity exercise in the 3-h postprandial state when carbohydrate use predominates. Rather, energy flux as determined by exercise intensity had the greatest influence on substrate partitioning. Oral contraceptive use did, however, result in an increase in total and plasma-derived fatty acid $R_{ao}$ and a decrease in the proportion of plasma

<table>
<thead>
<tr>
<th>Variable</th>
<th>Rest BOC</th>
<th>Rest IP</th>
<th>Rest HP</th>
<th>45% BOC</th>
<th>45% IP</th>
<th>45% HP</th>
<th>65% BOC</th>
<th>65% IP</th>
<th>65% HP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total, µmol kg$^{-1}$ min$^{-1}$</td>
<td>3.71±0.24</td>
<td>2.60±0.09‡</td>
<td>2.97±0.15‡</td>
<td>13.79±1.30*</td>
<td>13.80±1.36*</td>
<td>14.45±1.94*</td>
<td>16.10±1.77*</td>
<td>15.32±1.32*</td>
<td>15.92±0.87*</td>
</tr>
<tr>
<td>Plasma FFA, µmol kg$^{-1}$ min$^{-1}$</td>
<td>1.07±0.15</td>
<td>1.01±0.12</td>
<td>1.00±0.13</td>
<td>8.83±1.02*</td>
<td>7.54±0.83*</td>
<td>7.28±0.50*</td>
<td>10.11±1.02*</td>
<td>7.71±1.01*</td>
<td>10.41±0.82†</td>
</tr>
<tr>
<td>Other, µmol kg$^{-1}$ min$^{-1}$</td>
<td>2.64±0.28</td>
<td>1.50±0.15‡</td>
<td>1.98±0.15‡</td>
<td>4.96±0.96</td>
<td>6.26±1.05*</td>
<td>7.37±1.77*</td>
<td>6.00±1.40*</td>
<td>5.81±1.41*</td>
<td>5.51±0.97*</td>
</tr>
<tr>
<td>$R_{ao}$ oxidized, %</td>
<td>36.0±1.7</td>
<td>26.0±1.2‡</td>
<td>27.0±1.4‡</td>
<td>69.2±3.8*</td>
<td>55.2±2.4*</td>
<td>51.1±2.7†‡</td>
<td>83.2±5.1†‡</td>
<td>61.6±4.1‡*</td>
<td>69.5±3.5†‡</td>
</tr>
<tr>
<td>Fatty acid reesterification Total, µmol kg$^{-1}$ min$^{-1}$</td>
<td>2.98±0.36</td>
<td>4.89±0.96</td>
<td>4.54±0.64</td>
<td>6.31±0.85*</td>
<td>7.77±0.54*</td>
<td>10.59±1.52‡</td>
<td>5.62±0.90</td>
<td>11.27±1.52†‡</td>
<td>8.66±1.47*</td>
</tr>
<tr>
<td>% of Lipolysis Local, µmol kg$^{-1}$ min$^{-1}$</td>
<td>49.0±2.4</td>
<td>79.7±6.6</td>
<td>65.0±5.2</td>
<td>34.1±5.1*</td>
<td>40.9±3.9*</td>
<td>45.8±2.8*</td>
<td>28.8±4.9*</td>
<td>45.9±4.1†‡</td>
<td>34.8±3.3†‡</td>
</tr>
<tr>
<td>Plasma-derived, µmol kg$^{-1}$ min$^{-1}$</td>
<td>1.10±0.38</td>
<td>1.91±0.72</td>
<td>1.63±0.54</td>
<td>2.18±0.68</td>
<td>1.50±0.69</td>
<td>3.13±1.47</td>
<td>3.25±0.95</td>
<td>6.48±2.03†‡</td>
<td>4.05±1.28</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 8$ subjects. $R_{ao}$ rate of disappearance significantly different than *rest, †corresponding value at $45\% \, V\dot{O}_{2\text{peak}},$ and ‡BOC: $P < 0.05$. Main effects: IP and HP $R_{ao}$ oxidized < BOC; $P < 0.01$; IP and HP total fatty acid reesterification > BOC, $P < 0.01$; IP and HP % lipolysis reesterified > BOC, $P < 0.02$; IP and HP plasma-derived fatty acid reesterification > BOC, $P < 0.05$. 

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FFA $R_d$ that was oxidized compared with BOC. These results lead to general conclusions regarding 1) the availability and dynamics of individual plasma FFA in women during exercise, 2) the importance of endogenous ovarian hormones in the regulation of human lipid metabolism, and 3) the ability of oral contraceptive use to alter lipid metabolism. These general conclusions are subsequently discussed.

Availability and dynamics of individual plasma FFA in women. For the first time that we are aware, individual plasma FFA concentrations at multiple time points at rest and during controlled exercise bouts in young women are presented. The percent contribution of each individual plasma FFA to the total concentration agrees well with results of studies of men (25, 29, 33, 46, 47) and women (26, 27, 48). The absolute concentrations of the individual plasma FFA at rest agree closely with another report of 3- to 4-h postprandial subjects (47) and are 40–60% lower than seen in overnight fasted subjects (25, 27, 33, 46, 48), likely due to suppression of lipolysis by the prestudy dietary treatment.

The two- to fivefold increase in plasma FFA concentration from rest to exercise in all conditions was accompanied by a significant 21% increase in the plasma U/S in the BOC condition. This rise in the plasma U/S closely resembles the adipose tissue triglyceride U/S of 1.70–3.20 (30, 46–48) and suggests that $\beta$-adrenergic stimulation of lipolysis during exercise results in the liberation of FFA primarily from adipose tissue triglyceride. In fact, Mittendorfer et al. (46) found that the relative increase in individual fatty acid $R_a$ during epinephrine infusion in resting subjects closely matched the relative fatty acid content of adipose tissue triglyceride. Alternatively, a preferential uptake of saturated fatty acids during exercise could result in the observed rise in the plasma U/S. However, the only published study to examine working skeletal muscle individual fatty acid uptake to date has shown that exercising human forearm has a small preference for the uptake of the unsaturated fatty acids oleate and linoleate over that of the saturated fatty acid palmitate (28). This issue needs to be examined in large muscle groups, such as the leg, that are responsible for a greater proportion of whole body fatty acid uptake and oxidation during exercise. Preliminary evidence suggests that relative net leg individual fatty acid uptake during moderate-intensity exercise is proportional to relative individual plasma fatty concentration (Jacobs KA, Krauss RM, Fattor, JA, Horning MA, Friedlander AL, Bauer, T, Hagopian T, Wolfel EE, and Brooks GA, unpublished observation).

The importance of endogenous ovarian hormones in the regulation of human lipid metabolism. Whole body fatty acid $R_{ox}$ was not different between menstrual cycle phases at rest or during 60 min of exercise at either 45 or 65% $\text{VO}_2\text{peak}$. These findings corroborate results of previous investigations that found no effect of menstrual cycle phase on whole body fatty acid $R_{ox}$ during 20–90 min of exercise at 33–90% $\text{VO}_2\text{peak}$ (7, 37, 40, 56). The present report extends the results of those findings by showing for the first time that fluctuations in ovarian hormones across the normal menstrual cycle do not influence plasma FFA turnover, $R_{ox}$, or fatty acid $R_a$ at rest or during moderate-intensity exercise. Heiling and Jensen (34) performed the only other study to date to examine plasma FFA turnover during 60 min of exercise. However, they found no effect of phase on whole body fatty acid $R_{ox}$ and only demonstrated that relative net leg uptake was lower during early follicular phase compared with late follicular phase and luteal phase (34).
difference in whole body fatty acid oxidation (Rox) across the menstrual cycle. Using [9,10-3H]palmitate and [9,10-3H]oleate, they also found that plasma FFA Ra was not different between FP and LP at rest in the overnight fasted state. Additionally, plasma FFA Ra increased similarly in FP and LP during 3 h of somatostatin-induced hypoinsulinemia, indicating that the suppressive effects of insulin on lipolysis are not affected by fluctuations in ovarian hormone concentrations.

The literature is not entirely consistent with respect to the effect of menstrual cycle phase on substrate partitioning. Others (10, 63) have found higher whole body fatty acid oxidation in LP than FP during exercise, and the disparity of these results was initially explained by differences in subjects’ nutritional status. Campbell et al. (10) demonstrated that whole body fatty acid oxidation during 120 min of exercise at 70% V\text{O}_2\text{peak} was 17% higher in LP than FP in the overnight-fasted condition, but this difference was abolished when subjects were fed glucose before and during exercise. However, others have found no difference in whole body fatty acid oxidation across the normal menstrual cycle in overnight-fasted subjects (37), indicating that nutritional status does not completely explain the lack of agreement in the literature. Perhaps more important than the methodological differences between studies is the physiological variability of circulating ovarian hormone concentrations.

In well-controlled studies that have reported circulating ovarian hormone concentrations (3, 7, 10, 37, 56), average midluteal concentrations of plasma estradiol (range of 82–232 pg/ml) and progesterone (range of 8–40 ng/ml) have differed by as much as three- to fivefold among studies.

Due to the potential antiestrogen effects of progesterone, the ratio of circulating estradiol to progesterone concentrations (E/P) rather than absolute concentrations alone should be considered when assessing the potential regulatory roles of ovarian hormones (16, 37). The E/P expressed as a percent increases from ~7–8% in the early FP (3, 37, 56) to ~14–22% in the mid-FP (7, 37) due to a rise in estradiol and no change in progesterone. The E/P then drops to ~0.5–1.5% in the mid-LP due to a much greater increase in progesterone (~2- to 25-fold) than estradiol (~2- to 3-fold) (3, 7, 10, 37, 56). Interestingly, this approach would lead one to predict that the ability of estradiol to promote fatty acid mobilization and oxidation would be most evident in the mid-FP rather than the mid-LP. The E/P seems to explain the results of D’Eon et al. (17), who employed pharmacological control of serum ovarian hormone concentrations in eumenorrheic women and found that whole body fatty acid oxidation during 60 min of exercise at 60% V\text{O}_2\text{peak} was 50% greater under conditions of high estradiol-low progesterone (E/P = 16.5%) than either low estradiol-low progesterone (E/P = 3.6%) or high estrogen-high progesterone (E/P = 4.9%). However, Horton et al. (37) found no difference in whole body fatty acid oxidation during 90 min of exercise at 50% V\text{O}_2\text{peak} in nonpharmacologically controlled eumenorrheic women tested in the early FP, mid-FP, and mid-LP, indicating that the reason for the lack of complete agreement in the literature is likely multifactorial.

The lack of an effect of endogenous ovarian hormones on fatty acid mobilization or oxidation in the present study also does not agree with results of studies using animal models that have manipulated ovarian hormones by supplementing male or ovariectomized female rats with pharmacological doses of estrogen and progesterone. Those studies have found that, compared with treatments containing placebo or progesterone, estrogen treatment alone stimulates adipose lipolysis (31) and increases plasma FFA availability (41) and Ro\text{ox} during prolonged exercise (32). These results may be explained in part by the recent finding that estrogen treatment of ovariectomized female rats increases the expression of several proteins critical to the regulation of skeletal muscle lipid oxidation (11, 12).

The disagreement between human and animal studies is not surprising, given not only the species differences but also the vast differences in circulating ovarian hormone concentrations. Whereas circulating estradiol and progesterone concentrations may range from ~20 to 250 pg/ml and from 0.3 to 40 ng/ml, respectively, between the early FP and mid-LP of the normal human menstrual cycle (3, 7, 10, 37, 56), rats have been exposed to circulating estradiol and progesterone concentrations of ~45–1,700 pg/ml and 8–50 ng/ml, respectively (11, 12). Interestingly, studies on laboratory rodents suggesting that estradiol promotes the expression of proteins integral to fatty acid oxidation employed high-estrogen conditions with an E/P of only 0.6–3% (11, 12).

The ability of oral contraceptive use to alter lipid metabolism. We previously reported that 4 mo of triphasic oral contraceptive use increased whole body lipolytic rate during moderate-intensity exercise (14). The increase in lipolytic rate was not matched by an increase in whole body or plasma FFA oxidation (Fig. 3D and 4A). Whole body fatty acid oxidation at rest and during 60 min of exercise at both 45 and 65% V\text{O}_2\text{peak} was not affected by oral contraceptive use. These findings corroborate results of previous cross-sectional investigations that found no difference in whole body fatty acid oxidation during 30–90 min of exercise at 45–85% V\text{O}_2\text{peak} between women using mono- or multiphasic oral contraceptives for 12–98 mo and those not taking oral contraceptives (4, 6). Hagenfeldt et al. (26) showed that 3 mo of monophasic oral contraceptive use did not affect olate turnover at rest. The present report extends those findings by showing for the first time that oral contraceptive use does not influence plasma FFA turnover or Ro\text{ax} at rest or during moderate-intensity exercise.

To date, there are no definitive studies regarding the methodology used to quantify fatty acid reesterification. Most reports provide estimates of fatty acid reesterification based on equations first advanced by Wolfe at al. (62). Here we propose the following equations first advanced by Wolfe et al. (62):

\[
\text{Fatty acid reesterification} = \frac{\text{Local}}{\text{Plasma-derived}}
\]

Fig. 5. Model of the effect of exercise intensity on the partitioning of FA reesterification. Total FA rate of reesterification (R\text{s}) was derived from the difference between lipolytic rate (3 times glyceral rate of appearance) and whole body FA Ro\text{ox}. Local FA R\text{s} represents the reesterification of FAs that never left their site of origin. Plasma-derived R\text{s} represents the reesterification of FAs that exited their site of origin and were reesterified in another tissue (adipose, liver, or skeletal muscle).

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a slight modification of these equations to account for the reesterification of fatty acids within skeletal muscle that originate from the hydrolysis of IMTG and never entered the plasma pool. We feel that this modification is justified given the significant role of nonhepatic tissues, such as skeletal muscle, in the reesterification of fatty acids at rest (59), during a prolonged fast (39), and during exercise (24, 59).

Few reports exist concerning the dynamics of fatty acid reesterification during exercise. Total fatty acid $R_o$ of BOC at rest and during exercise (Table 4, Fig. 4B) agree well with previously reported values in 4- to 6-h postprandial women (22) and are $\sim 25$–50% lower than those reported in 12-h-fasted men (59, 62). Interestingly, it appears that exercise intensity plays a role in the partitioning of fatty acid reesterification (Fig. 5). The increase in total fatty acid $R_o$ from rest to exercise was primarily due to an increase in plasma-derived fatty acid $R_o$ and a majority of reesterification was plasma derived at rest and during exercise at 45% $\dot{V}O_2$ peak. However, plasma-derived fatty acid $R_o$ and its proportion of total fatty acid $R_o$ were lower at 65 than 45% $\dot{V}O_2$ peak in BOC, IP, and HP. Local fatty acid $R_o$ and its proportion to total fatty acid $R_o$, on the other hand, tended to be higher at 65 than 45% $\dot{V}O_2$ peak in all conditions and reached statistical significance in IP. These findings are supported by previous findings that plasma-derived fatty acid $R_o$ increases during 240 min of exercise at 40% $\dot{V}O_2$ peak (62), whereas local fatty acid $R_o$ increases during 60 min of exercise at 60% $\dot{V}O_2$ peak (59) in 12-h-fasted men. It has been suggested that local fatty acid $R_o$ is primarily regulated by the capacity to transport liberated FFA, which is a function of both adipose tissue blood flow and adequate albumin binding sites (62). Therefore, the shift in fatty acid $R_o$ from plasma-derived to local $R_o$ with increasing exercise intensity may be the result of a reduction in adipose tissue blood flow and/or albumin binding site availability.

The mismatch between the increase in whole body lipolytic rate during exercise (14) and the unchanged whole body and plasma FFA $R_{ox}$ with oral contraceptive use implicates an increase in total and plasma-derived $R_o$. Whether synthetic ovarian hormones stimulate adipose triglyceride lipolysis directly or indirectly is difficult to determine in the present study. It appears that, if the effect is indirect, it may be due to an increase in plasma cortisol with oral contraceptive use, as the other regulators of adipose triglyceride lipolysis (insulin, growth hormone, epinephrine, norepinephrine) were not different among BOC, IP, and HP. Cortisol on its own appears to provide most of the laboratory support for this study. We also thank Anne L. Friedlander for insightful advice related to the modification of the fatty acid reesterification equations.

Summary and conclusions. Endogenous ovarian hormones and exogenously administered synthetic hormones found in low-dose oral contraceptives do not exert a measurable influence on whole body fatty acid $R_{ox}$ or plasma FFA turnover or $R_{ox}$ at rest or during moderate-intensity exercise in the 3-h postprandial state when carbohydrate use predominates. The increase in whole body lipolytic rate during exercise induced by oral contraceptive use was not matched by an increase in fatty acid oxidation and instead resulted in an increase in reesterification. We conclude that the hierarchy of regulators of fatty acid oxidation is as follows: exercise intensity $>$ recent carbohydrate nutrition $>$ synthetic $>$ endogenous ovarian hormones.

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