Effects of oral contraceptives on glucose flux and substrate oxidation rates during rest and exercise

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SYNTHETIC STEROIDS COMMONLY used as oral contraceptives (OC) have been reported to alter glucose metabolism and insulin sensitivity in resting women (15, 26, 29, 38). These alterations in glucose metabolism induced by OC seem, in part, to be related to dose and type of OC (30, 37). For example, daily high-dose OC (50–150 μg ethinylestradiol, >1.0 mg progestin) has been associated with decreased glucose tolerance as evidenced by increased blood glucose and plasma insulin levels after an oral glucose load (15, 38), whereas low-dose monophasic or triphasic OC has been associated with lesser hyperinsulinemia (14). Studies of the individual steroid components suggest that the ethinyl estradiol in OC has little effect on circulating glucose or insulin levels, but the progestogen content of OC alters glucose tolerance in resting women (30). In addition, alterations in glucose tolerance were observed depending on type of progestogen used in OC (37). Studies on laboratory rodents have shown that estradiol treatment improves glucose tolerance by increasing insulin sensitivity of glucose uptake, whereas progesterone counteracts the influence of estradiol by decreasing insulin sensitivity of glucose uptake (7, 23).

A few well-controlled cross-sectional studies have investigated the influence of OC on substrate utilization in exercising women (1, 4). Bonen and associates (4) observed increases in free fatty acid and decreases in glucose levels during rest and exercise in women taking one of three different low-dose OCs for at least 1 year. Results of that study were interpreted to indicate that a shift toward lipid metabolism during mild exercise by skeletal muscle occurs in OC users compared with normally menstruating women. However, they observed no significant difference during rest and exercise between OC and control group in respiratory exchange ratio (RER) 3 h postprandial. More recently, Bemben et al. (1) observed significantly lower blood glucose levels in OC users during exercise at 50% of peak O2 uptake (VO2 peak). However, unlike Bonen et al. (4), Bemben et al. (1) found a significant decrease in RER during mild exercise in 8-h postabsorptive OC users. To our knowledge, there are no published reports that used stable isotope tracers to measure the influence of OC on glucose flux rates during rest and exercise in humans. Therefore, we undertook a longitudinal study on eight young women to test the hypothesis that synthetic steroids in OC would decrease blood glucose flux during exercise.

METHODS

Subjects. Eight healthy, moderately active women between the ages of 22 and 30 yr with normal menstrual cycles (24–32 days) were recruited from the University of California, Berkeley campus, community by posted notices and E-mail. Subjects exercised 2–6 h/wk (3.7 ± 0.1 h/wk) in activities such as weight training, walking, cycling, swimming, and surfing but were not elite endurance athletes. All subjects were nulliparous, reported having normal menstrual flows.

Suh, Sang-Hoon, Gretchen A. Casazza, Michael A. Horning, Benjamin F. Miller, and George A. Brooks. Effects of oral contraceptives on glucose flux and substrate oxidation rates during rest and exercise. J Appl Physiol 94: 285–294, 2003. First published September 20, 2002; 10.1152/japplphysiol.00693.2002.—We examined the effects of oral contraceptives (OC) on glucose flux and whole body substrate oxidation rates during rest (90 min) and two exercise intensities [60-min leg ergometer cycling at 45 and 65% peak O2 uptake (VO2 peak)]. Eight healthy, eumenorrheic women were studied during the follicular and luteal phases before OC and the inactive and high-dose phases after 4 mo of a low-dose, triphasic OC. Subjects were studied in the morning 3 h after a standardized (308 kcal) breakfast. There were significant reductions in glucose rates of appearance and disappearance during rest and exercise in humans. Therefore, we measure the influence of OC on glucose flux rates during rest or exercise. These results are interpreted to mean that, in women fed several hours before study, 1) OC decreases glucose flux, but not overall carbohydrate and lipid oxidation rates during moderate-intensity exercise; and 2) synthetic ovarian hormone analogs in the doses contained in OC have greater metabolic effects on glucose metabolism during exercise than do endogenous ovarian hormones.

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(for at least 6 mo), had not taken OC, and had not experi-
enced large weight, activity, or diet changes within the last 6
mo. Subjects had a percent body fat between 19 and 25%, a
$\dot{V}O_2$ peak between 38 and 54 ml·kg$^{-1}$·min$^{-1}$, normal lung
function (forced expiratory volume in 1 s of 70% or more) and
were injury and disease free as determined by health history
questionnaire and physical examination. Subjects provided
informed consent, and the study protocol was approved by
the University of California Committee for the Protection of
Human Subjects (CPHS 2000-8-30).

In our companion report using the same normally cycling
subjects on a controlled diet before taking OC (35), we found
no effect of menstrual phase on blood metabolite concentra-
tions or glucose flux rates. Therefore, data from the previous
report were pooled for use as comparisons to data obtained
on the same women taking OC.

Experimental design. After the initial screening interview
and physical examination, to determine $\dot{V}O_2$ peak subjects
performed continuous graded exercise tests in randomized
order in each phase of the menstrual cycle before starting the
OC treatment. Subjects were subsequently tested during the
effectively conducted early follicular (FP) and midluteal (LP) phases of the men-
cstral cycle before OC. Follicular phase testing occurred 3–9
days after the start of menses, when ovarian hormones are
low. Luteal phase testing occurred between days 18 and 24
after the start of menses and 4–9 days after ovulation. We
waited until 4–9 days after ovulation to test the subjects
when both ovarian hormones were high. Urinary luteinizing
hormone (LH) levels were measured with urine ovulation
kits (First Response, Carter Products) starting at day 10
after the start of menses until a positive test was achieved.
A positive test result indicated the surge in LH that occurred
–
within 48 h. Cycle phases were later con
fi
ed calibration gas (16% O2 and 4% CO2). Heart rate
was monitored continuously by a Quinton 759 electrocardio-
graph and blood pressure by stethoscope and sphygmoma-
nometer. $\dot{V}O_2$ peak tests were accepted as maximal if heart
rate was within 10% of predicted and RER values exceeded
1.1. The second screening test was done to ensure reliability
of the measures and evaluate the possibility of a menstrual
cycle phase effect on $\dot{V}O_2$ peak. Subjects were instructed to
maintain diet and physical activity level throughout the
entire experimental period. Body composition was deter-
mined by skinfold measurement (six skinfold sites with a
Harpenden skinfold caliper) (18). Three-day diet records
were collected twice before and with OC use to assess dietary
intake, physical activity, and body weight. We evaluated dietary and
nutrient composition. Analysis of dietary records was per-
formed by using the Nutritionist III program (N-squared
Computing, Salem, OR). Because of overlaps in subject cy-
cles, it was not always possible to conduct screening and
fluorescent trials in the same menstrual cycle.

Tracer protocol. Subjects were studied in a postabsorptive
state in the morning, and dietary intake was controlled for
the 24 h immediately preceding each of the eight isotope
tracer trials. Subjects rested the day before tracer trials and
were given a standardized daily diet [2,183 kcal; 63% carbo-
hydrate (CHO), 15% protein, 22% fat] to consume the day
before trials. As well, subjects took a standardized breakfast
(308 kcal; 75% CHO, 16% protein, 9% fat; cereal, milk, and
apple juice) in the laboratory 3 h before 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, as well as to mimic conditions in a nonlaboratory
environment. On the morning of the trial, a catheter was
placed in a hand or wrist vein to obtain “arterialized” blood
samples using the “heated hand vein” technique, and a fore-
arm venous catheter was placed in the contralateral arm for
continuous infusion of tracers. In previous studies in our
laboratory (12), arterial and arterialized blood glucose isoto-
opic enrichments in samples drawn simultaneously were not
different. After collection of background blood and expired
gas samples, a priming bolus of [6,6-2H]glucose (D2-glucose),
which was 125 times the resting minute infusion rate, was
given, and the subjects rested supine or semisupine for 90
min while the D2-glucose was continuously infused (Baxter
Travenol 6300 infusion pump). The glucose tracer was in-
fused at 1.6 mg/min. Infusion rates were increased to 4.8
mg/min and 6.4 mg/min during exercise at 45% and 65%
$\dot{V}O_2$ peak, respectively.

The isotope tracer infusion rates employed have been pre-
viously demonstrated by our laboratory to maintain stable
plasma isotopic enrichment for the measurement of sub-
strate kinetics throughout rest and the two exercise intensi-
ties (12, 13). Isotope tracers were obtained from Cambridge
Isotope Laboratories (Woburn, MA), diluted in 0.9% sterile
saline, pharmacologically tested for sterility and pyrogenicity
(University of California, San Francisco, School of Phar-
macy), and on the day of the experiment passed through a
0.2-μm Millipore filter (Nalgene, Rochester, NY) before infusion.

Blood sampling and analysis. Blood samples were taken at
0, 60, 75, and 90 min of rest and at 15, 30, 45, and 60 min of

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exercise. Blood samples for glucose isotopic enrichment and glucose and lactate concentrations were collected in an 8% perchloric acid solution and thoroughly mixed before centrifugation. Blood samples for determination of insulin and glucagon levels were collected in heparinized syringes, transferred to sterile vacutainers containing EDTA, and mixed before centrifugation. Aprotinin (4 mg/ml of blood) was added to the blood aliquot reserved for the determination of glucagon to prevent cross-reaction of glucagon fragments arising from proteolytic degradation. Samples were immediately chilled on ice before centrifugation at 2,800 g for 13 min. Supernatants were stored at either –20 or –80°C until analysis. Blood glucose (hexokinase, Sigma Chemical, St. Louis, MO) and lactate concentrations (lactate dehydrogenase; Ref. 16) were determined enzymatically. Plasma hormone concentrations were determined by 125I radioimmunoassay (Coat-A-Count kits; Diagnostic Products, Los Angeles, CA). Samples obtained from each subject in all trials were analyzed together. The intra-assay coefficients of variation ranged from 2 to 5%, and the sensitivities of the assays were 2.9 pmol/l for estradiol, 0.06 nmol/l for progesterone, 1.2 μIU/ml for insulin, and 13 pg/ml for glucagon. Hematocrit was measured at each sampling point by using a circular microcapillary tube reader (no. 2201, International Equipment) to ensure that metabolite concentrations and isotopic enrichments were not affected by changes in plasma volume. Subjects drank tap water ad libitum during each trial to maintain hydration status.

Isotopic enrichment analysis. Glucose isotopic enrichments were determined by using gas chromatography-mass spectrometry (GCMS) (GC model 5890 series II and MS model 5989A, Hewlett-Packard) of the penta-acetate derivative. In preparation for GCMS analysis, samples were neutralized with 2 N KOH and transferred to cation- (AG 50W-X8, 50–100 mesh H+ resin) and anion- (AG 1-X8, 100–200 mesh formate resin) exchange columns, and the glucose was eluted with deionized water. Samples were then lyophilized, resuspended in methanol, and transferred to a 1-ml GCMS vial. One hundred microliters of 2.1 acetic anhydride-pyridine solution were added to each vial, and each was heated at 60°C for 10 min. Samples were subsequently dried under nitrogen, resuspended in 100 μl of ethylacetate, and transferred to GCMS vials for analysis. For GCMS 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 35.1 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 with the use of methane gas, and selected ion monitoring was used to monitor ions mass-to-charge ratios 351.20 and 333.20 for [13C]glucose and D2-glucose, respectively.

Calculations. Glucose rates of appearance (Ra) and disappearance (Rd) and metabolic clearance rate (MCR) were calculated by using equations defined by Steele and modified for use with stable isotopes (34)

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

\[
Rd (mg \cdot kg^{-1} \cdot \text{min}^{-1}) = Ra - V[(C_2 - C_1)/(t_2 - t_1)]
\]

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

where F represents the isotopic infusion rate; V is the estimated volume distribution of glucose (180 ml/kg); C1 and C2 are concentrations at sampling times t1 and t2, respectively; and IE1 and IE2 are the glucose isotopic enrichments of D2-glucose at sampling times t1 and t2, respectively. Values for isotopic enrichment were corrected for baseline enrichments from background blood samples taken before infusion of the isotopes. Energy derived from total CHO, lipid, and glucose oxidation was calculated as described by Frayn (11)

\[
\%\text{Energy from CHO} = \frac{[\text{RER} - 0.707]}{0.293} \times 100
\]

\[
\%\text{Energy from lipid} = 100 - \frac{[\text{RER} - 0.707]}{0.293} \times 100
\]

Energy from CHO oxidation (kcal/min)

\[
=[(\% \text{CHO/100}) \times (V_{O2})] \times (5.05 \text{kcal/l O}_2)
\]

Energy from lipid oxidation (kcal/min)

\[
=[(1 - \% \text{CHO/100}) \times (V_{O2})] \times (4.7 \text{kcal/l O}_2)
\]

Energy expenditure (kcal/min)

\[
=[(\% \text{CHO/100}) \times (V_{O2}) \times (5.05 \text{kcal/l O}_2)]+
\[(1 - \% \text{CHO/100}) \times (V_{O2}) \times (4.7 \text{kcal/l O}_2)]
\]

where O2 uptake (V O2) is in liters per minute and body weight is in kilograms.

Statistics. Data are presented as means ± SE. Representative values for metabolite concentrations and glucose kinetics were averaged from the final 15 min (75, 90 min) of rest and 30 min (30, 45, 60 min) of exercise. Descriptive efforts to control prior activity and diet and to standardize time of day and menstrual cycle phase, cell sizes in ANOVA before OC varied because endocrine status criteria were not always met, mainly because of inconsistencies in progesterone rise after LH surge. Because there were no significant differences between resting values for the four trials in each phase before and with OC, the resting values were pooled to obtain one FP and one LP value before OC, and one IP and one HP value with OC. Additionally, for parameters in which there was no menstrual phase difference, FP and LP values were pooled to obtain a single before-OC value to compare with IP and HP values. Significance of differences among mean values in physical characteristics was determined by one-way ANOVA with repeated measures, followed by multiple comparisons (S-Plus 2000, Professional Release 2). Significance of differences among mean values representing metabolite and hormone concentrations as well as flux rates determined in the eight conditions were determined by using two-way ANOVA with repeated measures, followed by multiple comparisons. Statistical significance of mean differences was set at α = 0.05.

RESULTS

Subject characteristics. Physical characteristics of subjects before and with OC are listed in Table 1. Before OC use, subjects were weight stable with no changes in percent body fat, V O2 peak or lactate threshold between FP and LP (35). However, there was a small but significant increase in weight and percent body fat with OC (P < 0.05) (9). In addition, V O2 peak decreased (P < 0.05) 13–15% with OC, both in weight-corrected and uncorrected terms (9). Ergometric and physiological parameters at rest and during exercise at 45% and 65% V O2 peak are presented in Tables 2, 3, and 4, respectively. At rest and during exercise at either
intensity, there were no significant phase or OC effects on any of the variables in Tables 2–4, except for \( \dot{V}_{O_2}\text{peak} \) and for hematocrit in 45% trials. Before and with OC, subjects exercised at the same absolute workload; this resulted in them exercising at a greater percentage of their \( \dot{V}_{O_2}\text{peak} \) after 4 mo of OC, because of the fact that there was a significant decrease in \( \dot{V}_{O_2}\text{peak} \) with OC. Hematocrit decreased significantly only in 45% trials with OC. There were significant increases in \( V_{O_2} \), \( \dot{V}_{O_2}\text{peak} \), energy expenditure, energy from CHO and lipid, pulmonary minute ventilation, heart rate, and systolic blood pressure because of exercise at any intensity (\( P < 0.05 \)). Furthermore, significant intensity effects were observed on these variables, except for energy from lipid.

**Ovarian hormone concentrations.** As explained above, measured estradiol and progesterone values did not always meet the criteria values for FP and LP; hence, the number of subjects in FP and LP is less than eight. As well, subject numbers and hormone levels are slightly different than in our companion report (9) because \( \dot{V}_{O_2}\text{peak} \) assessment and isotope tracer trials could not always be determined during the same menstrual cycle. With the administration of OC, endogenous estradiol production was suppressed. Estradiol concentrations were lower at rest and during exercise with OC administration (FP vs. FP, \( P < 0.05 \)). In addition, compared with IP, estradiol and progesterone concentrations were lower in LP, with estradiol concentrations significantly lower at rest and during exercise of either intensity in HP (\( P < 0.05 \)). Estradiol and progesterone concentrations during rest and exercise of different intensities were significantly higher in LP compared with FP, IP, and HP (\( P < 0.05 \), Table 5). Increases in estradiol during exercise were intensity dependent in FP, LP, and IP (\( P < 0.05 \), Table 5). The overall pattern of response to exercise was similar in the four phases before and with OC.

**Blood glucose and lactate concentrations.** Blood glucose concentrations fell slightly (5–12%) in response to exercise (Fig. 1A). However, neither before nor with OC were changes significantly different among exercise conditions (Fig. 1A).

Blood lactate concentrations increased in the transition between rest and exercise of both intensities in all

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before OC</th>
<th>With OC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FP</td>
<td>LP</td>
</tr>
<tr>
<td>n</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Age, yr</td>
<td>24.9 ± 1.4</td>
<td>26.0 ± 1.8</td>
</tr>
<tr>
<td>Height, cm</td>
<td>165.6 ± 1.6</td>
<td>165.9 ± 2.2</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>61.4 ± 1.1</td>
<td>60.2 ± 1.4</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>23.3 ± 1.6</td>
<td>21.4 ± 1.5</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>14.4 ± 0.8</td>
<td>12.9 ± 0.7</td>
</tr>
<tr>
<td>Lean body mass, kg</td>
<td>47.0 ± 0.8</td>
<td>47.3 ± 1.1</td>
</tr>
<tr>
<td>( \dot{V}_{O_2}\text{peak} ), ml·kg⁻¹·min⁻¹</td>
<td>44.2 ± 1.8</td>
<td>42.9 ± 2.1</td>
</tr>
<tr>
<td>( \dot{V}_{CO_2}\text{peak} ), ml·kg⁻¹·min⁻¹</td>
<td>2.7 ± 0.1</td>
<td>2.5 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = \) no. of subjects; OC, oral contraceptives; FP, follicular phase; LP, luteal phase; IP, inactive phase; HP, high-dose phase; \( \dot{V}_{O_2}\text{peak} \), peak oxygen consumption. *Significantly different between FP and IP, \( P < 0.05 \); †significantly different between LP and HP, \( P < 0.05 \); ‡significantly different between FP and LP, \( P < 0.05 \); §significantly different between LP and IP, \( P < 0.05 \). Note: data on physical characteristics before OC (35) and OC effect on \( \dot{V}_{O_2}\text{peak} \) (9) have been previously reported.

### Table 2. Physiological parameters of subjects at rest before and with 4 mo of OC

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before OC</th>
<th>With OC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FP</td>
<td>LP</td>
</tr>
<tr>
<td>n</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>( V_{O_2} ), ml·kg⁻¹·min⁻¹</td>
<td>4.0 ± 0.1</td>
<td>4.0 ± 0.1</td>
</tr>
<tr>
<td>( \dot{V}_{CO_2} ), ml·kg⁻¹·min⁻¹</td>
<td>3.4 ± 0.1</td>
<td>3.5 ± 0.1</td>
</tr>
<tr>
<td>RER</td>
<td>0.85 ± 0.02</td>
<td>0.88 ± 0.01</td>
</tr>
<tr>
<td>EE, kcal/min</td>
<td>12 ± 0.0</td>
<td>12 ± 0.0</td>
</tr>
<tr>
<td>Energy from CHO, kcal/min</td>
<td>0.6 ± 0.1</td>
<td>0.7 ± 0.0</td>
</tr>
<tr>
<td>Energy from lipid, kcal/min</td>
<td>0.6 ± 0.1</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Minute ventilation, l/min</td>
<td>7 ± 0</td>
<td>8 ± 0</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>64 ± 2</td>
<td>67 ± 3</td>
</tr>
<tr>
<td>Blood pressure, mmHg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>108 ± 2</td>
<td>107 ± 2</td>
</tr>
<tr>
<td>Diastolic</td>
<td>69 ± 2</td>
<td>69 ± 2</td>
</tr>
<tr>
<td>Mean arterial</td>
<td>82 ± 2</td>
<td>81 ± 2</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>41 ± 1</td>
<td>41 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( V_{O_2} \), oxygen consumption; \( V_{CO_2} \), carbon dioxide production; RER, respiratory exchange ratio; EE, energy expenditure; CHO, carbohydrate; bpm, beats/min. Note: data on subjects before OC were previously reported (35).
conditions before and with OC in an intensity-dependent manner and remained elevated throughout the exercise in all conditions (Fig. 1B). The increase in blood lactate concentrations during exercise of either intensity was significant in all conditions before and with OC ($P < 0.05$), but there was no significant phase or OC effect on blood lactate response during rest or exercise of either intensity.

**Blood glucose kinetics.** Glucose $R_a$ increased significantly during exercise, for all eight exercise conditions compared with rest ($P < 0.05$), and values are presented as the average of the last 15 min of rest and 30 min of exercise (rest $< 45\% < 65\% V_{O2peak}$, Fig. 2A). Glucose $R_a$ fell during rest and exercise of both intensities in response to OC. Compared with before OC, there was 11% fall in glucose $R_a$ with OC during rest. Additionally, glucose $R_a$ decreased significantly (16 and 20%) during exercise at 45 and 65% $V_{O2peak}$ with OC, respectively ($P < 0.05$). There were no significant differences in glucose $R_d$ between IP and HP during rest or exercise of either intensity.

Responses of $R_a$ (Fig. 2B) to rest and exercise in all phases before and with OC were similar to those of $R_a$ except that there was no significant difference in glucose $R_d$ during exercise at 45% $V_{O2peak}$ between OC and IP. The similarity between our glucose $R_a$ and $R_d$ is consistent with the observed stable glucose concentrations and isotopic enrichments during exercise. The MCR of glucose (Fig. 2C) was similar to glucose $R_d$ among the eight trials except that there was no signific-

### Table 3. Ergometric and physiological parameters of subjects during exercise at 45% $V_{O2peak}$ before and with 4 mo of OC

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before OC</th>
<th>With OC</th>
<th>Before OC</th>
<th>With OC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FP</td>
<td>LP</td>
<td>IP</td>
<td>HP</td>
</tr>
<tr>
<td>n</td>
<td>7</td>
<td>6</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Workload, W</td>
<td>59.1 ± 5.8</td>
<td>58.2 ± 5.2</td>
<td>58.3 ± 5.2</td>
<td>58.3 ± 5.2</td>
</tr>
<tr>
<td>% $V_{O2peak}$</td>
<td>46.4 ± 0.6</td>
<td>47.8 ± 1.1</td>
<td>51.9 ± 1.3*</td>
<td>50.7 ± 1.1</td>
</tr>
<tr>
<td>$V_{O2}$, ml·kg$^{-1}$·min$^{-1}$</td>
<td>20.2 ± 0.9*</td>
<td>19.5 ± 1.2*</td>
<td>18.8 ± 0.8*</td>
<td>18.9 ± 1.0*</td>
</tr>
<tr>
<td>$VCO_2$, ml·kg$^{-1}$·min$^{-1}$</td>
<td>18.0 ± 0.9*</td>
<td>17.4 ± 1.0*</td>
<td>16.8 ± 0.6*</td>
<td>16.7 ± 0.8*</td>
</tr>
<tr>
<td>RER</td>
<td>0.90 ± 0.01</td>
<td>0.90 ± 0.01</td>
<td>0.90 ± 0.01</td>
<td>0.89 ± 0.01</td>
</tr>
<tr>
<td>EE, kcal/min</td>
<td>6.1 ± 0.3*</td>
<td>5.7 ± 0.3*</td>
<td>5.8 ± 0.3*</td>
<td>5.7 ± 0.3*</td>
</tr>
<tr>
<td>Energy from CHO, kcal/min</td>
<td>4.1 ± 0.3*</td>
<td>3.7 ± 0.2*</td>
<td>3.7 ± 0.2*</td>
<td>3.5 ± 0.1*</td>
</tr>
<tr>
<td>Energy from lipid, kcal/min</td>
<td>2.0 ± 0.1*</td>
<td>2.0 ± 0.4*</td>
<td>2.0 ± 0.2*</td>
<td>2.2 ± 0.3*</td>
</tr>
<tr>
<td>Minute ventilation, l/min</td>
<td>29 ± 1*</td>
<td>29 ± 1*</td>
<td>28 ± 1*</td>
<td>29 ± 1*</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>131 ± 4*</td>
<td>132 ± 3*</td>
<td>133 ± 4*</td>
<td>133 ± 4*</td>
</tr>
<tr>
<td>Blood pressure, mmHg</td>
<td>Systolic</td>
<td>130 ± 3*</td>
<td>129 ± 3*</td>
<td>127 ± 4*</td>
</tr>
<tr>
<td></td>
<td>Diastolic</td>
<td>68 ± 4</td>
<td>65 ± 2</td>
<td>60 ± 3</td>
</tr>
<tr>
<td></td>
<td>Mean arterial</td>
<td>89 ± 3</td>
<td>86 ± 2</td>
<td>82 ± 3</td>
</tr>
<tr>
<td></td>
<td>Hematocrit, %</td>
<td>43 ± 1</td>
<td>41 ± 1</td>
<td>39 ± 1b,e</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significantly different from resting conditions; $P < 0.05$; †significantly different between FP and IP, $P < 0.05$; ‡significantly different between LP and HP, $P < 0.05$; ‡significantly different between FP and HP, $P < 0.05$; ‡significantly different between FP and LP, $P < 0.05$. Note: data on subjects before OC were previously reported (35).

### Table 4. Ergometric and physiological parameters of subjects during exercise at 65% $V_{O2peak}$ before and with 4 mo of OC

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before OC</th>
<th>With OC</th>
<th>Before OC</th>
<th>With OC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FP</td>
<td>LP</td>
<td>IP</td>
<td>HP</td>
</tr>
<tr>
<td>n</td>
<td>7</td>
<td>5</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Workload, W</td>
<td>97.9 ± 6.8†</td>
<td>97.5 ± 6.6†</td>
<td>97.5 ± 6.7†</td>
<td>97.6 ± 6.7†</td>
</tr>
<tr>
<td>% $V_{O2peak}$</td>
<td>68.0 ± 0.8†</td>
<td>67.5 ± 0.8†</td>
<td>73.2 ± 1.3†</td>
<td>72.4 ± 1.9†</td>
</tr>
<tr>
<td>$V_{O2}$, ml·kg$^{-1}$·min$^{-1}$</td>
<td>29.4 ± 1.3†</td>
<td>28.7 ± 1.9†</td>
<td>26.8 ± 1.5†</td>
<td>26.9 ± 1.3†</td>
</tr>
<tr>
<td>$VCO_2$, ml·kg$^{-1}$·min$^{-1}$</td>
<td>27.2 ± 1.2†</td>
<td>26.8 ± 1.9†</td>
<td>25.2 ± 1.4†</td>
<td>25.0 ± 1.4†</td>
</tr>
<tr>
<td>RER</td>
<td>0.93 ± 0.01</td>
<td>0.93 ± 0.02</td>
<td>0.94 ± 0.01</td>
<td>0.93 ± 0.01</td>
</tr>
<tr>
<td>EE, kcal/min</td>
<td>8.9 ± 0.4†</td>
<td>8.5 ± 0.4†</td>
<td>8.9 ± 0.5†</td>
<td>8.3 ± 0.5†</td>
</tr>
<tr>
<td>Energy from CHO, kcal/min</td>
<td>6.7 ± 0.2†</td>
<td>6.7 ± 0.7†</td>
<td>6.7 ± 0.4†</td>
<td>6.4 ± 0.5†</td>
</tr>
<tr>
<td>Energy from lipid, kcal/min</td>
<td>2.2 ± 0.2*</td>
<td>1.8 ± 0.5*</td>
<td>1.6 ± 0.3*</td>
<td>1.9 ± 0.3*</td>
</tr>
<tr>
<td>Minute ventilation, l/min</td>
<td>47 ± 1*</td>
<td>47 ± 3*</td>
<td>46 ± 2*</td>
<td>46 ± 2*</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>169 ± 3†</td>
<td>169 ± 5†</td>
<td>167 ± 4†</td>
<td>167 ± 4†</td>
</tr>
<tr>
<td>Blood pressure, mmHg</td>
<td>Systolic</td>
<td>143 ± 4†</td>
<td>137 ± 2*</td>
<td>141 ± 5†</td>
</tr>
<tr>
<td></td>
<td>Diastolic</td>
<td>62 ± 2</td>
<td>66 ± 5</td>
<td>59 ± 2</td>
</tr>
<tr>
<td></td>
<td>Mean arterial</td>
<td>89 ± 1</td>
<td>89 ± 4</td>
<td>87 ± 3*</td>
</tr>
<tr>
<td></td>
<td>Hematocrit, %</td>
<td>43 ± 0</td>
<td>43 ± 1</td>
<td>40 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± SE. †Significantly different from resting conditions, $P < 0.05$; ‡significantly different from 45% trials, $P < 0.05$. Note: Data on subjects before OC were previously reported (35).
Table 5. Menstrual status and ovarian hormone concentrations before and with 4 mo of OC

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before OC</th>
<th>Rest</th>
<th>Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FP</td>
<td>LP</td>
<td>FP45</td>
</tr>
<tr>
<td>n</td>
<td>7</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Day of cycle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days past LH surge</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estradiol, pg/ml</td>
<td>27.06 ± 3.69</td>
<td>84.74 ± 5.77</td>
<td></td>
</tr>
<tr>
<td>Progesterone, ng/ml</td>
<td>0.39 ± 0.05</td>
<td>10.89 ± 1.46</td>
<td></td>
</tr>
</tbody>
</table>

| With OC                  |           |      |          |      |      |      |
| IP                       | 8         | 8    | 8        | 8   | 8    | 8    |
| HP                       |           |      |          |      |      |      |
| Estradiol, pg/ml         | 23.53 ± 4.43 | 13.38 ± 1.97 |
| Progesterone, ng/ml      | 0.29 ± 0.04 | 0.30 ± 0.04 |

Values are means ± SE. 45 and 65, exercise intensities, in % of VO2peak; LH, luteinizing hormone. *Significantly different from resting conditions, P < 0.05; b significantly different from 45% trials, P < 0.05; c significantly different between FP and LP at rest, 45%, or 65% trials, P < 0.05; f significantly different between FP and LP at rest, 45%, or 65% trials, P < 0.05; g significantly different between FP and LP at rest, 45%, or 65% trials, P < 0.05; h significantly different between LP and IP at rest, 45%, or 65% trials, P < 0.05. Note: data on subjects before OC were previously reported (35).

Fig. 1. Effect of oral contraceptives (OC) and exercise intensity on blood glucose (A) and lactate (B) concentrations. Square brackets denote concentration. Values are means ± SE of last 15 and 30 min for rest and exercise, respectively, for 5–7 women before OC (BOC) and for 8 women in all conditions with OC (WOC). IP, inactive phase; HP, high-dose phase; 45 and 65, exercise intensities in % of peak O2 uptake (VO2peak). See text for explanation of subject exclusion criteria. *Significantly different from resting conditions, P < 0.05; b significantly different from 45% trials, P < 0.05.

Significant difference in glucose MCR during exercise at 45% VO2peak between before OC and HP.

Insulin, glucagon, and insulin-to-glucagon ratio. At rest, there were significant increases in insulin concentrations in both IP and HP with OC, compared with before OC (P < 0.05, Fig. 3A). In all isotope trials, insulin concentrations decreased significantly in response to exercise compared with resting values (P < 0.05). There was a significant exercise intensity effect before OC (P < 0.05, rest > 45% > 65% VO2peak), but no significant phase, OC, or intensity effect was observed in other exercise conditions.

Glucagon concentrations increased significantly during exercise at 45% VO2peak in HP and during exercise at 65% VO2peak before and with OC (P < 0.05, Fig. 3B).

Insulin/glucagon ratio decreased significantly between rest and exercise in all phases before and with OC (Fig. 3C, P < 0.05). There was a phase effect in 45% trials before OC (P < 0.05, FP > LP), and an intensity effect was observed in FP (P < 0.05, rest > 45% > 65% VO2peak). However, no significant phase, OC, or intensity effect was observed in other conditions.

RER and substrate oxidation. There was an increase in RER in the transition between rest and exercise of both intensities in all phases, except for the 45% trials with OC administration (Tables 2–4). Values for RER during exercise were higher for the 65% trials compared with the 45% trials in all phases, but these values were not significantly different between phases.

At rest, most of energy was derived from CHO sources in all phases before and with OC (before 56% vs. with 66%), but no significant phase or OC effect was observed. During exercise in all isotope trials, there was a shift to a greater reliance on CHO sources, which was significant (P < 0.05, Tables 2–4). Energy derived from CHO during exercise at 45% VO2peak was 3% lower with OC compared to before OC (before 66% vs. with 63%), but the difference was not significant. During exercise at 65% VO2peak in all phases, >75% of the
energy used to do work was derived from CHO sources (before 77% vs. with 79%). The contribution of lipid to energy expenditure was 29–50% in all phases at rest (before 44% vs. with 34%). In all isotope trials, there

Fig. 2. Effect of OC and exercise intensity on glucose rate of appearance ($R_a$, $A$), rate of disappearance ($R_d$, $B$), and metabolic clearance rate (MCR, $C$). Values are means ± SE of last 15 and 30 min for rest and exercise, respectively, for 5–7 women BOC and for 8 women in all conditions WOC. See text for explanation of subject exclusion criteria. *Significantly different from resting conditions, $P < 0.05$; †significantly different from 45% trials, $P < 0.05$; ‡significantly different between BOC and LP, $P < 0.05$; $*$significantly different between BOC and HP, $P < 0.05$.

Fig. 3. Effect of OC and exercise intensity on insulin (A) and glucagon concentrations (B) and insulin-to-glucagon ratio (C). Values are means ± SE of last 15 and 30 min for rest and exercise, respectively, for 5–7 women BOC and for 8 women in all conditions WOC. FP, follicular phase; LP luteal phase. See text for explanation of subject exclusion criteria. *Significantly different from resting conditions, $P < 0.05$; †significantly different from 45% trials, $P < 0.05$; ‡significantly different between BOC and IP, $P < 0.05$; $*$significantly different between BOC and HP, $P < 0.05$. 
was a significant increase in energy derived from lipid in response to exercise compared with resting values ($P < 0.05$, Tables 2–4), but no significant phase, OC, or intensity effect was observed (before 34% vs. with 37% in 45% trials; before 23% vs. with OC 21% in 65% trials).

DISCUSSION

Previously, we were unable to demonstrate significant menstrual phase effects on blood glucose flux or whole body substrate utilization patterns in resting or exercising women on a controlled 3- to 4-h postabsorptive diet (35). However, our present results demonstrated that blood glucose flux was altered during exercise because of OC administration. In addition, results of the present study corroborated those of previous studies demonstrating direct relationships of exercise intensity with blood glucose flux and CHO oxidation (12, 13). However, the exponential rise in blood glucose flux during exercise of graded intensities was downregulated by OC.

Although the present investigation is the first utilizing a longitudinal design to examine the effects of OC on glucose flux, results are consistent with those of others, indicating that in men (12, 21, 24) and women (13, 39) glucose flux rates rise during exercise and as exercise intensity increases (Fig. 4). Our findings of a reduction in glucose $R_d$, and MCR after short-term administration of OC are consistent with those of Ruby et al. (25) and Carter et al. (8). Ruby et al. determined the effects of transdermal estradiol replacement on substrate turnover in amenorrheic women during 90 min of treadmill exercise at 65% $V_{O_2peak}$. Carter et al. administered oral estradiol (or placebo) to eight male subjects for 8 days and measured substrate turnover during 90 min of cycle ergometer exercise at 60% $V_{O_2peak}$. As in the present investigation, both studies found a reduction in glucose flux in response to exogenous estradiol administration. Because total CHO oxidation during exercise was unaffected by OC, OC administration must increase use of alternative CHO energy sources in skeletal muscle (e.g., glycogen and lactate) as a compensation to the reduction in glucose availability. OC reduced blood glucose flux during rest and exercise whether the results are related to overall metabolic rate, whether expressed on absolute (Fig. 4A) or relative (Fig. 4, B and C) basis. As noted in our companion report (9), OC reduced $V_{O_2peak}$ by 13%. This effect of OC on aerobic capacity increased the relative exercise intensity, an effect that usually increases glucose flux (5). However, as shown in Fig. 4C, when relative exercise intensity is considered, the effects of OC in suppressing glucose $R_d$ are especially notable.

The suppression of glucose flux by OC we observed was impressive considering our efforts to control energy intake and CHO nutrition. In our investigation, women were studied after a day of rest and controlled energy and CHO intake. Furthermore, our subjects consumed a standardized supper and took a prescribed breakfast in the laboratory; hence, we report data on rested, glycogen replete, and 3–4 h postabsorptive subjects. The two exercise tasks that we studied raised metabolic rate five to eight times above resting and resulted in 4- to 11-fold increments in CHO oxidation. In this setting, the overall contributions of CHO and lipid to total substrate oxidation were not different before and with OC (Tables 2–4). Still, glucose flux rates were suppressed by OC during rest and exercise.
(Fig. 2). OC decreased both glucose $R_a$ and $R_d$ (Fig. 2A and B), but glyemia was maintained (Fig. 1A). Although the mechanisms are unknown, it is apparent that both hepatic glucose production and peripheral glucose disposal were affected by OC. In particular, the decreases in glucose MCR (Fig. 2C) are likely reflective of effects of OC on peripheral insulin action (10, 19).

In comparing effects of endogenous and exogenous synthetic ovarian steroids on blood glucose kinetics, we pooled data obtained in our companion report (35) in which luteal and follicular phase differences were compared and found not to be significantly different. A retrospective power analysis of those results suggests that if the small mean difference observed were to hold up, data on 50 subjects would be required to establish statistical significance. Hence, for the present we are confident that under the controlled dietary conditions employed there were no physiologically significant effects of menstrual cycle variations on glucose flux.

Combined results in our companion (35) and present report indicate that OC have persistent as well as acute effects. Evidence for the persistence of OC effects is found in comparisons of inactive phase (IP, no synthetic steroids) results with those obtained in the same women during midfollicular (FP) menstrual phase before OC consumption. As shown in Table 5, estradiol and progesterone levels were low and not significantly different at rest and exercise at 65% $V_{O2\text{peak}}$ between FP and IP conditions. Yet glucose flux rates were lower after 4 mo of OC use (Figs. 2 and 4). We have no explanation for this observation of persistent metabolic effects of OC use.

In the present investigation, elevations in insulin levels (Fig. 3A) and no change in glucose $R_d$ (Fig. 2B) were observed at rest after 4 mo of OC use. Others (38) also observed that in the absence of altered glucose tolerance small doses of contraceptive steroids for 3 mo induced a significant elevation of fasting insulin levels with no significant difference in fasting blood glucose levels observed. Such observations suggest that a mild to moderate degree of insulin resistance exists in women using OC, necessitating compensatory increases of pancreatic insulin secretion to maintain normal glucose tolerance. The insulin resistance in response to OC may be caused by a postreceptor effect on insulin action (28). However, the common observations in OC users of insulin resistance to glucose challenge indicate a change in the balance between insulin secretion and insulin action. A greater pancreatic response to impaired insulin action on peripheral tissues likely serves to maintain glucose tolerance. Therefore, decreased insulin sensitivity at the cellular level in peripheral tissues is a possible explanation for the influence of OC on glucose metabolism even though a change in the metabolic clearance rate of insulin in the liver may also play a role (28).

Synthetic steroids were speculated to alter glucose metabolism depending on type of OC used or dose and duration of its administration. Although conflicting reports exist, deterioration of glucose tolerance with OC use has been observed by several investigators (18, 37). For example, Wynn et al. (37) examined the effect of six different combined OCs in terms of type and doses on glucose metabolism and observed differences in metabolic effects between combined OCs. Wynn et al. reported that glucose tolerance deteriorated in all OC groups containing estrone progestogens (nortestosterone-derived) or the gonane norgestrel but was unaltered by OC containing a pregnane progestogen (derived from progesterone). The OCs containing 75 μg or more of estrogen combined with an estrane progestogen caused the greatest deterioration in glucose tolerance associated with impaired insulin secretion. Lowering of the estrogen dose to 50 μg without altering the progestogen content of the OCs resulted in less deterioration of glucose tolerance and increased insulin secretion. Those results suggest the importance of the dose of estrogen and type of progestogen.

In summary, results of the present investigation contribute to the growing body of evidence on the relative effects of exercise, exercise training, carbohydrate nutrition, and endogenous and synthetic ovarian hormones on metabolic flux rates and substrate partitioning. Exercise increases glucose flux and oxidation in an intensity-dependent manner (3, 5, 12, 13, 21, 22). Endurance training decreases glucose flux and oxidation in both men (12) and women (13) during exercise of given absolute power outputs. However, in trained men and women, glucose flux is the same or greater at a given relative exercise intensity (3, 12, 22). Recent carbohydrate nutrition increases overall CHO oxidation (2, 7). Effects of endogenous ovarian hormones on glucose flux during exercise are subtle (6, 39) and overridden during exercise by CHO nutrition (35) or fluid-electrolyte-energy replacement beverages (6). Exogenous ovarian hormones, such as OC studied in this investigation, exert greater effects on glucose flux than do endogenous hormones as effects of OC can be observed in recently fed women. Furthermore, the effects of OC on glucose flux are persistent, being observable during days of the month when exogenous ovarian hormones are not provided. Finally, in contrast to the effects of acute and chronic exercise on increasing insulin action (10, 19), as shown in this report as well as previously (18, 30–32, 37), OCs dampen insulin action. In the future, it would be helpful to perform muscle biopsies to ascertain the mechanism of changes due to OC at a cellular level.

These results are interpreted to mean, in women fed several hours before study, that 1) OC decreases glucose flux, but not overall CHO and lipid oxidation rates during moderate-intensity exercise, and 2) synthetic ovarian hormone analogs in the doses contained in OC have greater metabolic effects on glucose metabolism during exercise than do endogenous ovarian hormones.

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REFERENCES


