J Appl Physiol 94: 285–294, 2003.
First published September 20, 2002; 10.1152/japplphysiol.00693.2002.

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

SANG-HOON SUH, GRETCHEN A. CASAZZA, MICHAEL A. HORNING, BENJAMIN F. MILLER, AND GEORGE A. BROOKS
Exercise Physiology Laboratory, Department of Integrative Biology, University of California, Berkeley, California 94720-3140

Submitted 29 July 2002; accepted in final form 17 September 2002

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 O_2 uptake ($\dot{V}O_{2\ peak}$)]. 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 exercise of both intensities with OC but not rest. There were no phase effects on substrate oxidation 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.

ovarian hormones; glucose kinetics; exertion; women; gender

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 significant 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 O_2 uptake ($\dot{V}O_{2\ peak}$). 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

Address for reprint requests and other correspondence: G. A. Brooks, Dept. of Integrative Biology, 3060 Valley Life Science Bldg, Univ. of California, Berkeley, Berkeley, CA 94720 (E-mail: gbrooks@socrates.berkeley.edu).

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.

(for at least 6 mo), had not taken OC, and had not experienced 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 $ml \cdot kg^{-1} \cdot min^{-1}$, 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 concentrations 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 early follicular (FP) and midluteal (LP) phases of the menstrual 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 confirmed by plasma estradiol and progesterone concentrations [estradiol <50 pg/ml, progesterone <1 ng/ml (FP); and estradiol >50 pg/ml, progesterone >3 ng/ml (LP)] (4, 6, 35). After the maximal exercise tests, four stable isotope tracer infusion trials were conducted within two sequential menstrual cycles, with each trial consisting of a 90-min rest period followed by a 60-min exercise protocol. Exercise tasks involved leg ergometer cycling at 45 and 65% $\dot{V}O_{2\ peak}$, separated by 3–5 days to complete the trials within a menstrual cycle. After completion of the menstrual cycle phase testing, each subject began taking the same triphasic OC (one pill per day) for four complete cycles (28 days per cycle). For *days 1–7*, each pill contained 0.035 mg ethinyl estradiol and 0.18 mg norgestimate; for *days 8–14* each pill contained 0.035 mg ethinyl estradiol and 0.215 mg norgestimate; for *days 15–21* each pill contained 0.035 mg ethinyl estradiol and 0.25 mg norgestimate (HP), and for *days 22–28* the pills were absent of synthetic hormones (IP). With monophasic OCs, the estradiol and progestin components remain constant throughout the pill cycle. In contrast, in triphasic OCs the amount of estradiol and/or progestin varies across the pill cycle with the intention of more closely mimicking the ovarian hormone variations that occur during the normal menstrual cycle. In addition, triphasic OCs contain lower per-cycle progestin levels to provide better cycle control and reduce the incidence of androgenic side effects such as alterations in carbohydrate and lipid metabolism. The same two maximal exercise tests and four stable isotope tracer infusion trials were subsequently conducted during the week of the inactive pills (IP) and during the week when the intake of synthetic ovarian hormones was high (HP).

Screening tests. $\dot{V}O_{2\ peak}$ during leg cycling was determined during a continuous graded exercise test on a bicycle ergometer (Monark Ergometric 839E, Vansbro, Sweden) beginning at 75 W and increasing 25 W every 3 min until voluntary cessation. Respiratory gases were continuously monitored via an open-circuit system (Ametek S-3A1 O_2 and Ametek CD-3A CO_2 analyzers, Pittsburgh, PA) and recorded every minute by an on-line, real-time personal computer-based mixing chamber system that we have used repeatedly (2, 12, 35, 36). In each trial, the open-circuit system was calibrated twice before rest and exercise by using room air and a certified calibration gas (16% O_2 and 4% CO_2). Heart rate was monitored continuously by a Quinton 759 electrocardiograph and blood pressure by stethoscope and sphygmomanometer. $\dot{V}O_{2\ peak}$ 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 determined 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 habits and monitor the subject's caloric intake and macronutrient composition. Analysis of dietary records was performed by using the Nutritionist III program (N-squared Computing, Salem, OR). Because of overlaps in subject cycles, it was not always possible to conduct screening and glucose flux 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% carbohydrate (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 forearm 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 isotopic enrichments in samples drawn simultaneously were not different. After collection of background blood and expired gas samples, a priming bolus of [6,6-^{2}H]glucose (D_2-glucose), which was 125 times the resting minute infusion rate, was given, and the subjects rested supine or semisupine for 90 min while the D_2-glucose was continuously infused (Baxter Travenol 6300 infusion pump). The glucose tracer was infused 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 previously demonstrated by our laboratory to maintain stable plasma isotopic enrichment for the measurement of substrate kinetics throughout rest and the two exercise intensities (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 Pharmacy), and on the day of the experiment passed through a 0.2-μm Millipore filter (Nalgen, 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

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 ^{125}I 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 331.20 and 333.20 for [^{12}C]glucose and D_2-glucose, respectively.

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

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

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

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

where F represents the isotopic infusion rate; V is the estimated volume distribution of glucose (180 ml/kg); C_1 and C_2 are concentrations at sampling times t_1 and t_2, respectively; and IE_1 and IE_2 are the glucose isotopic enrichments of D_2-glucose at sampling times t_1 and t_2, 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)

$$\%\text{Energy from CHO} = [(RER - 0.707)/0.293] \times 100$$

$$\%\text{Energy from lipid} = 100 - [(RER - 0.707)/0.293] \times 100$$

$$\text{Energy from CHO oxidation (kcal/min)} = [(\%\ CHO/100) \times (\dot{V}O_2)] \times (5.05\ kcal/l\ O_2)$$

$$\text{Energy from lipid oxidation (kcal/min)} = [(1 - \%\ CHO/100) \times (\dot{V}O_2)] \times (4.7\ kcal/l\ O_2)$$

$$\text{Energy expenditure (kcal/min)} = [(\%\ CHO/100) \times (\dot{V}O_2) \times (5.05\ kcal/l\ O_2)] + [(1 - \%\ CHO/100) \times (\dot{V}O_2) \times (4.7\ kcal/l\ O_2)]$$

where O_2 uptake ($\dot{V}O_2$) 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. Despite concerted 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 $\alpha = 0.05$.

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, $\dot{V}O_{2\ peak}$, 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, $\dot{V}O_{2\ peak}$ 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% $\dot{V}O_{2\ peak}$ are presented in Tables 2, 3, and 4, respectively. At rest and during exercise at either

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

Variable	Before OC		With OC	
	FP	LP	IP	HP
n	7	5	8	8
Age, yr	24.9 ± 1.4	26.0 ± 1.8	24.5 ± 1.3	24.5 ± 1.3
Height, cm	165.6 ± 1.6	165.9 ± 2.2	164.6 ± 1.7	164.6 ± 1.7
Weight, kg	61.4 ± 1.1	60.2 ± 1.4	61.9 ± 1.3*§	61.9 ± 1.4†‡
Body fat, %	23.3 ± 1.6	21.4 ± 1.5	24.5 ± 1.3*§	24.7 ± 1.3†‡
Fat mass, kg	14.4 ± 0.8	12.9 ± 0.7	15.2 ± 0.8*§	15.4 ± 0.8†‡
Lean body mass, kg	47.0 ± 0.8	47.3 ± 1.1	46.6 ± 0.8	46.5 ± 0.9
$\dot{V}O_{2peak}$				
ml·kg^{-1}·min^{-1}	44.2 ± 1.8	42.9 ± 2.1	37.0 ± 1.5*§	37.5 ± 1.2†‡
l/min	2.7 ± 0.1	2.5 ± 0.1	2.3 ± 0.1*§	2.3 ± 0.1†‡

Values are means ± SE; *n* = no. of subjects. OC, oral contraceptives; FP, follicular phase; LP, luteal phase; IP, inactive phase; HP, high-dose phase; $\dot{V}O_{2peak}$, 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 HP, $P < 0.05$; §significantly different between LP and IP, $P < 0.05$. Note: data on physical characteristics before OC (35) and OC effect on $\dot{V}O_{2peak}$ (9) have been previously reported.

intensity, there were no significant phase or OC effects on any of the variables in Tables 2–4, except for %$\dot{V}O_{2\,peak}$ 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 $\dot{V}O_{2\,peak}$ after 4 mo of OC, because of the fact that there was a significant decrease in $\dot{V}O_{2\,peak}$ with OC. Hematocrit decreased significantly only in 45% trials with OC. There were significant increases in $\dot{V}O_2$, $\dot{V}CO_2$, 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 $\dot{V}O_{2\,peak}$ 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 (HP vs. FP, $P < 0.05$). In addition, compared with IP, estradiol and progesterone concentrations were lower in HP, 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. 1*A*). However, neither before nor with OC were changes significantly different among exercise conditions (Fig. 1*A*).

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

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

Variable	Before OC		With OC	
	FP	LP	IP	HP
n	7	5	8	8
$\dot{V}O_2$, ml·kg^{-1}·min^{-1}	4.0 ± 0.1	4.0 ± 0.1	3.7 ± 0.1	3.8 ± 0.1
$\dot{V}CO_2$, ml·kg^{-1}·min^{-1}	3.4 ± 0.1	3.5 ± 0.1	3.4 ± 0.1	3.4 ± 0.1
RER	0.85 ± 0.02	0.88 ± 0.01	0.91 ± 0.01	0.89 ± 0.01
EE, kcal/min	1.2 ± 0.0	1.2 ± 0.0	1.1 ± 0.0	1.2 ± 0.0
Energy from CHO, kcal/min	0.6 ± 0.1	0.7 ± 0.0	0.8 ± 0.0	0.7 ± 0.0
Energy from lipid, kcal/min	0.6 ± 0.1	0.4 ± 0.1	0.3 ± 0.0	0.4 ± 0.0
Minute ventilation, l/min	7 ± 0	8 ± 0	7 ± 0	7 ± 0
Heart rate, bpm	64 ± 2	67 ± 3	67 ± 2	65 ± 2
Blood pressure, mmHg				
Systolic	108 ± 2	107 ± 2	105 ± 2	105 ± 1
Diastolic	69 ± 2	69 ± 2	64 ± 1	65 ± 1
Mean arterial	82 ± 2	81 ± 2	78 ± 1	79 ± 1
Hematocrit, %	41 ± 1	41 ± 1	38 ± 1	37 ± 1

Values are means ± SE. $\dot{V}O_2$, oxygen consumption; $\dot{V}CO_2$, 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).

Table 3. *Ergometric and physiological parameters of subjects during exercise at 45% $\dot{V}O_{2peak}$ before and with 4 mo of OC*

Variable	Before OC		With OC	
	FP	LP	IP	HP
n	7	6	8	8
Workload, W	59.1 ± 5.8	58.2 ± 5.2	58.3 ± 5.2	58.3 ± 5.2
% $\dot{V}O_{2peak}$	46.4 ± 0.6	47.8 ± 1.1	51.9 ± 1.3[e]	50.7 ± 1.1
$\dot{V}O_2$, ml·kg^{-1}·min^{-1}	20.2 ± 0.9[a]	19.5 ± 1.2[a]	18.8 ± 0.8[a]	18.9 ± 1.0[a]
$\dot{V}CO_2$, ml·kg^{-1}·min^{-1}	18.0 ± 0.9[a]	17.4 ± 1.0[a]	16.8 ± 0.6[a]	16.7 ± 0.8[a]
RER	0.90 ± 0.01	0.90 ± 0.01	0.90 ± 0.01	0.89 ± 0.01
EE, kcal/min	6.1 ± 0.3[a]	5.7 ± 0.3[a]	5.8 ± 0.3[a]	5.7 ± 0.3[a]
Energy from CHO, kcal/min	4.1 ± 0.3[a]	3.7 ± 0.2[a]	3.7 ± 0.2[a]	3.5 ± 0.1[a]
Energy from lipid, kcal/min	2.0 ± 0.1[a]	2.0 ± 0.4[a]	2.0 ± 0.2[a]	2.2 ± 0.3[a]
Minute ventilation, l/min	29 ± 1[a]	29 ± 1[a]	28 ± 1[a]	29 ± 1[a]
Heart rate, bpm	131 ± 4[a]	132 ± 3[a]	133 ± 4[a]	133 ± 4[a]
Blood pressure, mmHg				
Systolic	130 ± 3[a]	129 ± 3[a]	127 ± 4[a]	126 ± 2[a]
Diastolic	68 ± 4	65 ± 2	60 ± 3	61 ± 3
Mean arterial	89 ± 3	86 ± 2	82 ± 3	83 ± 3
Hematocrit, %	43 ± 1	41 ± 1	39 ± 1[b,e]	39 ± 1[c,d]

Values are means ± SE. [a]Significantly different from resting conditions; $P < 0.05$; [b]significantly different between FP and IP, $P < 0.05$; [c]significantly different between LP and HP, $P < 0.05$; [d]significantly different between FP and HP, $P < 0.05$; [e]significantly different between LP and IP, $P < 0.05$. 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. 1*B*). 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% $\dot{V}O_{2\ peak}$, Fig. 2*A*). 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% $\dot{V}O_{2\ peak}$ with OC, respectively ($P < 0.05$). There were no significant differences in glucose R_a between IP and HP during rest or exercise of either intensity.

Responses of R_d (Fig. 2*B*) 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% $\dot{V}O_{2\ peak}$ between before 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. 2*C*) was similar to glucose R_d among the eight trials except that there was no signif-

Table 4. *Ergometric and physiological parameters of subjects during exercise at 65% $\dot{V}O_{2peak}$ before and with 4 mo of OC*

Variable	Before OC		With OC	
	FP	LP	IP	HP
n	7	5	8	8
Workload, W	97.9 ± 6.8*†	97.5 ± 6.6*†	97.5 ± 6.7*†	97.6 ± 6.7*†
% $\dot{V}O_{2peak}$	68.0 ± 0.8†	67.5 ± 0.8†	73.2 ± 1.3†	72.4 ± 1.9†
$\dot{V}O_2$, ml·kg^{-1}·min^{-1}	29.4 ± 1.3*†	28.7 ± 1.9*†	26.8 ± 1.5*†	26.9 ± 1.3*†
$\dot{V}CO_2$, ml·kg^{-1}·min^{-1}	27.2 ± 1.2*†	26.81 ± 1.9*†	25.2 ± 1.4*†	25.0 ± 1.4*†
RER	0.93 ± 0.01*	0.93 ± 0.02	0.94 ± 0.01	0.93 ± 0.01
EE, kcal/min	8.9 ± 0.4*†	8.5 ± 0.4*†	8.2 ± 0.5*†	8.3 ± 0.5*†
Energy from CHO, kcal/min	6.7 ± 0.2*†	6.7 ± 0.7*†	6.7 ± 0.4*†	6.4 ± 0.5*†
Energy from lipid, kcal/min	2.2 ± 0.2*	1.8 ± 0.5*	1.6 ± 0.3*	1.9 ± 0.3*
Minute ventilation, l/min	47 ± 1*†	47 ± 3*†	46 ± 2*†	46 ± 2*†
Heart rate, bpm	169 ± 3*†	169 ± 5*†	167 ± 4*†	167 ± 4*†
Blood pressure, mmHg				
Systolic	143 ± 4*†	137 ± 2*	141 ± 5*†	136 ± 3*
Diastolic	62 ± 2	66 ± 5	59 ± 2	61 ± 2
Mean arterial	89 ± 1	89 ± 4	87 ± 3*	86 ± 2
Hematocrit, %	43 ± 0	43 ± 1	40 ± 1	41 ± 1*

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*

Variable	Rest		Exercise			
Before OC	FP	LP	FP45	LP45	FP65	LP65
n	7	5	7	6	7	5
Day of cycle			5.6 ± 0.8	22.0 ± 0.8	6.6 ± 0.8	20.2 ± 0.7
Days past LH surge				6.7 ± 0.5		6.2 ± 0.9
Estradiol, pg/ml	27.06 ± 3.69	84.74 ± 5.77[c]	37.31 ± 6.01	119.41 ± 6.71[c]	47.88 ± 6.74[a]	151.34 ± 23.13[a,c]
Progesterone, ng/ml	0.39 ± 0.05	10.89 ± 1.46	0.57 ± 0.10	14.46 ± 2.02[c]	0.62 ± 0.07	10.50 ± 2.51[c]
With OC	IP	HP	IP45	HP45	IP65	HP65
n	8	8	8	8	8	8
Day of cycle			5.6 ± 0.9	25.6 ± 0.7	7.5 ± 0.5	24.5 ± 0.5
Estradiol, pg/ml	23.53 ± 4.43[h]	13.38 ± 1.97[d,f,g]	26.78 ± 6.85[e,h]	13.55 ± 1.30[d,f,g]	43.59 ± 10.29[a,b,h]	20.81 ± 5.93[d,f,g]
Progesterone, ng/ml	0.29 ± 0.04[h]	0.30 ± 0.04[f]	0.33 ± 0.05[e,h]	0.30 ± 0.03[f,g]	0.55 ± 0.06[a,b,h]	0.47 ± 0.07[f]

Values are means ± SE. 45 and 65, exercise intensities, in % of $\dot{V}O_{2\,peak}$; LH, luteinizing hormone. [a]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, $P < 0.05$; [d]significantly different between IP and HP at rest, 45%, or 65% trials, $P < 0.05$; [e]significantly different between FP and IP at rest, 45%, or 65% trials, $P < 0.05$; [f]significantly different between LP and HP at rest, 45%, or 65% trials, $P < 0.05$; [g]significantly different between FP and HP 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).

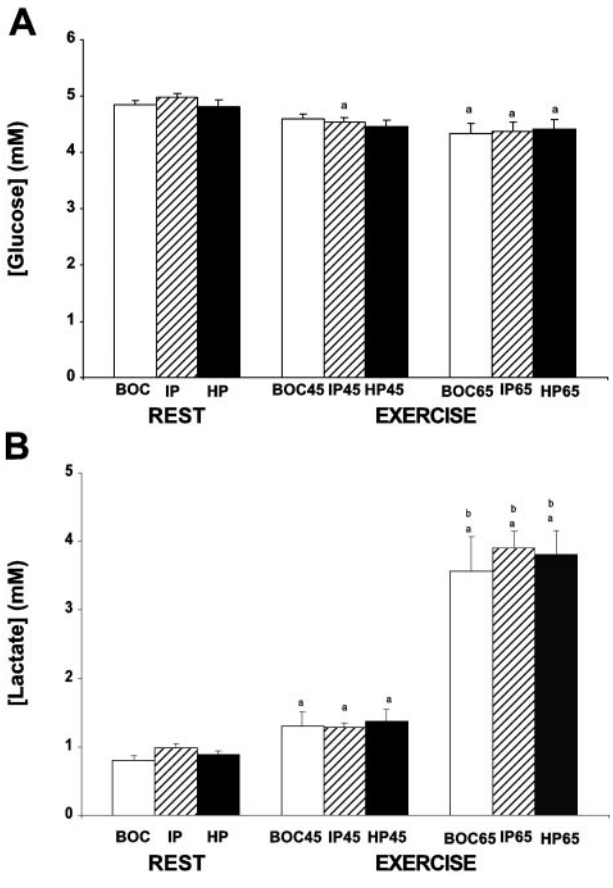

Fig. 1. Effect of oral contraceptives (OC) and exercise intensity on blood glucose (*A*) and lactate (*B*) concentrations. Square brackets denote concentration. Values are means ± SE of last 15 and 30 min for rest and exercise, respectively, for 5–7 women before OC (BOC) and for 8 women in all conditions with OC (WOC). IP, inactive phase; HP, high-dose phase; 45 and 65, exercise intensities in % of peak O_2 uptake ($\dot{V}O_{2\,peak}$). See text for explanation of subject exclusion criteria. [a]Significantly different from resting conditions, $P < 0.05$; [b]significantly different from 45% trials, $P < 0.05$.

icant difference in glucose MCR during exercise at 45% $\dot{V}O_{2\,peak}$ 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. 3*A*). 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% $\dot{V}O_{2\,peak}$), but no significant phase, OC, or intensity effect was observed in other exercise conditions.

Glucagon concentrations increased significantly during exercise at 45% $\dot{V}O_{2\,peak}$ in HP and during exercise at 65% $\dot{V}O_{2\,peak}$ before and with OC ($P < 0.05$, Fig. 3*B*).

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% $\dot{V}O_{2\,peak}$). 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% $\dot{V}O_{2\,peak}$ was 3% lower with OC compared to before OC (before 66% vs. with 63%), but the difference was not significant. During exercise at 65% $\dot{V}O_{2\,peak}$ in all phases, >75% of the

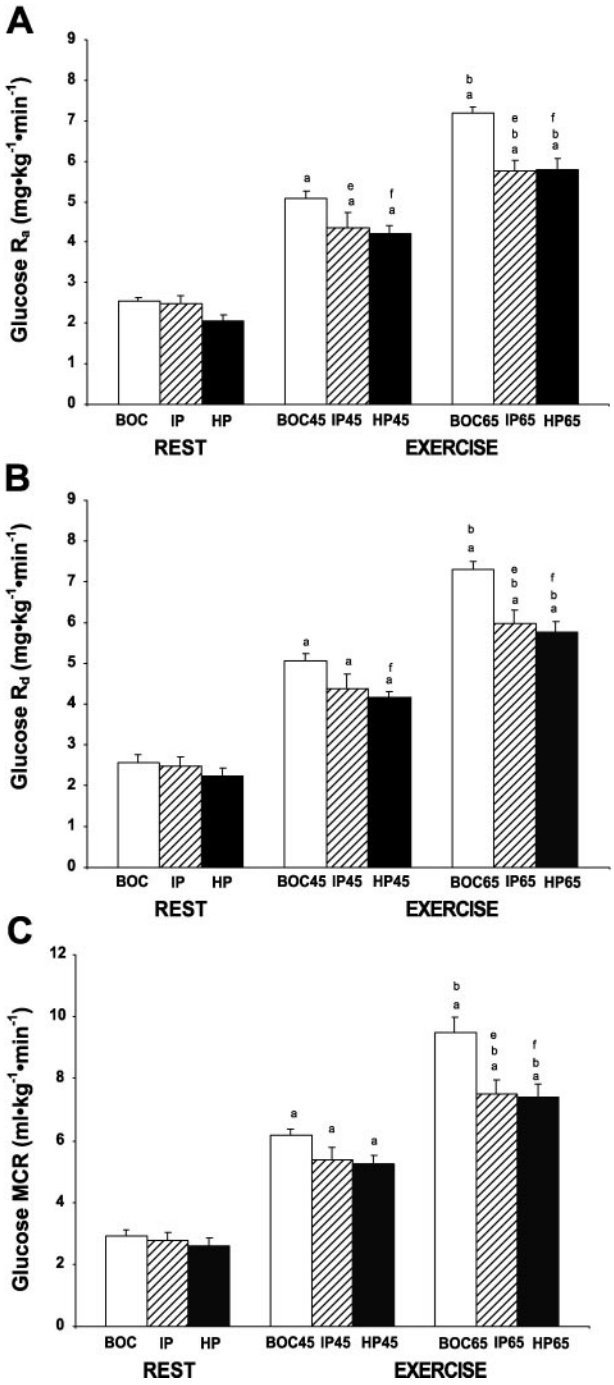

Fig. 2. Effect of OC and exercise intensity on glucose rate of appearance (R_a, *A*), rate of disappearance (R_d, *B*), and metabolic clearance rate (MCR, *C*). Values are means ± SE of last 15 and 30 min for rest and exercise, respectively, for 5–7 women BOC and for 8 women in all conditions WOC. See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

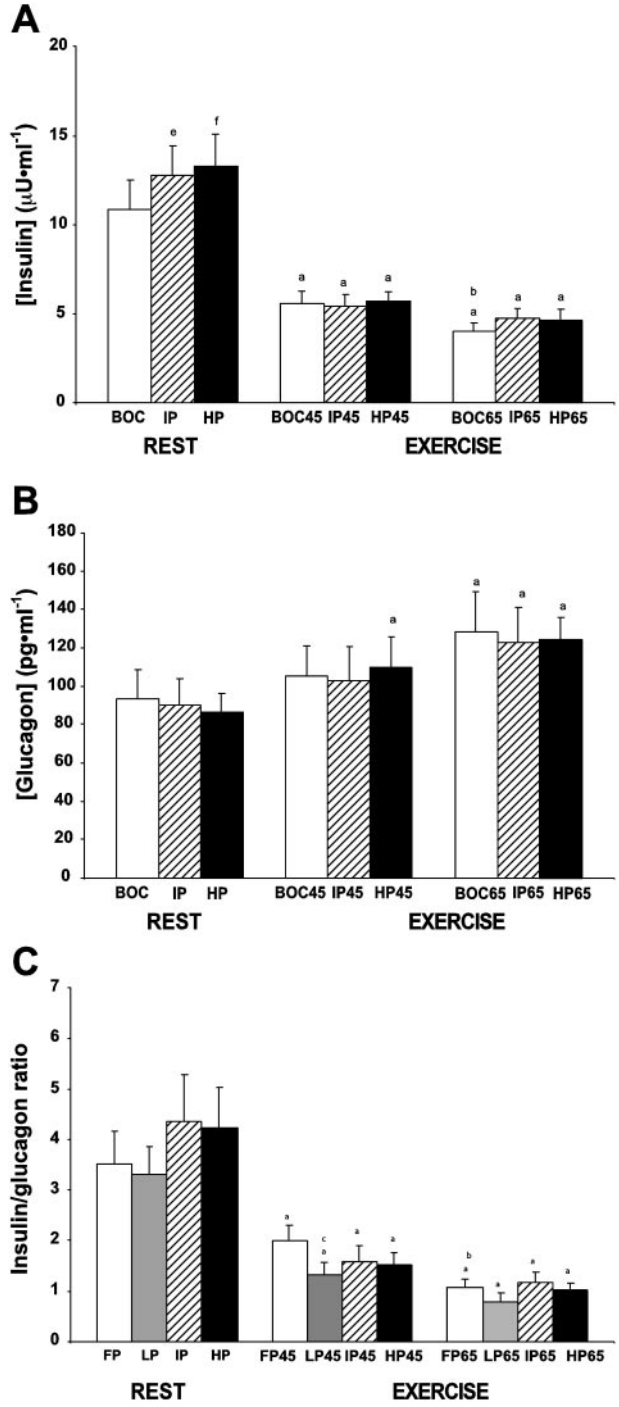

Fig. 3. Effect of OC and exercise intensity on insulin (*A*) and glucagon concentrations (*B*) and insulin-to-glucagon ratio (*C*). Values are means ± SE of last 15 and 30 min for rest and exercise, respectively, for 5–7 women BOC and for 8 women in all conditions WOC. FP, follicular phase; LP luteal phase. See text for explanation of subject exclusion criteria. [a]Significantly different from resting conditions, $P < 0.05$; [b]significantly different from 45% trials, $P < 0.05$; [c]significantly different between FP and LP, $P < 0.05$; [e]significantly different between BOC and IP, $P < 0.05$; [f]significantly different between BOC and HP, $P < 0.05$.

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

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 corroborate 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_a, 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% $\dot{V}O_{2\ peak}$. 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% $\dot{V}O_{2\ peak}$. 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. 4*A*) or relative (Fig. 4, *B* and *C*) basis. As noted in our companion report (9), OC reduced $\dot{V}O_{2\ peak}$ ~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. 4*C*, 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

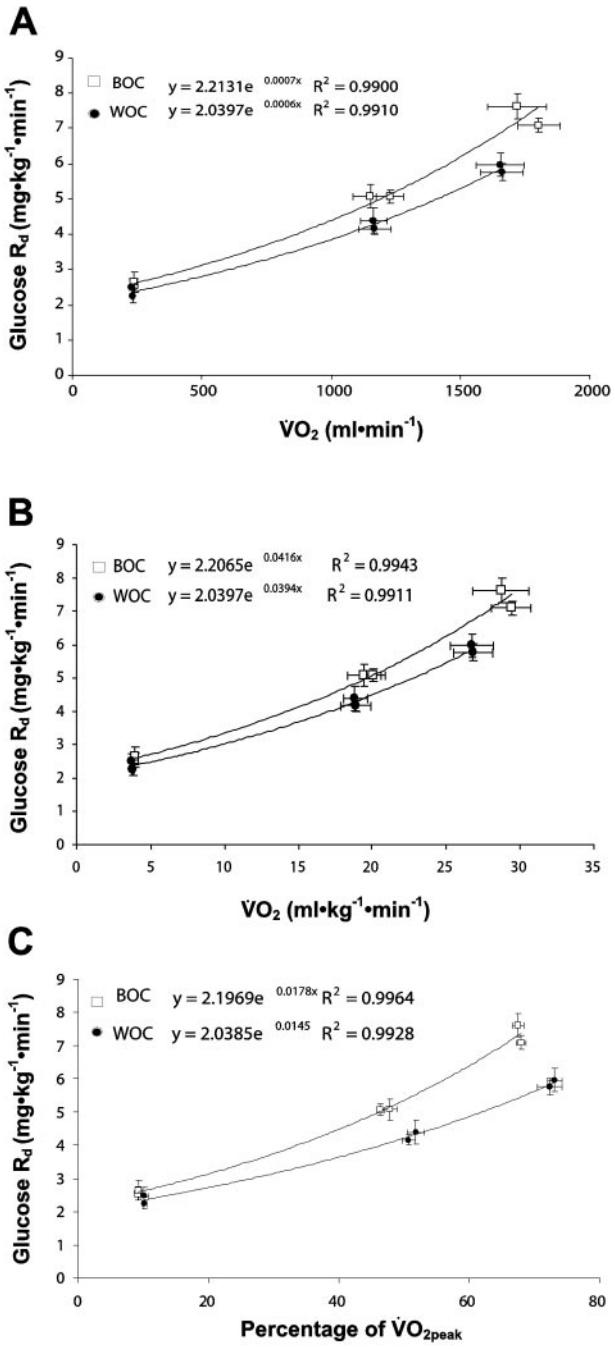

Fig. 4. Relationship of glucose R_d with absolute (*A*) and relative O_2 uptake ($\dot{V}O_2$) (*B*) and % of $\dot{V}O_{2\,peak}$ (*C*) in all phases before and with OC. Values are means ± SE of last 15 and 30 min for rest and exercise, respectively, for 5–7 women BOC and for 8 women in all conditions WOC. See text for explanation of subject exclusion criteria.

(Fig. 2). OC decreased both glucose R_a and R_d (Fig. 2, *A* and *B*), but glycemia was maintained (Fig. 1*A*). 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. 2*C*) 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% $\dot{V}O_{2\ peak}$ 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. 3*A*) and no change in glucose R_d (Fig. 2*B*) 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 types 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 estrane 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.

The authors thank the subjects for participating in this study. We also thank Karen Porto, Joe Vivo, and Zinta Zarins who provided much of the laboratory support throughout the study. We are also grateful to Jaimyoung Kwon who assisted with the statistical analysis.

This work was supported by National Institute of Arthritis and Musculoskeletal and Skin Diseases Grant AR-42906 and a gift from the Brian and Jennifer Maxwell Foundation.

REFERENCES

1. **Bemben DA, Boileau RA, Bahr JM, Nelson RA, and Misner JE.** Effects of oral contraceptives on hormonal and metabolic responses during exercise. *Med Sci Sports Exerc* 24: 434–441, 1992.
2. **Bergman BC and Brooks GA.** Respiratory gas-exchange ratios during graded exercise in fed and fasted trained and untrained men. *J Appl Physiol* 86: 479–487, 1999.
3. **Bergman BC, Butterfield GE, Wolfel EE, Lopaschuk GD, Casazza GA, Horning MA, and Brooks GA.** Muscle net glucose uptake and glucose kinetics after endurance training in men. *Am J Physiol Endocrinol Metab* 277: E81–E92, 1999.
4. **Bonen A, Haynes FW, and Graham TE.** Substrate and hormonal responses to exercise in women using oral contraceptives. *J Appl Physiol* 70: 1917–1927, 1991.
5. **Brooks GA and Mercier J.** Balance of carbohydrate and lipid utilization during exercise: the "crossover" concept. *J Appl Physiol* 76: 2253–2261, 1994.
6. **Campbell SE, Angus DJ, and Febbraio MA.** Glucose kinetics and exercise performance during phases of the menstrual cycle: effect of glucose ingestion. *Am J Physiol Endocrinol Metab* 281: E817–E825, 2001.
7. **Carrington LJ and Bailey CJ.** Effects of natural and synthetic estrogens and progestins on glycogen deposition in female mice. *Horm Res* 21: 199–203, 1985.
8. **Carter S, McKenzie S, Mourtzakis M, Mahoney DJ, and Tarnopolsky MA.** Short-term 17β-estradiol decreases glucose R_a but not whole body metabolism during endurance exercise. *J Appl Physiol* 90: 139–146, 2001.
9. **Casazza GA, Suh S-H, Miller BF, Navazio FM, and Brooks GA.** Effects of low-dose oral contraceptives on peak exercise capacity. *J Appl Physiol* 93: 1698–1702, 2002.
10. **Dela F, Mikines KJ, Von Linstow M, Secher NH, and Galbo H.** Effect of training on insulin-mediated glucose uptake in human muscle. *Am J Physiol Endocrinol Metab* 263: E1134–E1143, 1992.
11. **Frayn KN.** Calculation of substrate oxidation rates in vivo from gaseous exchange. *J Appl Physiol* 55: 628–634, 1983.
12. **Friedlander AL, Casazza GA, Horning MA, Huie MJ, and Brooks GA.** Training-induced alterations of glucose flux in men. *J Appl Physiol* 82: 1360–1369, 1997.
13. **Friedlander AL, Casazza GA, Horning MA, Huie MJ, Piacentini MF, Trimmer JK, and Brooks GA.** Training-induced alterations of carbohydrate metabolism in women: women respond differently from men. *J Appl Physiol* 85: 1175–1186, 1998.
14. **Gaspard UJ and Lefebvre PJ.** Clinical aspects of the relationship between oral contraceptives, abnormalities in carbohydrate metabolism, and the development of cardiovascular disease. *Am J Obstet Gynecol* 163: 334–343, 1990.
15. **Gossain VV, Sherma NK, Michelakis AM, and Rovner DR.** Effect of oral contraceptives on plasma glucose, insulin, and glucagon levels. *Am J Obstet Gynecol* 147: 618–623, 1983.
16. **Gutmann I and Wahlefeld AW.** L-(+)-Lactate determination with lactate dehydrogenase and NAD. In: *Methods of Enzymatic Analysis*, edited by Bergmeyer H. New York: Academic, 1974, p. 1464–1468.
17. **Jackson AS, Pollock ML, and Ward A.** Generalized equations for predicting body density of women. *Med Sci Sports Exerc* 12: 175–183, 1980.
18. **Kalkhoff RK.** Effects of oral contraceptive agents on carbohydrate metabolism. *J Steroid Biochem* 6: 949–956, 1975.
19. **King DS, Dalsky GP, Clutter WE, Young DA, Staten MA, Cryer PE, and Holloszy JO.** Effects of lack of exercise on insulin secretion and action in trained subjects. *Am J Physiol Endocrinol Metab* 254: E537–E542, 1988.
20. **Kjaer M, Farrell PA, Christensen NJ, and Galbo H.** Increased epinephrine response and inaccurate glucoregulation in exercising athletes. *J Appl Physiol* 61: 1693–1700, 1986.
21. **Kjaer M, Kiens B, Hargreaves M, and Richter EA.** Influence of active muscle mass on glucose homeostasis during exercise in humans. *J Appl Physiol* 71: 552–557, 1991.
22. **Kristiansen S, Gade J, Wojtaszewski JF, Kiens B, and Richter EA.** Glucose uptake is increased in trained vs. untrained muscle during heavy exercise. *J Appl Physiol* 89: 1151–1158, 2000.
23. **Matute ML and Kalkhoff RK.** Sex steroid influence on hepatic gluconeogenesis and glycogen formation. *Endocrinology* 92: 762–768, 1973.
24. **Romijn JA, Coyle EF, Sidossis LS, Gastaldelli A, Horowitz JF, Endert E, and Wolfe RR.** Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *Am J Physiol Endocrinol Metab* 265: E380–E391, 1993.
25. **Ruby BC, Robergs RA, Waters DL, Burge M, Mermier C, and Stolarczyk L.** Effects of estradiol on substrate turnover during exercise in amenorrhic females. *Med Sci Sports Exerc* 29: 1160–1169, 1997.
26. **Simon D, Senan C, Garnier P, Saint-Paul M, Garat E, Thibult N, and Papoz L.** Effects of oral contraceptives on carbohydrate and lipid metabolisms in a healthy population: the Telecom study. *Am J Obstet Gynecol* 163: 382–387, 1990.
27. **Skouby SO, Andersen O, Petersen KR, Mølsted-Pedersen L, and Kühl C.** Mechanism of action of oral contraceptives on carbohydrate metabolism at the cellular level. *Am J Obstet Gynecol* 163: 343–348, 1990.
28. **Skouby SO, Andersen O, Saurbrey N, and Kühl C.** Oral contraception and insulin sensitivity: in vivo assessment in normal women and women with previous gestational diabetes. *J Clin Endocrinol Metab* 64: 519–523, 1987.
29. **Skouby SO, Kühl C, Mølsted-Pedersen L, Petersen K, and Christensen MS.** Triphasic oral contraception: metabolic effects in normal women and those with previous gestational diabetes. *Am J Obstet Gynecol* 153: 495–500, 1985.
30. **Spellacy WN.** Carbohydrate metabolism during treatment with estrogen, progestogen, and low-dose oral contraceptives. *Am J Obstet Gynecol* 142: 732–734, 1982.
31. **Spellacy WN, Buhi WC, and Birk SA.** The effect of estrogens on carbohydrate metabolism: glucose, insulin, and growth hormone studies on one hundred and seventy-one women ingesting Premarin, mestranol, and ethinyl estradiol for six months. *Am J Obstet Gynecol* 114: 378–392, 1972.
32. **Spellacy WN, Buhi WC, and Birk SA.** Carbohydrate and lipid metabolic studies before and after one year of treatment with ethynodiol diacetate in "normal" women. *Fertil Steril* 27: 900–904, 1976.
33. **Spellacy WN, Buhi WC, and Birk SA.** Prospective studies of carbohydrate metabolism in "normal" women using norgestrel for eighteen months. *Fertil Steril* 35: 167–171, 1981.
34. **Steele R.** Influences of glucose loading and of injected insulin on hepatic glucose output. *Ann NY Acad Sci* 82: 420–430, 1959.
35. **Suh S-H, Casazza GA, Horning MA, Miller BF, and Brooks GA.** Luteal and follicular glucose fluxes during rest and exercise in 3-h postabsorptive women. *J Appl Physiol* 93: 42–50, 2002.
36. **Trimmer JK, Casazza GA, Horning MA, and Brooks GA.** Recovery of $^{13}CO_2$ during rest and exercise after [1-^{13}C]acetate, [2-^{13}C]acetate, and $NaH^{13}CO_3$ infusions. *Am J Physiol Endocrinol Metab* 281: E683–E692, 2001.
37. **Wynn V, Adams PW, Godsland I, Melrose J, Niththyananthan R, Oakley NW, and Seed M.** Comparison of effects of different combined oral-contraceptive formulations on carbohydrate and lipid metabolism. *Lancet* 1: 1045–1049, 1979.
38. **Yen SSC and Vela P.** Effects of contraceptive steroids on carbohydrate metabolism. *J Clin Endocrinol Metab* 28: 1564–1570, 1968.
39. **Zderic TW, Coggan AR, and Ruby BC.** Glucose kinetics and substrate oxidation during exercise in the follicular and luteal phases. *J Appl Physiol* 90: 447–453, 2001.