Health Promotion and Physical Activity
ISSN 2544-9117 ▪ 2025; 30 (1): 10-25
doi: 10.55225/hppa.638

HPPA • 2025; 30 (1): 10-25

Oral contraceptives do not impact metabolic and cardiorespiratory response during acute high-intensity rowing interval exercise – a pilot study

Sara A. Garcia[1] A-G, Alyssa A. Olenick[2] C-E,G, Nathan T. Jenkins[1] A,G, Regis C. Pearson[3] A,C-E,G

[1] University of Georgia, Department of Kinesiology, Integrative Cardiovascular Physiology Laboratory, USA
[2] University of Colorado Anschutz Medical School, Department of Endocrinology and Metabolism, USA
[3] Defense Centers for Public Health – Aberdeen, Injury Prevention Branch, USA

Abstract

Purpose: Increasing prevalence of oral contraceptive (OC) use in physically active females highlights the need for sex-specific exercise metabolism research. This pilot study investigated the influence of chronic OC use on the exercising metabolic and respiratory response during a rowing high-intensity interval exercise (HIIE) protocol in young, healthy, moderately active females.

Methods: Fifteen females [21.9 (3.7) years] were categorized by OC (n = 6) vs. non-oral contraceptive use (NOC) (n = 9). HIIE was four sets of 3 min maximal effort intervals on the rowing ergometer with 3 min rest between intervals and performed during the follicular phase (days 2–10 or inactive pills) of participants' menstrual cycles. To confirm comparable physical profiles of participants, we collected body fat percentage, fat-free mass, bone mineral density, blood pressure, aerobic fitness, muscular strength and endurance, flexibility, and microvascular function.

Results: Groups were similar in all measures of physical profiles ($P > 0.05$). Our pilot study showed that OC use or NOC use did not influence the exercising metabolic and cardiorespiratory response to HIIE in young, healthy, moderately active females. Fat oxidation, carbohydrate oxidation, metabolic flexibility, blood lactate concentration, blood glucose, and cardiorespiratory response were similar between groups ($P > 0.05$).

Conclusion: These findings suggest that there was no significant difference in exercising metabolic and cardiorespiratory response between OC and NOC users.

Original article

Keywords

- hormonal contraceptives
- metabolic flexibility
- female
- high-intensity interval exercise

Contribution

A – Preparation of the research project
B – Assembly of data
C – Conducting of statistical analysis
D – Interpretation of results
E – Manuscript preparation
F – Literature review
G – Revising the manuscript

Corresponding author

Sara A. Garcia
e-mail: saragar0012@gmail.com
University of Georgia
Department of Kinesiology
Integrative Cardiovascular Physiology Laboratory
330 River Road, Athens, Georgia, USA

Article info

Article history

- Received: 2025-04-15
- Accepted: 2025-06-24
- Published: 2025-06-30

Publisher

University of Applied Sciences in Tarnow
ul. Mickiewicza 8, 33-100 Tarnow, Poland

Financing

This research was cofunded by the University of Georgia Graduate School's Master's Fellow Award.

Conflict of interest

NTJ reports consultancies with Renaissance Periodization, LLC, separate from the submitted work. RCP – The views expressed in this paper are those of the author(s) and do not necessarily reflect the official policy of the Department of Defense, Department of the Army, US Army Medical Department, or the US government. The mention of any nonfederal entity and/or its products is not to be construed or interpreted, in any manner, as federal endorsement of that nonfederal entity or its products.

Introduction

Hormones, such as estrogen and progesterone, are essential for female health and metabolism. In females with regular mensturation, these hormones undergo monthly cyclical changes that are divided into two main phases: the follicular phase and the luteal phase. The follicular phase occurs approximately during the first 14 days of the menstrual cycle, and it is characterized by low levels of progesterone and estrogen during menstruation, followed by a steady rise in estrogen leading to ovulation.[1,2,3] The luteal phase occurs approximately during days 15 to 28 of the menstrual cycle and is characterized by high levels of both progesterone and estrogen.[1,2,3] Hormonal oral contraceptives (OC), however, reduce hormonal fluctuations by lowering natural estradiol and/or progestin levels, creating hormone-controlled phases. These phases include the 'active pill phase,' which lasts for three weeks and consists of equally dosed hormones or hormones that increase weekly for three weeks. After the active phase, the 'inactive' or 'placebo phase' follows, which lasts for a week and does not contain any hormones. This week allows for a natural rise in estrogen before repeating the 28-day pill cycle. However, these hormone levels are significantly lower than the natural eumenorrheic hormone levels throughout the month, potentially affecting exercise metabolism.[4]

Around half of elite females athletes report using hormonal contraceptives,[5,6] with 68.1% of those reporting OC use.[5] Hormonal OCs are widely accepted as a legitimate form of medication with the World Anti-Doping Agency not prohibiting its use in elite sport.[7] Contrary, only ~14% of US females, aged 15–49, use OCs.[8] The prevalence of OC use in athletic females highlights the need for sex-specific research in exercise metabolism.[9,10] Multiple narrative and systematic reviews have addressed OC use and exercise performance, concluding OC use results in no effect, or trivial reductions, on exercise performance compared to naturally menstruating females.[11-16] Interestingly, OC use appears to have small but potentially clinically meaningful effects on cardiometabolic health markers, with reports of increased plasma triglyceride levels possibly driven by increased high-density lipoprotein cholesterol at rest, but with no or minor effects on LDL cholesterol, HOMA-IR, and plasma glucose.[17,18] However, these differences do not appear to extend to whole-body exercising substrate oxidation, or differ during cycling exercise compared to naturally menstruating females.[19,20] While OC use may have minor effects on exercise performance, the known effects on metabolism are limited at this time.[14]

Metabolic flexibility is a measure of an individual's ability to match substrate demand to availability and serves to efficiently maintain fuel homeostasis.[21,22] Since estrogen is a powerful regulator of fat metabolism in females, it is plausible that metabolic flexibility may be influenced by changes in estrogen and progesterone from OC use.[23] Lower concentrations of female sex hormones during OC use may have a negative impact on metabolic flexibility.[22,25] Recently, we showed exercising fat and carbohydrate oxidation varies by oral contraceptive use dependent on the phase of menstruation.[26] While differences were not found during exercise, fat and carbohydrate oxidation was higher during recovery in naturally menstruating females compared to OC users.[26] These recovery differences between OC users and naturally menstruating females are speculated to be due to differences in pulmonary ventilation variables but need confirmation.[26]

Estrogen and progesterone also induce physiological effects that could influence cardiovascular and respiratory function. For example, progesterone stimulates minute ventilation.[27,28] Interestingly, OC use does not appear to influence maximum oxygen uptake,[16] but seems to negatively influence training adaptations in pulmonary oxygen uptake kinetics.[29] These alterations can pose significant effects on exercise responses, especially during high-intensity exercise modalities. Further investigation of how OC use affects exercising respiratory function is warranted.

This pilot study investigated the influence of chronic OC use on the exercising metabolic and respiratory response during a high-intensity interval rowing protocol in young, healthy, moderately active females. Specifically, we assessed measures of metabolic flexibility during high-intensity interval exercise (HIIE) in the inactive pill phase of the menstrual cycle for OC and in the follicular phase for non-oral contraceptive (NOC) users. We hypothesized that chronic OC use would not impact measures of metabolic flexibility or respiratory response during HIIE compared to NOC users, but may negatively impact the cardiorespiratory response to HIIE.

Methods

Ethical approval

This pilot study was approved by the WCG Institutional Review Board (study no. 1323806, approved on 24JAN2022), with written informed consent being obtained prior to any experimental procedures. This pilot study conformed to the standards set by the Declaration of Helsinki, except for registration in a database. This

research was carried out fully in accordance to the ethical standards of the *International Journal of Exercise Science*.[30]

Participant characteristics

Fifteen healthy, non-smoking, moderately active, 18- to 35-year-old adult females were recruited and completed the pilot study. Participants were classified as moderately active based on their cardiorespiratory fitness and performance characteristics. Average VO_{2peak} values fall within normative ranges for moderately active females aged 20–29, as reported by the American College of Sports Medicine (ACSM).[31] In addition, participants demonstrated average to above-average muscular endurance and flexibility, which are also consistent with ACSM normative performance standards.[31] Body composition measures reflect typical values for healthy, non-sedentary young adult women.[32] Participants self-reported if they were experiencing a normal regular menstrual cycle, i.e., menstruation approximately every 22–35 days, for at least 12 months without use of birth control or took mono- or triphasic-based oral contraceptive pills for at least 12 months, prior to pilot study enrollment. Self-reported menstrual cycle regularity has been validated and has moderate agreement with true cycle length.[33] All NOC participants completed visits within days 2–10 following the self-reported onset of their menstrual cycle.[34] For participants who were on OC pills, testing visits took place during days 2–7 of the placebo week of their medication.[35]

Exclusion criteria included biological male sex, weight loss or gain exceeding 5% in the past three months, plans to begin a weight loss or exercise program during the pilot study, age outside of 18–35 years, those with chronic hypertension (systolic BP ≥ 140 mmHg or diastolic BP ≥ 90 mmHg), diabetes, or other chronic diseases, thyroid medication, NOC medication, lipid-lowering medication, blood pressure medication, antipsychotics, supplement use (other than a daily multivitamin/mineral), or tobacco use. If a potential participant reported supplement use, they were instructed to follow a 2-weak washout phase before testing. No participants reported supplementation use; thus, no washout period was warranted.

Study design and protocol

Participants completed two trials consisting of a test day 1 visit and a test day 2 (HIIE) visit. The goal of the test day 1 visit was to confirm similar fitness levels between groups. Testing visits took place at least 48 hours apart and all visits were completed within 6 days. Participants were asked to avoid exercise for 48 hours, and caffeine and alcohol 24 hours before visits. All participants were instructed to follow their normal dietary routine, other than the specific guidance given below, for the duration of the pilot study.

Test Day 1: Visit

Participants received a pre-visit meal recommendation that consisted of 50% carbohydrate, 35% fat, and 15% protein, equating to 25% of the estimated resting energy expenditure.[36] Participants were given guidance on food selection to meet the prescribed energy content and macronutrient composition for recommended meals. Participants were requested to consume the pre-visit meal two hours prior to arriving for testing.

First, participants' height and weight (500KL Eye-Level Digital Medical Scale, Health O Meter, n.d., China), resting blood pressure (5 Series Upper Arm Blood Pressure Monitor, Omron Healthcare, Inc., Vietnam), and body composition (via dual-energy X-ray absorptiometry, Horizon® DXA System, Hologic, Inc., Marlborough, MA, USA) were measured. After those measurements, participants completed a 24-hour food recall[37] and the modified 16-question Quality-of-Life Scale (QOLS).[38] Muscular strength of the quadriceps muscle was then assessed using a Biodex System 4 Dynamometer (Model 850-230, Biodex Medical Systems, Inc., Shirley, NY, USA) by measuring the maximal voluntary isometric contraction of the dominant leg.[39] Participants then completed a standard sit-and-reach test using the Baseline Sit N' Reach Trunk Flexibility Box (Fabrication Enterprises Inc., White Plains, NY, USA) to assess the flexibility of the lower back and hamstrings.[40] Upper body muscular endurance was then measured as the maximal number of modified pushups that could be completed in a continuous effort.[41] Adequate rest (10–15 minutes) was provided between assessments to ensure recovery and reduce the influence of fatigue on subsequent assessments.

To conclude this visit, participants completed a maximal exercise test to determine the highest rate of oxygen consumption (VO_{2peak}) on a rower ergometer (AssaultRowerElite, Assault Fitness, Carlsbad, CA, USA). Prior to test start, participants were given a familiarization brief. Participants were instructed on proper rowing form, according to the four-phase technique: catch, drive, finish, and recovery. Following familiarization, participants were fitted with a mask to collect respiratory gasses for analysis via indirect calorimetry (True-One 2400, Parvo Medics, Sandy, UT, USA), a heart rate monitor (Polar, Polar Electro Inc., Lake Success, NY, USA), and two muscle oxygen monitors (Moxy Monitor, Hutchinson, MN, USA). One Moxy Monitor was placed

on the right Vastus Lateralis, approximately 2/3 of the way down from the greater trochanter to the patella, and the second was placed on their Biceps Brachii, approximately 1/2 of the way from the humeral head to the coronoid fossa. Additionally, Moxy Monitors were secured using elastic pre-wrap and an elastic bandage to reduce transient light. Participants started with a pace of 3:00 (500 meters every 3 minutes) for 3 minutes, followed by a pace increase every 2 minutes until participants could no longer sustain the prescribed pace (Suppl., Table S1). When participants could not keep a given pace, they were asked to complete a 1-minute all-out effort before cessation. At the cessation of exercise, subjects were asked to report their perceived exertion (RPE) rating, and blood lactate concentration was measured (Lactate Plus, Nova Biomedical, Waltham, MA, USA). VO_{2peak} was confirmed by satisfying three of the following requirements: (i) an RER ≥ 1.10, (ii) a plateau in oxygen consumption (change < 100 mL × min^{-1} in the last 30 s stage), (iii) a maximum heart rate ≥ 85% of the age-predicted maximal heart rate, (iv) RPE ≥ 18, and (v) blood lactate concentration ≥ 7 mmol.[42]

Test Day 2: High-intensity interval exercise visit

The evening before this visit, participants received a dinner recommendation that consisted of 50% carbohydrate, 35% fat, and 15% protein, equating to 30% of their estimated resting energy expenditure.[36] For this visit, participants were instructed to eat their pre-fasting meal the evening prior to their visit, and they arrived after an overnight fast (~10–12 hours). Upon arrival at the lab, the participant's body weight was measured.

As a marker of microvascular function, resting microvascular reactivity was measured in the forearm muscles using continuous wave near-infrared spectroscopy (CW-NIRS) (Portamon, Artinis Medical Systems, Elst, Netherlands).[43] Participants were asked to lay supine, and the right arm was extended and positioned at an angle of ~80° from the torso. A rapid inflation pneumatic cuff (Hokanson SC5, D.E; Hokanson Inc., Bellevue, WA, USA) was positioned immediately proximal to the olecranon process to provide a stimulus of forearm ischemia. The CW-NIRS probe was placed distal to the occlusion cuff on the flexors of the forearm. Following 2 minutes of continuous baseline recording, the forearm cuff was inflated (~220 mmHg) for 5 minutes. Upon cuff deflation, recording continued for 3 minutes. CW-NIRS signals were sampled at 10 Hz, and laser diodes were sampled at three wavelengths (905, 850, and 760 nm) corresponding to the absorption wavelengths of oxygenated hemoglobin.

Participants then completed a bout of HIIE on a rower ergometer (AssaultRowerElite, Assault Fitness, Carlsbad, CA, USA). Participants were fitted with a mask to collect respiratory gases, a heart rate monitor, and two muscle oxygen monitors, as described above. Participants began with a warmup at a rate of perceived exertion of 11/20 for 3 minutes.[44] Participants received verbal feedback during the warmup, to keep their heart rate at approximately 55% of their age-predicted max heart rate (estimated max heart rate = 220 – age). The warm-up was followed by a 3 minute rest period. There were 4 high-intensity bouts. Each high-intensity bout was 3 minutes long followed by a 3 minute rest bout. During the high-intensity bouts, participants were instructed to perform with maximum effort. During rest, participants were instructed to remain seated on the rower. Blood lactate concentration (Lactate Plus, Nova Biomedical, Waltham, MA, USA), and blood glucose concentration (OneTouch UltraMini, LifeScan, Inc., Milpitas, CA, USA) were measured via fingerstick at baseline, the end of every high and low bout, immediately post-, 3 minutes post-, and 10 minutes post-exercise. RPE was additionally taken at all these time points.

Data analyses

Moxy Monitor data, e.g., muscle oxygen saturation percent (SmO_2%), was exported and analyzed as 10-second averages. SmO_2% data were averaged for the duration of each interval (H1–H4) and recovery periods (L1–3 and 10-min recovery). One participant's (NOC group) heart rate data was removed due to improper reading.

Microvascular reactivity CW-NIRS signals were analyzed using an electronic spreadsheet and were assessed by comparing changes in O_2Hb during the reactive hyperemia phase.[43] Time for O_2Hb signal to reach 50% of peak post-occlusion hyperemia level ($T_{1/2}$) is reported as a marker for microvascular function.[45]

The macronutrient oxidation rate was assessed for the entire HIIE bout using equations developed by Frayn:[46]

$$\text{Fat (g/min)} = [1.67 \times VO_2\ (\text{L/min})] - [1.67 \times VCO_2\ (\text{L/min})]$$

$$\text{Carbohydrate (g/min)} = [4.55 \times VCO_2\ (\text{L/min})] - [3.21 \times \times VO_2\ (\text{L/min})].$$

Oxidation values calculated as negative values were replaced with 0.00000001. Macronutrient oxidation rates were averaged for the duration of each interval. Total grams of substrate oxidized were calculated by multiplying the average rate of substrate oxidation by time duration and summed for total exercise (H1–H4), recovery (L1–3 and 10-min recovery), and full session.

Metabolic flexibility between stages, e.g., H1 to L1, was calculated as the absolute difference. Total metabolic flexibility was calculated as the average absolute differences from each stage across the entire HIIE bout.

Statistical analyses

Student's *t*-tests were conducted to assess the statistical significance between groups (OC vs. NOC) on demographics, VO_{2peak}, lower body muscular strength, upper body muscular endurance, flexibility, microvascular function, 24-hour food recall, QOLS, calculations of total fat and carbohydrate oxidized during HIIE, and total metabolic flexibility. A one-way repeated-measures ANOVA was conducted to assess the statistical significance between groups (OC vs. NOC) for HIIE data with Tukey HSD post hoc analysis. Effect size was determined by partial eta squared (η_p^2), where a value of 0.01 represents a small effect, 0.06 represents a medium effect and > 0.14 represents a large effect. Assumptions of normality and homogeneity of variance were verified for all outcome measures using Shapiro-Wilk's Tests ($P > 0.05$), visually examining Q-Q plots, and Levene's Tests ($P > 0.05$). Statistical significance was accepted at $P \leq 0.05$. Data presented as means ±SD. All statistical analyses were performed with SPSS Statistics version 28.0 (IBM Corp., Armonk, NY, USA).

Power analysis

Due to the exploratory nature of this pilot study and the lack of data on exercise metabolism during HIIE, an a priori power analysis was based on studies with similar outcome measures. Previous comparisons of exercising metabolic response used 8 OC vs. 8 NOC[47] and 11 OC vs. 10 NOC.[25] Previous comparisions of exercising cardiorespiriatory response use 8 OC vs. 8 NOC[48] and 6 OC vs 10 NOC.[49] Therefore, to detect differences between groups of similar magnitudes across the pilot study's outcomes, we aimed to recruit 8 females per group (16 total). Our final participant total was 15 females, 6 OC vs. 9 NOC.

Results

Participant demographics

Fifteen healthy, non-smoking, moderately active females completed both visits (Table 1). Participants' weight did not significantly change during their time in the pilot study (OC: $P = 0.534$, NOC: $P = 0.247$). One OC participant did not complete a 24-hour food recall, and one NOC participant's data was removed due to incomplete recording. Participants consumed similar macronutrient percentages between groups (OC: n = 5, 46.16 (11.40)% CHO, 35.00 (13.49)% fat, and 18.84 (3.38)% protein vs. NOC: n = 8, 51.33 (10.67)% CHO, 29.14 (8.38)% fat, and 19.53 (5.46)% protein, $P = 0.05$), but OC participants reported a higher caloric intake compared to NOC participants (OC: 1946.7 (248.67) kcals/day vs. NOC: 1499.49 (347.38) kcals/day, $P = 0.030$).

Table 1. Participant demographics

Category	OC (n = 6)	NOC (n = 9)	*P*-value	Effect size (η_p^2)
Age (y)	21.67 (3.33)	22.00 (4.15)	0.872	0.002
Weight (kg)	67.50 (9.61)	64.67 (13.80)	0.671	0.014
BMI (kg/m^2)	23.87 (2.69)	24.19 (3.54)	0.853	0.003
Body Fat (%)	29.17 (6.63)	27.74 (5.06)	0.645	0.017
Fat-Free Mass (kg)	47.43 (5.62)	46.27 (7.66)	0.757	0.008
Waist-to-Hip Ratio	0.75 (0.06)	0.75 (0.02)	0.967	0.000
Bone Mineral Density (g/cm^2)	1.12 (0.12)	1.12 (0.15)	0.986	0.000
Systolic Blood Pressure (mmHg)	121.00 (20.90)	113.33 (9.60)	0.350	0.067
Diastolic Blood Pressure (mmHg)	73.50 (15.04)	71.00 (6.16)	0.659	0.015
VO_{2peak} (L/min)	2.41 (0.44)	2.35 (0.42)	0.785	0.006
VO_{2peak} (ml/kg/min)	36.25 (7.94)	37.03 (6.69)	0.839	0.003
Quadriceps Torque (Nm/kg FFM)	3.90 (1.01)	3.84 (0.68)	0.894	0.001
Push-ups (repetitions)	32.00 (9.94)	30.44 (12.21)	0.800	0.005
Sit-n-Reach (in)	20.38 (1.17)	20.64 (2.33)	0.806	0.005
Microvascular Function ($T_{1/2}$)	10.06 (3.12)	11.70 (5.4)	0.515	0.033
Quality of Life Scale	**103.33 (1.21)**	**96.33 (6.14)**	**0.017**	**0.033**

Note: OC – oral contraception users; NOC – non-oral contraception users; η_p^2 – partial eta squared; y – years; kg – kilogram; m – meters; % – percent; g – gram; cm – centimeter; mmHg – millimeters of mercury; L – liters; min – minute; Nm – newton-meter; FFM – fat-free mass; in – inches; $T_{1/2}$ – halftime.

Metabolic flexibility

Metabolic flexibility for absolute fat oxidation (g/min) had a a significant time effect during HIIE [time: F(1, 18) = 16.413, $P < 0.01$, $\eta_p^2 = 0.558$], but no group or interaction effects [group: F(1, 18) = 0.282, $P = 0.605$, $\eta_p^2 = 0.021$; time × group: F(1,18) = 0.590, $P = 0.804$, $\eta_p^2 = 0.043$]. Metabolic flexibility for relative fat oxidation (g/kg/min) also had a significant time effect during HIIE [time: F(1, 18) = 4.825, $P = 0.049$, $\eta_p^2 = 0.897$], but no group or interaction effects [group: F(1, 18) = 0.483, $P = 0.499$, $\eta_p^2 = 0.036$; time × group: F(1, 18) = 0.800, $P = 0.637$, $\eta_p^2 = 0.590$]. Lastly, metabolic flexibility for fat oxidation relative to fat-free mass (g/kg FFM/min) also had a significant time effect during HIIE [time: F(1, 18) = 6.177, $P = 0.030$, $\eta_p^2 = 0.917$], but no group or interaction effects [group: F(1, 18) = 0.471, $P = 0.504$, $\eta_p^2 = 0.035$; time × group: F(1, 18) = 0.817, $P = 0.628$, $\eta_p^2 = 0.959$]. No differences were found in absolute fat (g/min) [t(13) = 0.531, $P = 0.605$, $\eta_p^2 = 0.021$; Figure 1c], relative to body weight fat (g/kg/min) [t(13) = 0.695, $P = 0.499$, $\eta_p^2 = 0.036$; Figure 1f], or relative to fat-free mass fat (g/kg FFM/ min) [t(13) = 0.687, $P = 0.504$, $\eta_p^2 = 0.035$, Figure 1i] average total metabolic flexibility.

When looking at absolute carbohydrate metabolic flexibility (g/min), there was a significant time effect during HIIE [time: F(1, 18) = 27.256, $P < 0.001$, $\eta_p^2 = 0.677$], but no group or interaction effects [group: F(1, 18) = 1.025, $P = 0.330$, $\eta p_2 = 0.073$; time × group: F(1, 18) = 0.379, $P = 0.943$, $\eta_p^2 = 0.028$]. Similarly, metabolic flexibility relative to total body weight carbohydrate metabolic flexibility (g/kg/min), had a significant time effect during HIIE [time: F(1, 18) = 28.707, $P < 0.001$, $\eta_p^2 = 0.688$], but no group or interaction effects [group: F(1, 18) = 0.273, $P = 0.610$, $\eta_p^2 = 0.021$; time × group: F(1, 18) = 0.427, $P = 0.918$, $\eta_p^2 = 0.032$]. Lastly, when looking at relative to fat-free mass carbohydrate metabolic flexibility (g/kg FFM/min), there was also a significant time effect during HIIE [time: F(1, 18) = 30.177, $P < 0.001$, $\eta_p^2 = 0.699$], but no group or interaction effects [group: F(1, 18) = 0.540, $P = 0.475$, $\eta_p^2 = 0.040$; time × group: F(1, 18) = 0.446, $P = 0.907$, $\eta_p^2 = 0.033$]. There were also no differences in absolute carbohydrate (g/min) [t(13) = −1.013, $P = 0.330$, $\eta_p^2 = 0.073$; Figure 2c], relative to body weight carbohydrate (g/kg/min) [t(13) = −0.523, $P = 0.610$, $\eta_p^2 = 0.020$; Figure 2f], or relative to fat-free mass carbohydrate (g/kg FFM/ min) [t(13) = −0.735 $P = 0.475$, $\eta_p^2 = 0.040$; Figure 2i] average total metabolic flexibility.

Fat oxidation

When looking at absolute fat oxidation (g/min), there was a significant time effect during HIIE [time: F(1, 20) = 9.186, $P = 0.023$, $\eta_p^2 = 0.958$; Figure 1a], but no group or interaction effects [group: F(1, 20) = 0.410, $P = 0.533$, $\eta_p^2 = 0.031$; time × group: F(1, 20) = 0.522, $P = 0.816$, $\eta_p^2 = 0.566$; Figure 1a]. No differences were found in total fat oxidized (g) for the entire HIIE [t(13) = 0.694, $P = 0.905$, $\eta_p^2 = 0.035$], for the exercising periods of HIIE [t(13) = 0.713, $P = 0.828$, $\eta_p^2 = 0.036$], or for the rest periods of HIIE [t(13) = 0.371, $P = 0.125$, $\eta_p^2 = 0.010$]; Figure 1b.

When fat oxidation was quantified relative to total body weight (g/kg/min), there was a significant time effect during HIIE [time: F(1, 20) = 9.765, $P = 0.021$, $\eta_p^2 = 0.961$; Figure 1d], but no group or interaction effects [group: F(1, 20) = 0.626, $P = 0.443$, $\eta_p^2 = 0.046$; time × group: F(1, 20) = 0.629, $P = 0.749$, $\eta_p^2 = 0.611$; Figure 1d]. No differences were found in total body weight relative fat oxidized (g/kg) for the entire HIIE [t(13) = 0.787, $P = 0.943$, $\eta_p^2 = 0.045$], for the exercising periods of HIIE [t(13) = 0.829, $P = 0.816$, $\eta_p^2 = 0.050$], or for the rest periods of HIIE [t(13) = 0.332, $P = 0.257$, $\eta_p^2 = 0.008$]; Figure 1e.

When fat oxidation was quantified relative to fat-free mass (g/kg FFM/min), there was a significant time effect during HIIE [time: F(1, 20) = 12.596, $P = 0.013$, $\eta_p^2 = 0.969$; Figure 1g], but no group or interaction effects [group: F(1, 20) = 0.638, $P = 0.439$, $\eta_p^2 = 0.047$; time × group: F(1, 20) = 0.647, $P = 0.738$, $\eta_p^2 = 0.618$; Figure 1g]. No differences were found in total fat-free mass relative fat oxidized (g/kg FFM) for the entire HIIE [t(13) = 0.791, $P = 0.827$, $\eta_p^2 = 0.046$], for the exercising periods of HIIE [t(13) = 0.827, $P = 0.697$, $\eta_p^2 = 0.050$], or for the rest periods of HIIE [t(13) = 0.363, $P = 0.201$, $\eta_p^2 = 0.010$]; Figure 1h.

Carbohydrate oxidation

When looking at absolute carbohydrate oxidation (g/min),there was a significant time effect during HIIE [time: F(1, 20) = 13.969, $P = 0.011$, $\eta_p^2 = 0.972$; Figure 2a], but no group or interaction effects [group: F(1, 20) = 0.010, $P = 0.923$, $\eta_p^2 = 0.001$; time × group: F(1, 20) = 2.923, $P = 0.156$, $\eta_p^2 = 0.880$; Figure 2a]. No differences were found in total carbohydrate oxidized (g) for the entire HIIE [t(13) = −0.004, $P = 0.682$, $\eta_p^2 = 0.000$], for the exercising periods of HIIE [t(13) = −0.515, $P = 0.503$,

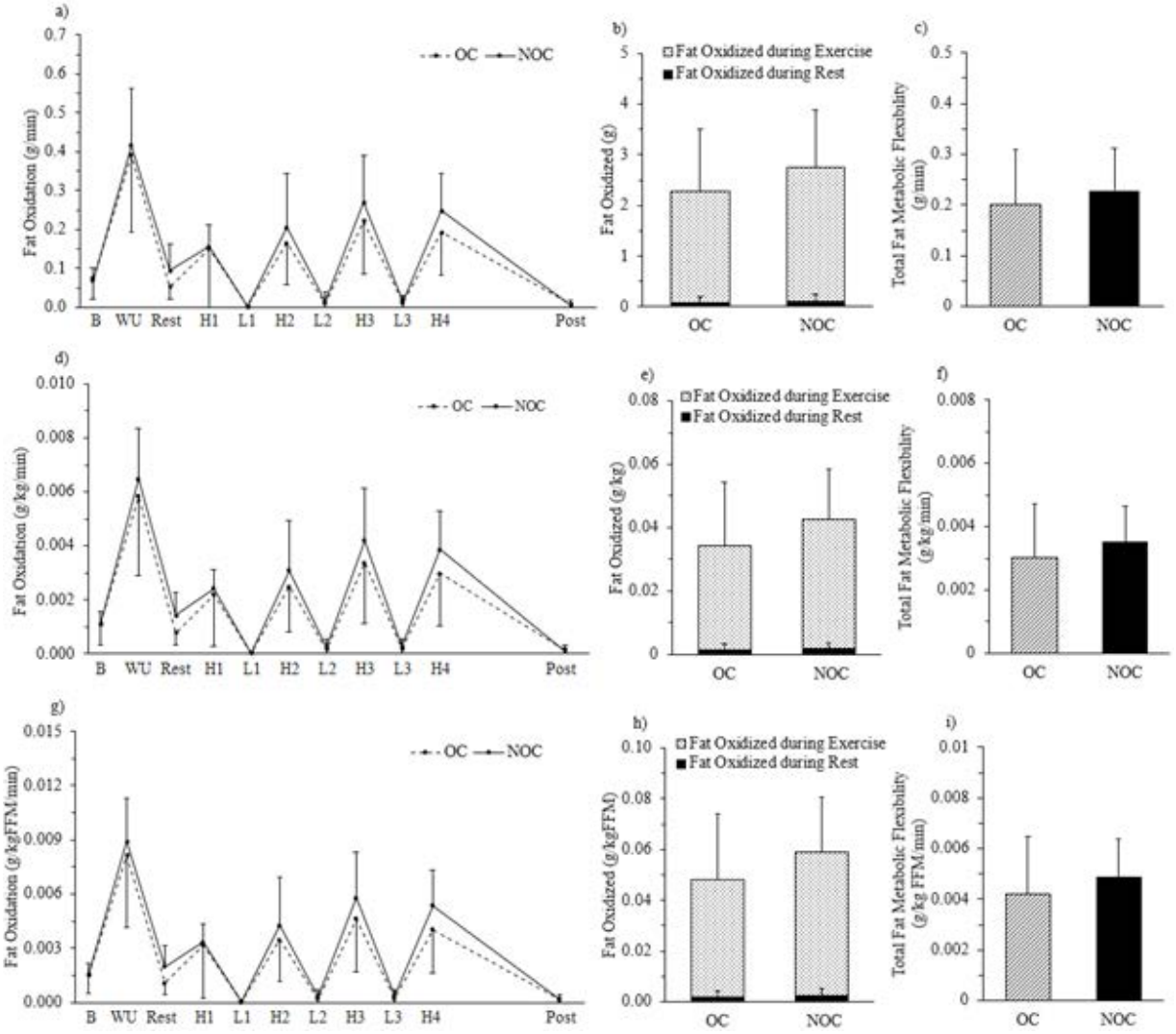

Figure 1 Fat oxidation response during HIIE

Note: OC – oral contraception users; NOC – non-oral contraception users; B – baseline; WU – warm-up; Rest – 3-minute rest; H1–4 – high effort bout; L1–3 – low effort bout (rest); Post – 10 minutes post-exercise; g – gram; kg – kilogram; FFM – fat-free mass; min – minute.

$\eta_p^2 = 0.020$], or for the rest periods of HIIE [t(13) = 0.567, $P = 0.862$, $\eta_p^2 = 0.024$]; Figure 2b.

When carbohydrate oxidation was quantified relative to total body weight (g/kg/min), there was a significant time effect during HIIE [time: F(1, 20) = 28.726, $P = 0.003$, $\eta_p^2 = 0.986$; Figure 2d], but no group or interaction effects [group: F(1, 20) = 0.090, $P = 0.768$, $\eta_p^2 = 0.007$; time × group: F(1, 20) = 4.391, $P = 0.083$, $\eta_p^2 = 0.917$; Figure 2d]. No differences were found in total body weight relative carbohydrate oxidized (g/kg) for the entire HIIE [t(13) = 0.372, $P = 0.968$, $\eta_p^2 = 0.011$], for the exercising periods of HIIE [t(13) = −0.113, $P = 0.830$, $\eta_p^2 = 0.001$], or for the rest periods of HIIE [t(13) = 0.904, $P = 0.652$, $\eta_p^2 = 0.059$]; Figure 2e.

For carbohydrate oxidation quantified relative to fat-free mass (g/kg FFM/min), there was a significant time effect during HIIE [time: F(1, 20) = 35.483, $P = 0.002$, $\eta_p^2 = 0.989$; Figure 2g], but no group or interaction effects [group: F(1, 20) = 0.034, $P = 0.858$, $\eta_p^2 = 0.003$; time × group: F(1, 20) = 3.664, $P = 0.111$, $\eta_p^2 = 0.902$; Figure 2g]. No differences were found in total fat-free mass relative carbohydrate oxidized (g/kg FFM) for the entire HIIE [t(13) = 0.269, $P = 0.985$, $\eta_p^2 = 0.006$], for the exercising periods of HIIE [t(13) = −0.281, $P = 0.749$, $\eta_p^2 = 0.006$], or for the rest periods of HIIE [t(13) = 0.903, $P = 0.617$, $\eta_p^2 = 0.059$]; Figure 2h.

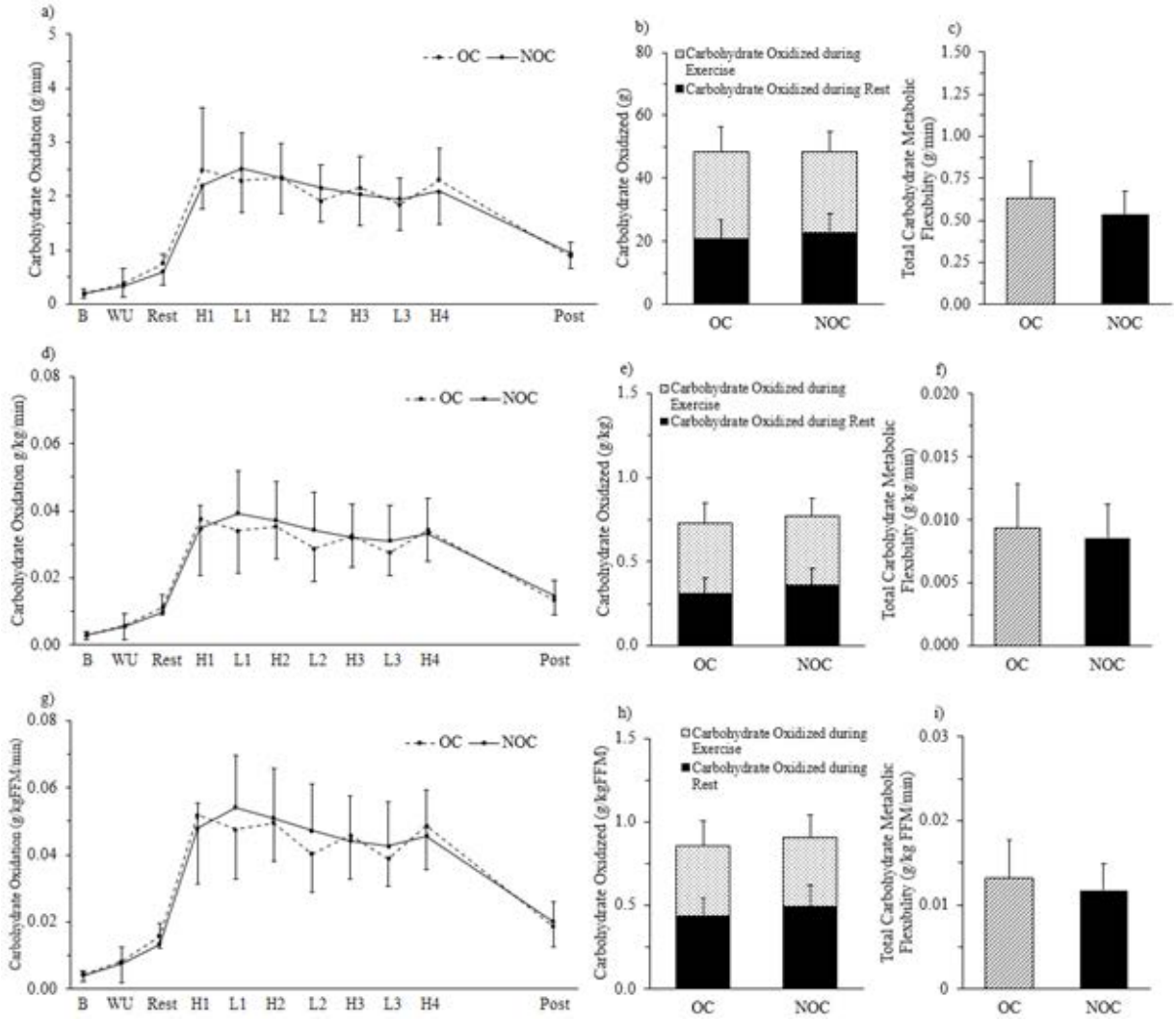

Figure 2 Carbohydrate oxidation response during HIIE

Note: OC – oral contraception users; NOC – non-oral contraception users; B – baseline; WU – warm-up; Rest – 3-minute rest; H1–4 – high effort bout; L1–3 – low effort bout (rest); Post – 10 minutes post-exercise; g – gram; kg – kilogram; FFM – fat-free mass; min – minute.

Blood lactate and glucose response

There were no significant time, group, or interaction effects for blood lactate concentration (mmol/L) during HIIE [time: F(1, 18) = 5.854, $P = 0.052$, $\eta_p^2 = 0.929$; group: F(1, 18) = 0.004, $P = 0.953$, $\eta_p^2 < 0.001$; time × group: F(1, 18) = 0.752, $P = 0.670$, $\eta_p^2 = 0.628$; Figure 3a]. There were also no significant time, group, or interaction effects for blood glucose (mg/dL) during HIIE [time: F(1, 18) = 3.786, $P = 0.078$, $\eta_p^2 = 0.872$; group: F(1, 18) = 0.001, $P = 0.975$, $\eta_p^2 < 0.001$; time × group: F(1, 18) = 2.653, $P = 0.148$, $\eta_p^2 = 0.827$; Figure 3b].

Respiratory response

Absolute VO_2 (L/min) had a significant time effect during HIIE [time: F(1, 20) = 44.498, $P = 0.001$, $\eta_p^2 = 0.991$; Figure 4a], but no group or interaction effects [group: F(1, 20) = = 0.002, $P = 0.963$, $\eta_p^2 = 0.000$; time × group: F(1, 20) = 0.216, $P = 0.977$, $\eta_p^2 = 0.351$; Figure 4a]. Similarly, body weight relative VO_2 (ml/kg/min) had a significant time effect during HIIE [time: F(1, 20) = 5.633, $P = 0.055$, $\eta_p^2 = 0.934$; Figure 4b], but no group or interaction effects [group: F(1, 20) = 0.142, $P = 0.712$, $\eta_p^2 = 0.011$; time × group: F(1, 20) = 0.211, $P = 0.979$, $\eta_p^2 = 0.346$; Figure 4b].

VO_2 relative to fat-free mass (ml/kg FFM/min) also had a significant time effect during HIIE [time: F(1, 20) = 103.876, $P < 0.001$, $\eta_p^2 = 0.996$; Figure 4c], but no group or interaction effects [group: F(1, 20) = 0.326, $P = 0.578$, $\eta_p^2 = 0.024$; time × group: F(1, 20) = 0.167, $P = 0.990$, $\eta_p^2 = 0.295$; Figure 4c].

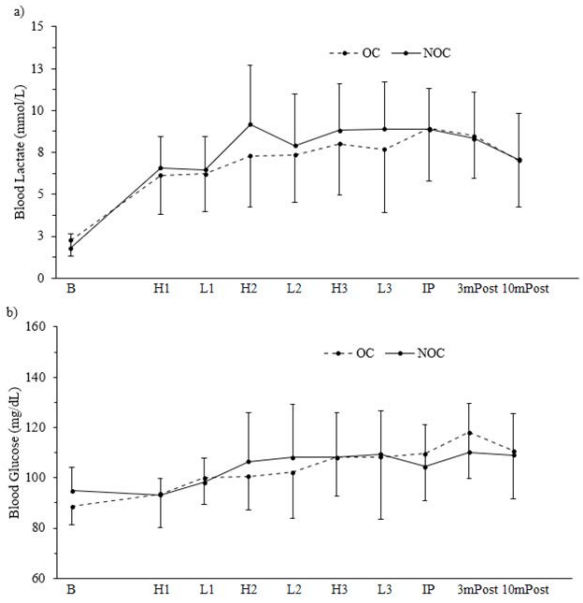

Figure 3 Blood lactate and glucose response during HIIE

Note: OC – oral contraception users; NOC – non-oral contraception users; B – baseline; H1–4 – high effort bout; L1–3 – low effort bout (rest); IP – immediate post-exercise; 3mPost – 3 minutes post-exercise; 10 mPost – 10 minutes post-exercise; mmol – millimole; mg – milligram; L – liter; dL – deciliter.

When looking at absolute VCO_2 (L/min) during HIIE, there was a significant time effect [time: F(1, 20) = 32.968, $P = 0.002$, $\eta_p^2 = 0.988$; Figure 4d], but no group or interaction effects [group: F(1, 20) = 0.000, $P = 0.997$, $\eta_p^2 = 0.000$; time × group: F(1, 20) = 1.005, $P = 0.546$, $\eta_p^2 = 0.715$; Figure 4d]. There was also a significant time effect for respiratory exchange ratio (RER) during HIIE [time: F(1, 20) = 30.698, $P = 0.002$, $\eta_p^2 = 0.987$; Figure 4e], but no group or interaction effects [group: F(1, 20) = 0.020, $P = 0.891$, $\eta_p^2 = 0.002$; time × group: F(1, 20) = 0.474, $P = 0.846$, $\eta_p^2 = 0.542$; Figure 4e]. Ventilation (VE, L/min) also had a significant time effect during HIIE [time: F(1, 20) = 64.383, $P < 0.001$, $\eta_p^2 = 0.994$; Figure 4f], but no group or interaction effects [group: F(1, 20) = 0.367, $P = 0.555$, $\eta_p^2 = 0.027$; time × group: F(1, 20) = 1.799, $P = 0.300$, $\eta_p^2 = 0.818$; Figure 4f].

Fractional content of expired oxygen (F_EO_2) also displayed a significant time effect, as well as a group interaction effect during HIIE [time: F(1, 20) = 90.760, $P < 0.001$, $\eta_p^2 = 0.996$; time × group: F(1, 20) = 6.609, $P = 0.042$, $\eta_p^2 = 0.943$; Figure 4g], but no group effect [group: F(1, 20) = 0.533, $P = 0.478$, $\eta_p^2 = 0.039$; Figure 4g]. Post-hoc analysis revealed no other group differences, $P > 0.05$. Lastly, there was a significant time effect for fractional content of expired carbon dioxide (F_ECO_2) during HIIE [time: F(1, 20) = 12.101, $P = 0.014$, $\eta_p^2 = 0.968$; Figure 4h], but no group or interaction effects [group: F(1, 20) = 0.671, $P = 0.428$, $\eta_p^2 = 0.049$; time × group: F(1, 20) = 0.993, $P = 0.551$, $\eta_p^2 = 0.713$; Figure 4h].

Skeletal muscle oxygenation, rate of perceived exertion, and heart rate response

There was a significant time effect for biceps brachii SmO_2% during HIIE [time: F(1, 20) = 13.043, $P = 0.012$, $\eta_p^2 = 0.970$; Suppl., Figure S1a], but no group or interaction effects [group: F(1, 20) = 0.028, $P = 0.870$, $\eta_p^2 = 0.002$; time × group: F(1, 20) = 0.933, $P = 0.580$, $\eta_p^2 = 0.700$; Suppl., Figure S1a]. There was a no significant time, group, or interaction effects for vastus lateralis SmO_2% during HIIE [time: F(1, 20) = 3.627, $P = 0.113$, $\eta_p^2 = 0.901$; group: F(1, 20) = 0.016, $P = 0.900$, $\eta_p^2 = 0.001$; time × group: F(1, 20) = 0.717, $P = 0.696$, $\eta_p^2 = 0.642$; Suppl., Figure 1b]. There was a significant time effect for rating of perceived exertion during HIIE [time: F(1, 16) = 31.181, $P < 0.001$, $\eta_p^2 = 0.977$; Suppl., Figure S1c), but no group or interaction effects [group: F(1, 16) = 0.052, $P = 0.823$, $\eta_p^2 = 0.004$; time*group: F(1, 16) = 0.508, $P = 0.815$, $\eta_p^2 = 0.404$; Suppl., Figure S1c). There was a significant time effect for heart rate (bpm) during HIIE [time: F(1, 20) = 909.866, $P < 0.001$, $\eta_p^2 = 1.000$; Suppl., Figure S1d], but no group or interaction effects [group: F(1, 20) = 0.260, $P = 0.619$, $\eta_p^2 = 0.021$; time × group: F(1, 20) = 1.213, $P = 0.490$, $\eta_p^2 = 0.802$; Suppl., Figure S1d].

Discussion

The current pilot study examined the effects of prolonged OC use on exercising metabolic and cardiorespiratory responses to HIIE in young, healthy, moderately active females, comparing them to those who experience a natural menstrual cycle. Our primary finding – that there were no significant differences in metabolic or cardiorespiratory responses to HIIE between OC and NOC users – provides further evidence that hormonal contraceptive use does not impair exercise performance or physiology in this population. One of the strengths of our pilot study is sample groups had

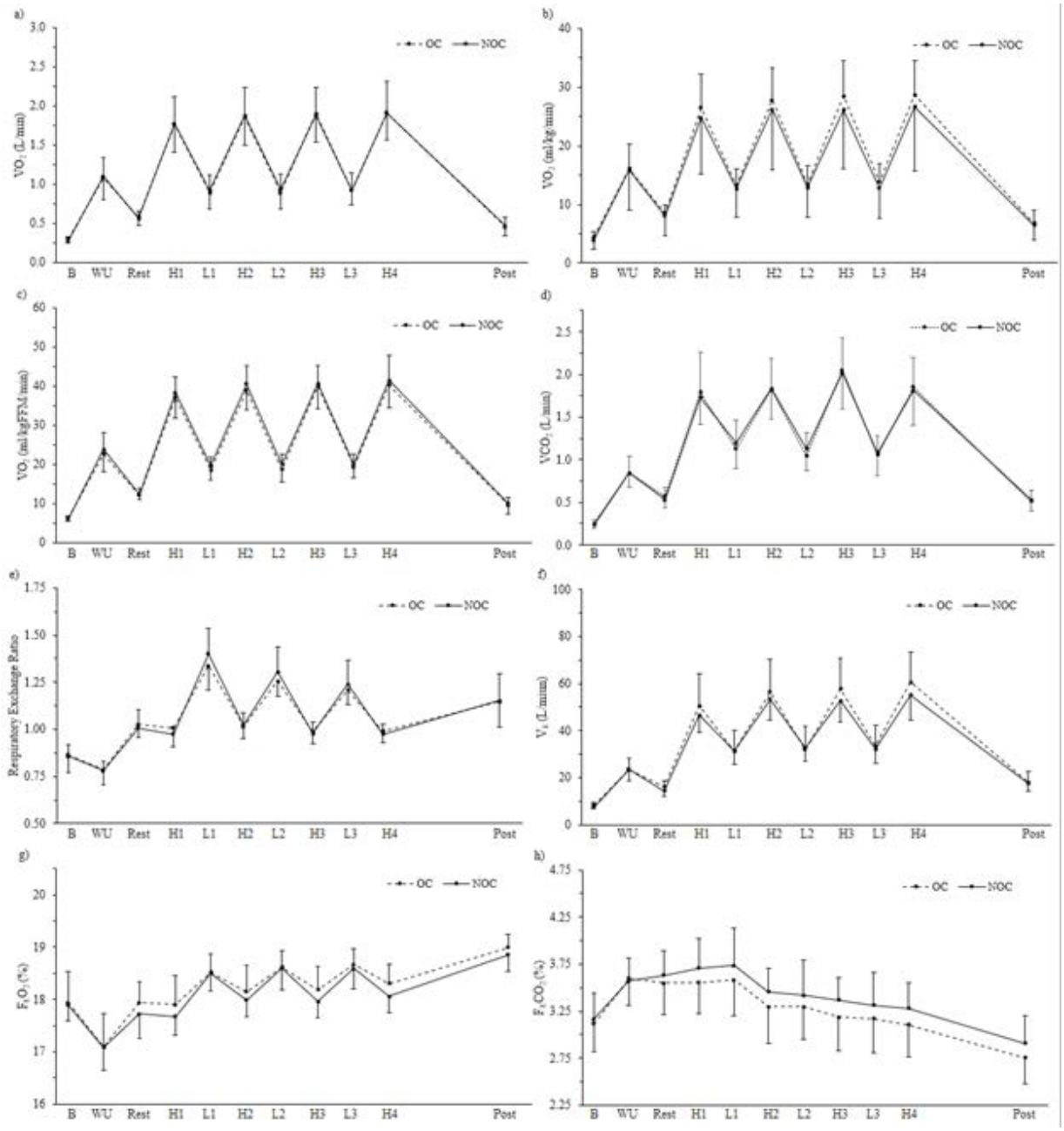

Figure 4. Respiratory response during HIIE

Note: OC – oral contraception users; NOC – non-oral contraception users; B – baseline; WU – warm-up; Rest – 3-minute rest; H1–4 – high effort bout; L1–3 – low effort bout (rest); Post – 10 minutes post-exercise; VO_2 – volume of oxygen; VCO_2 – volume of carbon dioxide; V_E – ventilation; F_EO_2 – fractional content of expired oxygen; F_ECO_2 – fractional content of expired carbon dioxide; ml – milliter; L – liter; min – minute; kg – kilogram; FFM – fat-free mass.

a similar fitness status, thus eliminating any confounding effects of fitness or body composition on exercise outcomes. This design feature strengthens the validity of our comparisons. Additionally, these findings are consistent with previous research indicating that OC use has little to no negative impact on overall exercise metabolism.[11-16] However, they do contradict the hypothesis that OC use may affect female cardiorespiratory response to exercise.

Oral contraceptive use can affect lipid kinetics and triglyceride mobilization and may decrease glucose kinetics in females during exercise.[20,24,50-53] However, these effects do not seem to extend to the whole-body oxidation level.[19,20,24,50-53] It is worth noting that studies

examining the impact of menstrual cycle or OC use on substrate oxidation during exercise are generally conducted at low to moderate exercise intensities in the range of 30–60% VO_{2max}.[24,50,53-55] Our findings extend this work to higher-intensity interval exercise, where substrate oxidation demands are rapidly fluctuating. Our previous work indicated that there are no significant differences in whole-body substrate oxidation during cycling-based HIIE between OC and NOC users.[26] Our data also indicate that there are no differences in substrate oxidation between OC and NOC users during HIIE. These results further support OC use does not significantly impact whole-body exercise metabolism at higher intensities. In addition, our data suggest that metabolic flexibility is similar across the follicular phase and inactive pill phase for both OC and NOC users. This suggests that the capacity to adapt substrate oxidation in response to exercise intensity transitions is preserved in OC users. Lastly, our data here suggests that OC use does not negatively impact exercise metabolic flexibility, which has been previously observed by our group.[26] Taken together, our findings are consistent with current literature and suggest that OC use does not have any negative effects on exercising metabolic flexibility or HIIE metabolism.

The effects of OC use on exercise blood lactate concentrations are not yet fully understood. Studies have shown that OC users may experience higher levels of blood lactate concentration during exercise compared to NOC users.[20,35] Other studies have found no differences in blood lactate concentrations across different menstrual phases and OC cycles.[23,56] Our group has shown no significant differences in blood lactate concentration response to HIIE between females taking OCs and those not.[26] Our present data further suggests that OC use does not lead to elevated blood lactate concentrations during exercise. Additionally, we found no differences in blood glucose levels between the two groups during HIIE. These findings suggest that OC use does not impair glycemic control or promote anaerobic stress during high-intensity work. Given the inverse relationship between blood lactate concentration and fat oxidation,[57,58] our pilot study's lack of differences in blood glucose levels is reasonable. NOC and OC groups had similar responses to HIIE that were not impacted by OC use. This pilot data helps dispute concerns that OC use may hinder metabolic recovery or increase glycolytic reliance during an acute bout of HIIE during the follicular and inactive pill phases.

The impact of OC use on cardiorespiratory response to exercise is also not fully understood. Studies comparing OC and NOC users across various exercise protocols have shown mixed results.[59] Some studies suggest no differences in outcomes such as VO_2, VCO_2, VE, RER, and heart rate.[25,35,49,60-62] However, another study found that OC users may not reach a plateau during VO_{2max} testing,[49] which is otherwise observed in NOC users. Additionally, OC use may lead to lower absolute VO_{2max} after training.[11] Elevated VE/VCO_2 values have been observed in OC users during rowing exercise, but no other differences in cardiorespiratory response were found[61,62] which may result in lower cardiorespiratory efficiency during higher hormonal phases.[54] We only found elevated VE/VO_2 in the recovery period following HIIE in OC users compared to NOC users. The lack of differences in other cardiorespiratory variables suggests that OC use, at least in the inactive pill phase, may not affect the cardiorespiratory response during HIIE. Importantly, our data suggest that OC users can maintain comparable aerobic efficiency and recovery to NOC users during an acute bout of HIIE. Future studies should investigate the response of HIIE training in OC users across different phases of the menstrual and contraceptive pill cycles as well as confirm these findings.

Our pilot study had limitations. First, the OC group reported significantly greater calorie intake in the 24-hour dietary recall, which may have contributed to greater glycogen storage and more energy for testing sessions. Future studies should control lead-in dietary intake. Second, we were unable to complete blood hormone analysis for the current dataset. Assessments of estrogen and progesterone in our participants to address individual differences and responses would have been informative. Moreover, knowledge of hormone concentrations across the menstrual cycle could have been used as a factor in our statistical analysis or to assess relationships between hormone levels and metabolic responses. In future research, the role of circulating estrogen and progesterone should be evaluated in the metabolic and cardiorespiratory response in females during exercise. Third, our pilot study may be underpowered, due to our final participant total of 15 females (6 OC vs. 9 NOC). Therefore, future studies should confirm and expand our findings. Lastly, the current pilot study only assessed the impact of the menstrual cycle vs. contraceptive use during the early follicular and inactive pill phases. Future studies should assess this impact across the entire menstrual and contraceptive pill cycles.

Conclusion

Our pilot study showed there were no differences between OC vs. NOC users during HIIE in exercising metabolic or cardiorespiratory response in young, healthy,

moderately active females. All measures, including fat oxidation, carbohydrate oxidation, metabolic flexibility, blood lactate concentration, blood glucose concentrations, and cardiorespiratory fitness, were similar between the two groups. These findings have practical implications for exercise professionals and health practitioners, as they suggest that oral contraceptive use does not compromise metabolic or cardiovascular response to high-intensity interval exercise. This supports the inclusion of OC users in high-intensity training programs without the need for cycle-specific modifications, at least during the early follicular and inactive pill phases. Future research should investigate the response to HIIE across the full menstrual cycle and oral contraceptive cycles as well as in other contraceptive types.

Acknowledgements

Authors would like to thank Dr. Kevin M. McCully for assistance in data interpretation and Sarah E. Watson for help with data collection.

References

[1] Birch K. Circamensal rhythms in physical performance. *Biol Rhythm Res*. 2000;31(1):1-14. doi: 10.1076/0929-1016(200002)31:1;1-0;FT001.

[2] Reilly T. The menstrual cycle and human performance: An overview. *Biol Rhythm Res*. 2000;31(1):29-40. doi: 10.1076/0929-1016(200002)31:1;1-0;FT029.

[3] Janse de Jonge X, Thompson B, Han A. Methodological recommendations for menstrual cycle research in sports and exercise. *Med Sci Sports Exerc*. 2019;51(12):2610-2617. doi: 10.1249/MSS.0000000000002073.

[4] Christin-Maitre S. History of oral contraceptive drugs and their use worldwide. *Best Pract Res Clin Endocrinol Metab*. 2013;27(1):3-12. doi: 10.1016/j.beem.2012.11.004.

[5] Martin D, Sale C, Cooper SB, Elliott-Sale KJ. Period prevalence and perceived side effects of hormonal contraceptive use and the menstrual cycle in elite athletes. *Int J Sports Physiol Perform*. 2018;13(7):926-932. doi: 10.1123/ijspp.2017-0330.

[6] Oxfeldt M, Dalgaard LB, Jorgensen AA, Hansen M. Hormonal contraceptive use, menstrual dysfunctions, and self-reported side effects in elite athletes in Denmark. *Int J Sports Physiol Perform*. 2020;15(10):1377-1384. doi: 10.1123/ijspp.2019-0636.

[7] World Anti-Doping Agency. *WADA's 2025 Prohibited List now in force*. https://www.wada-ama.org/en/news/wadas-2025-prohibited-list-now-force. Accessed September 12, 2024.

[8] Daniels K, Abma JC. *Current contraceptive status among women aged 15–49: United States, 2017–2019*. Hyattsville, MD: National Center for Health Statistics; 2020.

[9] Oosthuyse T, Strauss JA, Hackney AC. Understanding the female athlete: Molecular mechanisms underpinning menstrual phase differences in exercise metabolism. *Eur J Appl Physiol*. 2023;123(3):423-450. doi: 10.1007/s00421-022-05090-3.

[10] Lebrun CM. Effect of the different phases of the menstrual cycle and oral contraceptives on athletic performance. *Sports Med*. 1993;16(6):400-430. doi: 10.2165/00007256-199316060-00005.

[11] Lebrun CM, Petit MA, McKenzie DC, Taunton JE, Prior JC. Decreased maximal aerobic capacity with use of a triphasic oral contraceptive in highly active women: a randomised controlled trial. *Br J Sports Med*. 2003;37(4):315-320. doi: 10.1136/bjsm.37.4.315.

[12] Burrows M, Peters CE. The influence of oral contraceptives on athletic performance in female athletes. *Sports Med*. 2007;37(7):557-574. doi: 10.2165/00007256-200737070-00001.

[13] Thompson B, Almarjawi A, Sculley D, Janse de Jonge X. The effect of the menstrual cycle and oral contraceptives on acute responses and chronic adaptations to resistance training: A systematic review of the literature. *Sports Med*. 2020;50(1):171-185. doi: 10.1007/s40279-019-01219-1.

[14] Elliott-Sale KJ, McNulty KL, Ansdell P, et al. The effects of oral contraceptives on exericse performance in women: A systematic review and meta-analysis. *Sports Med*. 2020;50(10):1785-1812. doi: 10.1007/s40279-020-01317-5.

[15] Nolan D, McNulty KL, Manninen M, Egan B. The effect of hormonal contraceptive use on skeletal muscle hypertrophy, power and strength adaptations to resistance exercise training: A systematic reveiw and multilevel meta-analysis. *Sports Med*. 2024;54(1):105-125. doi: 10.1007/s40279-023-01911-3.

[16] Schumpf LF, Braun C, Peric A, et al. The influence of the menstrual cycle and hormonal contraceptives on cardiorespiratory fitness in physically active women: A systematic review and meta-analysis. *Heliyon*. 2023;9(6):e17049. doi: 10.1016/j.heliyon.2023.e17049.

[17] Wang Q, Wurtz P, Auro K, et al. Effects of hormonal contraception on systemic metabolism: cross-sectional and longitudinal evidence. *Int J Epidemiol*. 2016;45(5):1445-1457. doi: 10.1093/ije/dyw147.

[18] Silva-Bermudez LS, Toloza FJK, Perez-Matos MC, de Souza RJ, Banfield L, Vargas-Villanueva A, Mendivil CO. Effects of oral contraceptives on metabolic parameters in adult premenopausal women: A meta-analysis. *Endocr Connect*. 2020;9(10):978-998. doi: 10.1530/EC-20-0423.

[19] Williams JS, Stone JC, Masood Z, Bostad W, Gibala MJ, MacDonald MJ. The impact of natural menstrual cycle and oral contraceptive pill phase on substrate

oxidation during rest and acute submaximal aerobic exercise. *J Appl Physiol*. 2023;135(3):642-654. doi: 10.1152/japplphysiol.00111.2023.

[20] Suh S-H, Casazza GA, Horning MA, Miller BF, Brooks GA. Effects of oral contraceptives on glucose flux and substrate oxidation rates during rest and exercise. *J Appl Physiol.* 2003;4(1):285-294. doi: 10.1152/japplphysiol.00693.2002.

[21] Smith RL, Soeters MR, Wüst RCI, Houtkooper RH. Metabolic flexibility as an adaptation to energy resources and requirements in health and disease. *Endocr Rev*. 2018;39(4):489-517. doi: 10.1210/er.2017-00211.

[22] Storlien L, Oakes ND, Kelley DE. Metabolic flexibility. *Proc Nutr Soc*. 2004;63(2):363-368. doi: 10.1079/PNS2004349.

[23] Hackney AC. Menstrual cycle hormonal changes and energy substrate metabolism in exercising women: A perspective. *Int J Environ Res Public Health*. 2021;18(19):10024. doi: 10.3390/ijerph181910024.

[24] Isacco L, Thivel D, Pelle AM, et al. Oral contraception and energy intake in women: impact on substrate oxidation during exercise. *Appl Physiol Nutr Metab*. 2012;37(4):646-656. doi: 10.1139/h2012-031.

[25] Isacco L, Thivel D, Pereira B, Duclos M, Boisseau N. Maximal fat oxidation, but not aerobic capacity, is affected by oral contraceptive use in young healthy women. *Eur J Appl Physiol*. 2015;115(5):937-945. doi: 10.1007/s00421-014-3075-7.

[26] Olenick AA, Pearson RC, Jenkins NT. Impact of aerobic fitness status, menstrual cycle phase, and oral contraceptive use on exercise substrate oxidation and metabolic flexibility in females. *Appl Physiol Nutr Metab*. 2023;49(1):93-104. doi: 10.1139/apnm-2023-0101.

[27] Schoene RB, Robertson HT, Pierson DJ, Peterson AP. Respiratory drives and exercise in menstrual cycles of athletic and nonathletic women. *J Appl Physiol Respir Environ Exerc Physiol*. 1981;50(6):1300-1305. doi: 10.1152/jappl.1981.50.6.1300.

[28] Dombovy ML, Bonekat HW, Williams TJ, Staats BA. Exercise performance and ventilatory response in the menstrual cycle. *Med Sci Sports Exerc*. 1987;19(2):111-117.

[29] Schaumberg MA, Stanley J, Jenkins DG, et al. Oral contraceptive use influences on-kinestic adaptations to sprint interval training in recreationally-active women. *Front Physiol*. 2020;11:629. doi: 10.3389/fphys.2020.00629.

[30] Navalta JW, Stone WJ, Lyons TS. Ethical issues relating to scientific discovery in exercise science. *Int J Exerc Sci*. 2019;12(1):1-8. doi: 10.70252/EYCD6235.

[31] American College of Sports Medicine. *ACSM's Guidelines for Exercise Testing and Prescription*. 11th ed. Wolters Kluwer; 2021.

[32] Heyward VH, Wagner DR. *Applied Body Composition Assessment*. 2nd ed. Human Kinetics; 2004.

[33] Jukic AMZ, Weinberg CR, Wilcox AJ, McConnaughey DR, Hornsby P, Baird DD. Accuracy of reporting of menstrual cycle length. *Am J Epidemiol*. 2008;167(1):25-33. doi: 10.1093/aje/kwm265.

[34] Sims ST, Heather AK. Myths and methodologies: Reducing scientific design ambiguity in studies comparing sexes and/or menstrual cycle phases. *Exp Physiol*. 2018;103(10):1309-1317. doi: 10.1113/ep086797.

[35] Mattu AT, Iannetta D, MacInnis MJ, Doyle-Baker PK, Murias JM. Menstrual and oral contraceptive cycle phases do not affect submaximal and maximal exercise responses. *Scand J Med Sci Sports*. 2020;30(3):472-484. doi: 10.1111/sms.13590.

[36] Mifflin MD, St Jeor ST, Hill LA, Scott BJ, Daugherty SA, Koh YO. A new predictive equation for resting energy expenditure in healthy individuals. *Am J Clin Nutr*. 1990;51(2):241-247. doi: 10.1093/ajcn/51.2.241.

[37] Subar AF, Thompson FE, Kipnis V, et al. Comparative validation of the Block, Willett, and National Cancer Institute food frequency questionnaires: The Eating at America's Table Study. *Am J Epidemiol*. 2001;154(12):1089-1099. doi: 10.1093/aje/154.12.1089.

[38] Burckhardt CS, Anderson KL. The Quality of Life Scale (QOLS): Reliability, validity, and utilization. *Health Qual Life Outcomes*. 2003;1:60. doi: 10.1186/1477-7525-1-60.

[39] Sosnoff JJ, Voudrie SJ, Ebersole KT. The effect of knee joint angle on torque control. *J Mot Behav*. 2010;42(1):5-10. doi: 10.1080/00222890903269237.

[40] Liemohn W, Sharpe G, Wasserman JF. Criterion related validity of the Sit-and-Reach Test. *J Strength Cond Res*. 1994;8(2):91-94.

[41] Baumgartner TA, Oh S, Chung H, Hales D. Objectivity, reliability, and validity for a Revised Push-Up Test protocol. *Meas Phys Educ Exerc Sci*. 2002;6(4):225-242. doi: 10.1207/S15327841MPEE0604_2.

[42] Wasserman K, Hansen JE, Sue DY, Sringer WW, Whipp BJ. *Principles of exercise testing and interpretation: Including pathophysiology and clinical applications*. 4th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2004.

[43] Willingham TB, Southern WM, McCully KK. Measuring reactive hyperemia in the lower limb using near-infrared spectroscopy. *J Biomed Optics*. 2016;21(9):091302. doi: 10.1117/1.JBO.21.9.091302.

[44] Borg GA. Psychophysical bases of perceived exertion. *Med Sci Sports Exerc*. 1982;14(5):377-381.

[45] Cho AR, Lee HJ, Kim HJ, et al. Microvascular reactivity measured by dynamic near-infrared spectroscopy following induction of general anesthesia in health patients: Observation of age-related change. *Int J Med Sci*. 2021;18(5):1096-1103. doi: 10.7150/ijms.52433.

[46] Frayn KN. Calculation of substrate oxidation rates in vivo from gaseous exchange. *J Appl Physiol Respir Environ Exerc Physiol*. 1983;55(2):628-634. doi: 10.1152/jappl.1983.55.2.628.

[47] Bemben DA, Boileau RA, Bahr JM, Nelson RA, Misner JE. Effects of oral contraceptives on hormonal and

metabolic responses during exercise. *Med Sci Sports Exerc*. 1992;24(4):434-441.

[48] Quinn KM, Billaut F, Bulmer AC, Minahan CL. Cerebral oxygenation declines but does not impair peak oxygen uptake during incremental cycling in women using oral contraceptives. *Eur J Appl Physiol*. 2018;118(11):2417-2427. doi: 10.1007/s00421-018-3968-y.

[49] Gordon D, Scruton A, Barnes R, Baker J, Prado L, Merzbach V. The effects of menstrual cycle phase on the incidence of plateau at VO_{2max} and associated cardiorespiratory dynamics. *Clin Physiol Funct Imaging*. 2018;38(4):689-698. doi: 10.1111/cpf.12469.

[50] Bonen A, Haynes FW, Graham TE. Substrate and hormonal responses to exercise in women using oral contraceptives. *J Appl Physiol*. 1991;70(5):1917-1927. doi: 10.1152/jappl.1991.70.5.1917.

[51] Casazza GA, Jacobs KA, Suh S-H, Miller BF, Horning MA, Brooks GA. Menstrual cycle phase and oral contraceptive effects on triglyceride mobilization during exercise. *J Appl Physiol*. 2004;97(1):302-309. doi: 10.1152/japplphysiol.00050.2004.

[52] Casazza GA, Suh SH, Miller BF, Navazio FM, Brooks GA. Effects of oral contraceptives on peak exercise capacity. *J Appl Physiol*. 2002;93(5):1698-1702. doi: 10.1152/japplphysiol.00622.2002.

[53] Jacobs KA, Casazza GA, Suh SH, Horning MA, Brooks GA. Fatty acid reesterification but not oxidation is increased by oral contraceptive use in women. *J Appl Physiol*. 2005;98(5):1720-1731. doi: 10.1152/japplphysiol.00685.2004.

[54] Barba-Moreno L, Cupeiro R, Romero-Parra N, Janse de Jonge XAK, Peinado AB. Cardiorespiratory responses to endurance exercise over the menstrual cycle and with oral contraceptive use. *J Strength Cond Res*. 2022;36(2):392-399. doi: 10.1519/JSC.0000000000003447.

[55] Berend JZ, Brammeier MR, Jones NA, Holliman SC, Hackney AC. Effect of the menstrual cycle phase and diet on blood lactate responses to exercise. *Biol Sport*. 1994;11(4):241-248.

[56] De Souza MJ, Maguire MS, Rubin KR, Maresh CM. Effects of menstrual phase and amenorrhea on exercise performance in runners. *Med Sci Sports Exerc*. 1990;22(5):575-580. doi: 10.1249/00005768-199010000-00006.

[57] Broskey NT, Zou K, Dohm GL, Houmard JA. Plasma lactate as a marker for metabolic health. *Exerc Sport Sci Rev*. 2020;48(3):119-124. doi: 10.1249/jes.0000000000000220.

[58] Brooks GA. Lactate: link between glycolytic and oxidative metabolism. *Sports Med*. 2007;37(4-5):341-343. doi: 10.2165/00007256-200737040-00017.

[59] D'Souza AC, Wageh M, Williams JS, et al. Menstrual cycle hormones and oral contraceptives: A multimethod systems physiology-based review of their impact on key aspects of female physiology. *J Appl Physiol*. 2023;135(6):1284-1299. doi: 10.1152/japplphysiol.00346.2023.

[60] Lynch NJ, Nimmo MA. Effects of menstrual cycle phase and oral contraceptive use on intermittent exercise. *Eur J Appl Physiol Occup Physiol*. 1998;78(6):565-572. doi: 10.1007/s004210050462.

[61] Vaiksaar S, Jürimäe J, Mäestu J, et al. No effect of menstrual cycle phase and oral contraceptive use on endurance performance in rowers. *J Strength Cond Res*. 2011;25(6):1571-1578. doi: 10.1519/JSC.0b013e3181df7fd2.

[62] Vaiksaar S, Jürimäe J, Mäestu J, et al. Phase of oral contraceptive cycle and endurance capacity of rowers. *Percept Mot Skills*. 2011;113(3):764-772. doi: 10.2466/05.06.Pms.113.6.764-772.

Supplementary materials

Table S1. Rower VO_2peak Protocol

Pace	Time
3:00	3 minutes
2:45	2 minutes
2:30	2 minutes
2:15	2 minutes
2:00	2 minutes
1:50	2 minutes
1:45	2 minutes
1:40	2 minutes
1:35	2 minutes
1:32	2 minutes
1:30	As long as possible

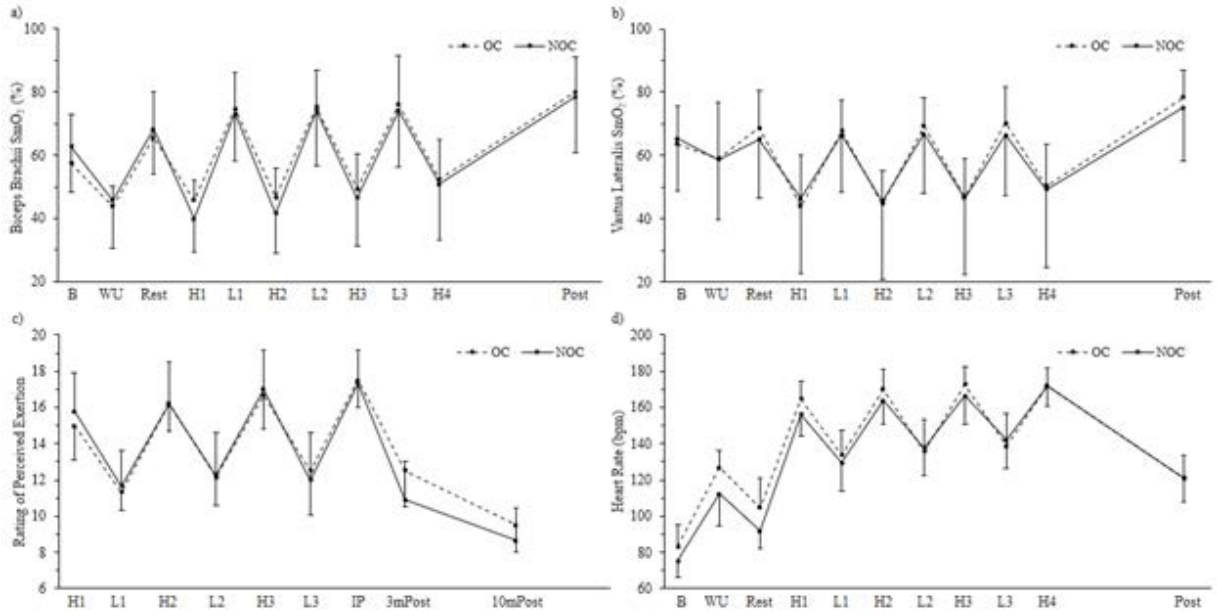

Figure S1. SmO_2%, rate of perceived exertion, and hear rate response during HIIE

Note: OC – oral contraception users; NOC – non-oral contraception users; B – baseline; WU – warm-up; Rest – 3-minute rest; H1–4 – high effort bout; L1–3 – low effort bout (rest); IP – immediate post-exercise; 3mPost – 3 minutes post-exercise; 10mPost – 10 minutes post-exercise; Post – 10 minutes post-exercise; SmO_2 – muscle oxygen saturation percent; % – percent; bpm – beats per minute; heart rate OC n = 6, NOC n = 8.

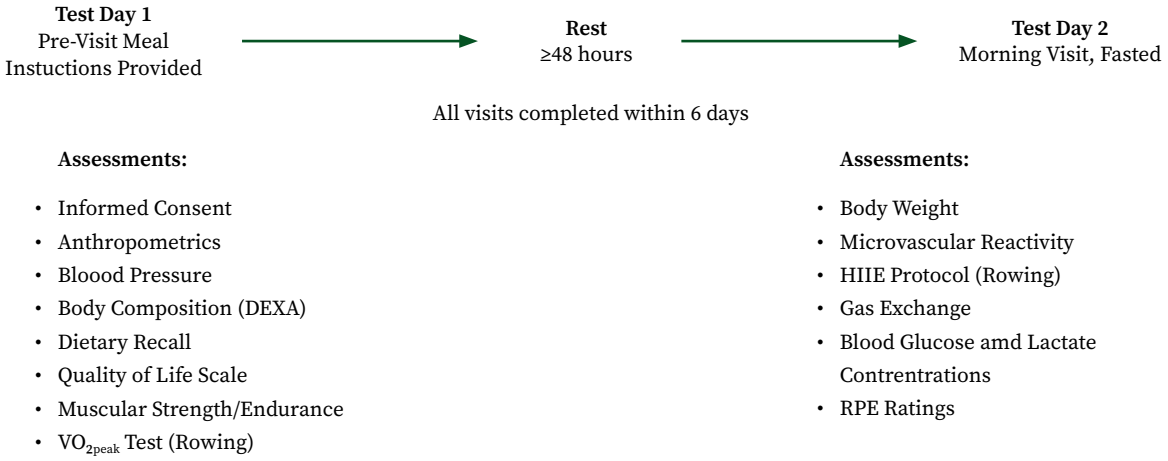

Figure S2. Overview of experimental design and testing procedures