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Running title: Oral contraceptives and training adaptation

Abstract

Purpose: Oral contraceptive (OC) use reduces peak aerobic capacity ($\dot{V}O_{2peak}$), however, whether it also influences adaptations to training has yet to be determined. This study aimed to examine the influence of OC use on peak performance [peak power output (PPO)] and physiological adaptations [$\dot{V}O_{2peak}$ and peak cardiac output ($\dot{Q}_{peak}$)] following sprint interval training (SIT) in recreationally-active women.

Methods: Women taking an OC (n=25) or experiencing natural regular menstrual cycles (MC; n=16) completed an incremental exercise test to assess $\dot{V}O_{2peak}$, PPO, and $\dot{Q}_{peak}$ before, immediately after, and four weeks following 12 sessions of SIT. The SIT consisted of 10, one-minute efforts at 100-120% PPO in a 1:2 work:rest ratio.

Results: Though $\dot{V}O_{2peak}$ increased in both groups following SIT (both $p<0.001$), the MC group showed greater improvement (OC +8.5%; MC +13.0%; $p=0.010$). Similarly, $\dot{Q}_{peak}$ increased in both groups, with greater improvement in the MC group (OC +4.0%; MC +16.1%; $p=0.013$). PPO increased in both groups (OC +13.1%; MC +13.8%; NS). All parameters decreased four weeks after SIT cessation, but remained elevated from pre-training levels; the OC group showed more sustained training effects in $\dot{V}O_{2peak}$ (OC -4.0%; MC -7.7%; $p=0.010$).

Conclusion: SIT improved peak exercise responses in recreationally-active women. However, OC use dampened $\dot{V}O_{2peak}$ and $\dot{Q}_{peak}$ adaptation. A follow-up period indicated that OC users had spared $\dot{V}O_{2peak}$ adaptations, suggesting that OC use may influence the time course of physiological training adaptations. Therefore, OC use should be verified, controlled for, and considered when interpreting physiological adaptations to exercise training in women. **Key words:** aerobic capacity; athletic performance; cardiac output; detraining; female; ovarian hormones

Introduction

Exogenous hormones introduced through oral contraceptive (OC) use may influence endurance exercise performance by reducing maximal exercise capacity (8, 24, 27), increasing fat-mass (5) and changing the metabolic (23), thermoregulatory (36), cardiovascular (12) and ventilatory (9) responses to exercise. While OC use has been shown to reduce maximal aerobic capacity ($\dot{V}O_{2max}$) in both highly trained (24) and recreationally active (8, 27) women, whether physiological, cardiovascular and performance adaptations to endurance exercise training are influenced by OC use remains unclear.

Near-maximal to maximal interval training, classified as either high-intensity interval training (80-100% peak heart rate) or sprint interval training (SIT) (target at or above 100% maximal aerobic capacity) (42) has been extensively studied in both trained and untrained men, with results showing rapid improvements in peak aerobic capacity ($\dot{V}O_{2peak}$) and endurance performance in as little as two weeks (1, 7, 18). Relatively few studies have investigated adaptations to SIT in women (1, 14, 15, 39, 41). Of these, only one (41) controlled for menstrual cycle phase, by measuring $\dot{V}O_{2peak}$ in the follicular phase (determined by onset of menstruation), yet did not verify serum ovarian hormone concentrations and excluded OC users. Elevated oestradiol and progestin levels in OCs attenuate submaximal cardiovascular responses to exercise (25), potentially by altering fluid retention mechanisms and blood volume changes (37), and may therefore alter the responses to exercise training in recreationally active women. Whether elevated exogenous oestradiol and progestin levels in OCs may alter responses to exercise training and maintenance of adaptations following training in recreationally-active women remains to be determined.

To date, only one study has investigated maintenance of training adaptations following SIT in women (31). Two weeks following completion of SIT, the authors found significant decreases in $\dot{V}O_{2max}$ towards baseline, with only 24% of the $\dot{V}O_{2max}$ improvements retained. OC use, menstrual status and/or menstrual cycle phase were not considered/reported.

Therefore, the primary aim of this study was to assess the influence of OC use, compared to natural menstruation, on peak physiological, cardiovascular and performance adaptations to SIT in recreationally-active women while stringently verifying ovarian hormone concentrations. The secondary aim was to investigate the influence of OC use, compared to natural menstruation, on the sustainability of gained adaptations following a four-week follow-up.

Methods

Overview

Following a baseline assessment of serum hormone levels and $\dot{V}O_{2peak}$, peak power output (PPO), peak cardiac output ($\dot{Q}_{peak}$), peak stroke volume (SV_{peak}), peak heart rate (HR_{peak}), peak rating of perceived exertion (RPE_{peak}), peak respiratory quotient ($R\dot{Q}$) and minute ventilation ($\dot{V}e\dot{V}CO_{2slope}$), participants with either natural menstrual cycles (no current hormone contraception) or using an OC completed a four-week SIT program with reassessment of all measures following completion of the training program and after a four-week follow-up period.

Participants

Healthy, recreationally-active (regularly completing at least 150 minutes of self-reported moderate to vigorous physical activity per week, but not currently training for, or competing at

state or national level sport competition) women, who were either long-term (minimum six months uninterrupted) monophasic combined OC users (n=25) or experiencing regular natural menstrual cycles (MC; n=22) participated in the study. All experimental procedures were approved by the Human Research Ethics Committee of The University of Queensland, ethical clearance #2012001438, and all participants provided written informed consent.

Nutrition, hydration and exercise control measures

Prior to all experimental trials, participants were required to: (a) complete a 24 h food diary and consume, as closely as possible, the same types and quantities of food and beverages the day before testing; (b) fast overnight (≥8 h); (c) consume a standardised moderate carbohydrate (1.5 $g \cdot kg^{-1}$ body mass carbohydrate) pre-trial meal 1 h prior to arrival at the laboratory for testing; (d) abstain from caffeine, alcohol and other stimulants and depressants for 24 h, as well as record any additional medications or supplements; and (e) maintain a euhydrated state, avoid hot, humid conditions and record the volume of water consumed.

Participants were encouraged to maintain their normal physical activity levels throughout the study; however, were asked to refrain from strenuous physical activity for 24 h prior to each trial to ensure maximal effort. A physical activity questionnaire was completed prior to each exercise testing session to monitor participants' activity levels throughout the testing and training period. On the days of testing, participants were requested to arrive at the laboratory in a rested state. A pre-trial preparation checklist was completed and signed by participants upon arrival at the laboratory to confirm compliance to pre-testing requirements.

Hormone verification and testing

All MC participants completed a menstrual cycle diary adapted from Prior, Vigna and Alojada (30) for three consecutive cycles to determine average cycle length, calculated as the number of days between the onset of consecutive menses. The menstrual diary determined approximate days of follicular and luteal phases, and ovulation (28). Participants taking an OC mapped their cycle based on their pill packaging, with day one of the cycle coinciding with the first inactive (sugar) pill of the package; if a participant reported missing two or more consecutive pills in one cycle, testing was delayed by one cycle until adherence was confirmed.

Urinary ovulation prediction testing was performed during the experimental cycle to verify cycle phase and ovulatory status in the MC group and confirm cycle control by exogenous hormones in the OC group. Participants were provided a home urine ovulation prediction testing kit (Discover® 7-Day Pregnancy Planning kit, Church and Dwight Australia Pty Ltd.) and instructed to follow the manufacturer's directions to perform ovulation prediction testing for seven consecutive days during one cycle. Participants visually inspected the test strip and the result was confirmed by the lead researcher via photographic record. Two days following the urinary luteinising hormone surge, ovulation was assumed to have occurred, with the mid-luteal phase beginning approximately six to eight days following ovulation. An absence of the luteinising hormone surge during the menstrual cycle (non-OC use) indicated absence of ovulation. In this case, testing was delayed (n=3) by a further cycle until a positive ovulation prediction test was recorded. If three consecutive non-ovulatory cycles (n=0) were experienced by participants in the MC group, participants were excluded from the study.

MC participants performed testing during the estimated mid-luteal phase, six to eight days following a positive ovulation prediction test ovulation (29). OC participants performed testing in the final two weeks of the active pill phase (days 15 to 28). On the day of the trial, venous blood (12 mL) was sampled from an antecubital vein for later measurement of serum hormone concentrations. These methods are described in more detail in Schaumberget al. (34).

Body composition

Height and body mass were measured using a stadiometer (Seca, Birmingham, UK) and electronic scales (A&D Mercury, Pty Ltd., Thebarton, AUS), respectively. Body composition was assessed by dual-energy x-ray absorptiometry (Hologic Discovery W, QDR 4500A, Waltham, Mass., USA). Scans were analysed using software (APEX version 3.3) provided by the manufacturer (Hologic, Bedford, Va., USA) and according to the manufacturer's instructions. The coefficients of variation (CV) in our laboratory for whole body mass, lean body mass, fat mass and body fat percentage are 0.1%, 0.4%, 1.2% and 1.2%, respectively.

Measurement of peak aerobic capacity and peak power output

A $\dot{V}O_{2peak}$ familiarisation session was completed prior to the first experimental trial to minimise any learning effects and ensure participant familiarity with the protocol. The $\dot{V}O_{2peak}$ protocol involved participants performing a five-minute self-selected warm up prior to a continuous incremental (25 $W \cdot min^{-1}$) exercise test on an electronically-braked cycle ergometer (Lode Excalibur Sport, Quinton) to determine $\dot{V}O_{2peak}$, ventilatory threshold, $R\dot{Q}$, $\dot{V}e\dot{V}CO_{2slope}$ and PPO. Before each test, the O_2 and CO_2 analysers were calibrated as recommended by the manufacturer. Participants continued until volitional fatigue, whereby the required cadence could

not be maintained despite strong verbal encouragement. Heart rate and rate of perceived exertion (6) were recorded each minute and respiratory gas exchange was continuously recorded via automated indirect calorimetry (Parvo Medics' TrueOne® 2400 Indirect Calorimetry System, Utah, USA) for calculation of ventilatory parameters. For the incremental test, data was averaged in 15-second epochs. $\dot{V}O_{2peak}$ was defined as the highest $\dot{V}O_2$ value attained during a 15-second period (33, 38).

Measurement of cardiovascular parameters

During exercise, heart rate (HR), stroke volume (SV) and cardiac output ($\dot{Q}$) were measured continuously using impedance cardiology (PhysioFlow®, Manatec Biomedical, France) (10, 32); this method has been described elsewhere (10). Two sets of electrodes (Skintact FS-50, Leonhard Lang Gmbh, Austria) - one transmitting, one sensing - were applied above the supra-clavicular fossa at the left base of the neck, and along the xiphoid process. Another two electrodes were used to monitor a single electrocardiographic signal (ECG; CM5 position). Blood pressure was assessed (Digital blood pressure monitor, UA-767, A&D Instruments Ltd., UK) as part of standard calibration process for the PhysioFlow® prior to the incremental exercise test. HR, SV, and $\dot{Q}$ data were sampled at 15-second intervals (38). The coefficient of variation for SV and $\dot{Q}$ during repeated cycle ergometer $\dot{V}O_{2peak}$ tests in healthy, fit men, assessed using the PhysioFlow® has been reported as 3.6 and 3.4%, respectively (22).

Sprint interval training protocol

Participants completed three supervised SIT sessions per week for four weeks, with a minimum of 36 h between sessions. Following a five-minute standardised warm-up at an intensity of 50 W

and a self-selected revolutions per minute (RPM), participants completed the SIT protocol comprising one minute of work followed by two minutes of passive recovery in a 1:2 work:rest ratio (19, 31). The work interval intensity was self-selected at the maximal sustainable effort between 100-120% of PPO determined in the baseline peak exercise test. Participants completed 10 one-minute repetitions, totalling 10 minutes of work per session, with a total time commitment of 40 minutes per session. Peak heart rate, , RPE, average power output and PPO were recorded for each interval and used to calculate protocol compliance. All exercise sessions were completed on an air- and magnetically-braked cycle ergometer (Wattbike Ltd, Nottingham, England). Following completion of each SIT session participants completed a five-minute active cool-down on the cycle ergometer at a self-selected intensity.

Follow-up period

Following completion of the four-week SIT program, participants were instructed to return to the physical activity levels they were undertaking previous to the SIT protocol. Physical activity was monitored via a questionnaire (Active Australia Survey, Australian Institute of Health and Welfare, 2003) following the duration of the follow-up period. Participants completed testing procedures identical to baseline four weeks following completion of the SIT training program. Participants were excluded from the follow-up assessment if they commenced or ceased an OC in the preceding month or fell pregnant.

Blood sampling, storage and analysis

Venous blood was collected into prepared vacuum tubes containing K3EDTA or micronised silica until centrifugation. The serum tubes (micronised silica) were allowed to clot at room

temperature, and the plasma tubes (K3EDTA) were stored on ice. After 30 min, samples were centrifuged at 1100 x G for 10 min at 4° C. Serum and plasma was removed, placed into separate 0.4 mL aliquots and stored at -80° C until later analysis. Plasma samples were analysed for oestradiol, progesterone and testosterone, whilst serum samples were analysed for sex-hormone binding globulin (SHBG) using a Cobas e411 electrochemilumescence immunoassay autoanalyser (Roche Diagnostics, Germany) and manufacturer-recommended Elecsys assays. Manufacturer-supplied reagents were used, and instruments calibrated according to the manufacturer's instructions. The CVs in our laboratory for oestradiol-II, progesterone, testosterone and SHBG are 3.1%, 5.1%, 4.8% and 3.1%, respectively.

Statistical analysis

A sample size calculation indicated that to detect a 3.5 $mL \cdot kg^{-1} \cdot min^{-1}$ change in $\dot{V}O_{2peak}$ (1 MET) with a SD of 3.5 $mL \cdot kg^{-1} \cdot min^{-1}$, alpha=0.05 and power=80% (ES=1), and 30% participant withdrawal prior to post-testing, a total of 44 participants would be required (22 participants per group) (Power and Sample Size Software, Vanderbilt University, TN). As session attendance was 100%, data were analysed per-protocol using Microsoft Excel® 2007 and SPSS® (version 22.0, SPSS, Inc., Chicago, IL). Normality of distribution was tested using the Kolmogorov-Smirnov test; when not normally distributed, data were log-transformed and re-checked for normality of distribution. Analyses included standard descriptive statistics, Pearson's and Spearman's correlation coefficients, paired t-test, mixed-model one-way and two-way repeated measures analysis of variance (ANOVA) (with a main effect for training x group). To locate the source of significant differences, the Bonferroni post-hoc test was used. Homogeneity of variance was confirmed using Mauchly's test of sphericity. When the assumption of sphericity

was violated ($p<0.05$), the F-statistic was adjusted using the Greenhouse-Geisser correction. Where Mauchly's test of sphericity was not found to be significant, post-hoc analyses assumed sphericity (40).Magnitude-based inferences (4, 21) calculated the between-trial standardised differences or effect sizes [ES, 95% confidence interval (CI)] using the pooled standard deviation (11) and standard threshold values (3). All tests were two-tailed and statistical significance was set at $p<0.05$. Parametric results are given as the mean, standard deviation and 95% confidence interval (CI), [mean±SD (95% CI)]; non-parametric results are given as the median and interquartile range and 95% CI, [median (IQR) (95% CI)] unless stated otherwise.

Results

Participants

Participant recruitment and retention is displayed in Figure 1. Six of the 22 participants recruited to the MC group were excluded from analysis on the basis of potential luteal phase deficiency (LPD), i.e. they did not satisfy the mid-luteal serum progesterone criterion of >6 $ng.mL^{-1}$ on the day of testing. These participants completed the intervention and a sub-analysis of the data are presented in the supplemental content (see Table, Supplemental Digital Content, Luteal phase deficient participant demographics, control parameters, body composition, serum hormone concentrations and peak exercise parameters at baseline, following training and after a four-week follow-up period, http://links.lww.com/MSS/A819). Therefore, 16 participants who met the progesterone criterion and therefore exhibited normal menstrual function were included in the MC group for analysis. All 25 participants recruited to the OC group were taking a monophasic combined oestradiol and progestin formulation, with a low ethinyl oestradiol (20-30 μg) and a second or third generation progestin. There were variations in androgenic (n=5), anti-androgenic

(n=5) and non-androgenic (n=15) formulations (calculated using the method of Greer et al. (20)) subsequent analyses confirmed androgenicity of OC type (indicative of progestin type and oestradiol ratio) did not influence baseline characteristics or outcome measures.

There were no differences in participant demographics at baseline between groups (p=0.574-0.988; Table 1). Physical activity, energy intake and body composition parameters were not different within or between groups, at any time point. At baseline, the MC group had significantly higher oestradiol, progestogen and free androgen index (all $p<0.001$), and significantly lower sex-hormone-binding globulin ($p<0.001$) concentrations compared to the OC group. There was no difference between groups for total testosterone (p=0.192). Of the participants identified as LPD, it is interesting to note that these participants were younger, had longer menstrual cycles, lower body mass and body fat indices, and higher free androgen index than both the OC and MC groups (see Table, Supplemental Digital Content, Luteal phase deficient participant demographics, control parameters, body composition, serum hormone concentrations and peak exercise parameters at baseline, following training and after a four-week follow-up period, http://links.lww.com/MSS/A819).

Adherence to protocol

Of the 25 OC participants and 16 MC participants who undertook the training protocol, all participants completed all 12 training sessions and all 120 intervals (i.e. 100% attendance). There were three minor adverse events (one participant fainted during a training session, and one participant had two separate asthma incidents requiring basic first aid). Target power output was achieved in 79.0% of intervals in the OC group and 73.3% of intervals in the MC group; there

was no difference between groups. There were also no between-group differences in mean rating of perceived exertion, heart rate, or power output; results and p-values are presented in Table 2.

Peak aerobic capacity

There was no significant difference between the MC and OC groups for $\dot{V}O_{2peak}$ at baseline [t(39)=-0.278;p=0.783]. Following training, $\dot{V}O_{2peak}$ increased from baseline in both groups [OC; t(24)=-5.108; p<0.001, MC; t(15)=-11.760; p<0.001] and remained significantly increased from baseline at follow-up in both groups [OC; t(22)=-3.840; p=0.001, MC; t(12)=-5.049; p<0.001]; data are presented in Table 2. The MC group showed greater improvement in $\dot{V}O_{2peak}$ following training compared to the OC group [OC +8.5% vs. MC +13.0%; F(1,45)=7.322; p=0.010], but also a greater decline at follow-up [OC -4.0% vs. MC -7.7%; F(1,40)=6.610; p=0.014]. Standardised between-group differences for within-group changes (Cohen's D), presented in Figure 2, demonstrated that the OC group had a likely lower $\dot{V}O_{2peak}$ adaptation to training [-0.22±0.18 (-0.40- -0.04); 0/40/60% higher/trivial/lower than MC]. When $\dot{V}O_{2peak}$ was adjusted for body mass and lean body mass, the above significant relationships remained true.

Peak power output

There was no between-group difference for PPO at baseline [t(39)=-0.127; p=0.899], post-training [t(39)=-0.283; p=0.779] or follow-up [t(35)=-0.053; p=0.958]. PPO increased following training in both groups [OC; t(24)=-15.371; p<0.001, MC; t(15)=-9.249; p<0.001]. At follow-up, PPO decreased from post-training in both groups [OC; t(22)=5.061; p<0.001, MC; t(13)=3.085; p=0.009], but remained above baseline [OC; t(22)=-9.148; p<0.001, MC; t(13)=-5.737; p<0.001] . There was no difference between groups [F(1.776,72.820)=0.048; p=0.938] at any time point;

data are presented in Table 2. Standardised between-group differences for within-group changes (Cohen's D), presented in Figure 2, demonstrated a trivial between-group difference in PPO adaptation to training [-0.04±0.19 (-0.24-0.15); 1/94/5% higher/trivial/lower than MC].

Peak cardiac output

There was no significant between-group difference in peak cardiac output ($\dot{Q}_{peak}$) at baseline (p=0.385) or follow-up (p=0.804), but the MC group demonstrated higher $\dot{Q}_{peak}$ following training (p=0.002). Following training, $\dot{Q}_{peak}$ increased in both the OC [t(24)=-3.348; p=0.003] and MC [t(15)=-6.742; p<0.001] groups, and returned to pre-training values at follow-up (OC; t(22)=-0.986; p=0.335, MC; t(13)=-1.735;p=0.107]. There was a significant group x time interaction for the OC group compared to the MC group following training [OC group +4.0% vs. MC group +16.1%; F(1,39)=6.711, p=0.013]; data are presented in Table 2. There was also a significant group x time interaction for the MC groups vs. the LPD sub-group following training [MC group +16.1% vs. LPD sub-group +6.3%; F(1,20)=5.328, p=0.032]; data are presented in the supplemental content (see Table, Supplemental Digital Content, Luteal phase deficient participant demographics, control parameters, body composition, serum hormone concentrations and peak exercise parameters at baseline, following training and after a four-week follow-up period, http://links.lww.com/MSS/A819). Standardised between-group differences for within-group changes (Cohen's D) demonstrated that the OC group had a likely lower $\dot{Q}_{peak}$ adaptation to training [-0.51±0.39 (-0.90- -0.12); 0/6/94% higher/trivial/lower than MC] compared to the MC group (Figure 2).

Peak stroke volume

There was no significant between-group differences in SV_{peak} at any time point [baseline; $t(37)$=-0.004; p=0.997, post-training; $t(38)$=-1.176; p=0.247, or follow-up; $t(31)$=-1.576; p=0.125]. Following training, SV_{peak} increased in the MC group [$t(15)$=-3.794; p=0.002], and remained elevated from pre-training at follow-up [$t(11)$=-2.643; p=0.023]; but did not change in the OC group [post-training; $t(22)$=-1.694; p=0.104, follow-up $t(19)$=-0.583; p=0.566]. There was no significant group x time interaction for the OC group compared to the MC group following training [$F(1,37)$=1.055, p=0.311]; data are presented in Table 3. Standardised between-group differences for within-group changes (Cohen's D) demonstrated that the OC group had a possibly lower SV_{peak} adaptation to training [-0.28±0.48 (-0.76- 0.20); 3/34/63% higher/trivial/lower than MC] compared to the MC group (Figure 2).

Respiratory quotient

The MC group had a higher $R\dot{Q}$ compared to the OC group at all time points [baseline; $t(39)$=-2.151; p=0.038, post-training; $t(39)$=-2.533; p=0.015, follow-up; $t(33)$=-2.342; p=0.025]. Following training, $R\dot{Q}$ increased in the OC [$t(24)$=-2.273; p=0.032] but not the MC [$t(15)$=-1.831; p=0.087) group, and returned to pre-training values at follow-up [OC; $t(21)$=-0.405; p=-.690, MC; $t(12)$=-0.158; p=0.877]. There was no significant group x time interaction for $R\dot{Q}$ [$F(1.972, 59.146)$=0.129, p=0.877]; data are presented in Table 3. Standardised between-group differences for within-group changes (Cohen's D) demonstrated that OC use compared to normal menstrual function had a possibly trivial effect on $R\dot{Q}$ adaptation to training [0.02±0.52 (-0.50-0.54); 24/56/20% higher/trivial/lower than MC] (Figure 2).

Minute ventilation

There was no significant between-group difference in $\dot{V}e\dot{V}CO_{2slope}$ at each time point [baseline; $t(39)$=-1.128; p=0.266, post-training; $t(39)$=1.741; p=0.090, follow-up; $t(33)$=0.552; p=0.585]. There were no changes in $\dot{V}e\dot{V}CO_{2slope}$ following training [OC; $t(24)$=-0.460; p=0.650, MC; $t(15)$=0.249; p=0.807] or at follow-up [OC; $t(21)$=-0.855; p=0.402, MC; $t(12)$=-1.2780; p=0.225]. There was no significant group x time interaction for $\dot{V}e\dot{V}CO_{2slope}$ [$F(1.551, 46.539)$=1.132, p=0.319]; data are presented in Table 3. Standardised between-group differences for within-group changes (Cohen's D) demonstrated that OC use compared to normal menstrual function had a possibly trivial effect on $\dot{V}e\dot{V}CO_{2slope}$ adaptation to training [0.09±0.45 (-0.36-0.54); 31/59/10% higher/trivial/lower than MC] (Figure 2).

Peak heart rate and rating of perceived exertion

There was no significant difference between MC and OC group for HR_{peak} or RPE_{peak} at each of the three time points (all p>0.05). There were no differences in HR_{peak} or RPE_{peak} pre-, post- or de-training in the OC and MC-groups (all p>0.05). There was no significant group x time interaction for HR_{peak} [$F(1.738, 71.258)$=0.089, p=0.891] or RPE_{peak} [$F(1.970, 80.780)$=1.981, p=0.145].

Discussion

The present study assessed the influence of OC use on peak physiological adaptations ($\dot{V}O_{2peak}$, PPO, $\dot{Q}_{peak}$, SV_{peak}, $R\dot{Q}$, HR_{peak}, RPE_{peak}, and $\dot{V}e\dot{V}CO_{2slope}$) to four weeks of SIT in recreationally-active women under stringently-controlled ovarian hormone conditions. Additionally, the maintenance of these adaptations during a four-week follow-up period after

SIT was assessed. This study found that OC use, compared to normal menstruation, dampened $\dot{V}O_{2peak}$ and $\dot{Q}_{peak}$ adaptations to SIT in recreationally-active women, but did not influence PPO or other adaptations. Interestingly, OC use appeared to protect against the loss of peak physiological and performance adaptations during the four-week follow-up period. This is the first study to investigate the influence of OC use on peak exercise adaptations to training.

The magnitude of improvement in $\dot{V}O_{2peak}$ in the OC (8.5%), MC (13.0%), and LPD (13.6%) groups in the present study are similar to those previously reported in recreationally-active women for whom hormone levels were not established. Indeed, several SIT studies in recreationally-active women have found that two to eight weeks of SIT comprising four to 10 bouts of 30 seconds to four minutes in duration (variable intensity) with one to four minutes' rest have elicited significant ($p<0.05$) improvements of 6.5-14.0% in $\dot{V}O_{2max}$ (1, 14, 15, 31, 39, 41). Of these, only one study (41), excluding OC users, attempted to control for menstrual cycle phase by testing in the follicular phase (determined by onset of menstruation), yet did not verify menstrual cycle phase using hormone measures. With up to 57% of reproductive-aged women in worldwide reporting OC use (16), it is likely that the majority of participants within these studies were taking an OC. Therefore, the findings of the present study suggest that the $\dot{V}O_{2peak}$ adaptation reported within these previous studies, where no consideration of OC use or ovarian hormone concentrations in the methodology is apparent, were likely influenced by OC use. However, it must be noted that there is a wide array of hormone contraceptives in use. As this study specifically investigation low-dose combined OCs, with 20-30 μg ethinyl estradiol and a second or third generation progestin, this conclusion cannot yet be drawn for higher dose formulations and OCs that use earlier or later generation progestins. Furthermore, while not

statistically significant, it is important to note that, on average, the OC group lost weight over the duration of the study, while the MC group and LPD sub-group gained a small amount of weight. When variables of interest were adjusted for body mass and lean body mass, the interactions remained true. Therefore, it is unlikely that these small, non-significant changes in body mass influenced the outcomes of the study. The significant improvement demonstrated by the LPD sub-group, despite their lower body mass provide compelling evidence that the lower $\dot{V}O_{2peak}$ adaptation in the OC group is an actual finding and not an artefact of the small non-significant differences in body mass between groups.

Normative data developed for women must also be interpreted carefully, and the threshold improvement for $\dot{V}O_{2peak}$ as a marker of cardiorespiratory fitness and/or health may need to consider OC use or ovarian hormone status during interpretation. Furthermore, OC status should be considered by coaches and sports scientist when interpreting physiological responses to training blocks, and the utilisation of measures and standards that are not influenced by ovarian hormone status should be a priority within female-specific populations.

In the one study reporting repeated measures of $\dot{V}O_{2peak}$ during a follow-up period, Ready and colleagues (31) observed that only 24% of the improvements in $\dot{V}O_{2peak}$ following training were maintained after a two-week follow-up. However, the authors did not consider/report OC use or menstrual status. Results of the present study suggest that while OC use dampens $\dot{V}O_{2peak}$ adaptation following a training intervention, it may also minimise the loss of the training effect for the parameter. $\dot{V}O_{2peak}$ returned towards baseline after follow-up in both groups; however, OC users retained 93% of $\dot{V}O_{2peak}$ adaptations to training, compared to naturally-menstruating

women, who retained 41% of the $\dot{V}O_{2peak}$ adaptation to training. It is important to note that while the OC group demonstrated lower $\dot{V}O_{2peak}$ adaptations to training, and therefore may have been expected to exhibit lower decline towards baseline, both groups had similar percentage improvements in $\dot{V}O_{2peak}$ at follow-up (both approximately 4.4%). Therefore, OC use may alter the time course of training adaptations, and the use of OC during lower training periods, tapering or injury, may indeed be a useful practise to minimise the negative effects of detraining. Inclusion of longer training and follow-up periods with intermediate testing would shed further light on this phenomenon.

Furthermore, it must also be noted that participants in the present study were already physically active, and were encouraged to maintain their habitual levels of physical activity throughout training and follow-up, and merely refrain from performing SIT during the follow-up phase. Therefore, results from the present study may not be comparable to studies where habitual exercise was completely ceased. Furthermore, while not statistically significant, there was an average of 18 min/week difference in physical activity levels between the OC and MC groups at follow-up. Additionally, the LPD group reported similar physical activity to the OC group at baseline, and only slightly higher physical activity than the MC group at follow-up. These observations align with the changes in $\dot{V}O_{2peak}$ following detraining (MC; +7.9% and LPD; +8.4%, compared with OC; +3.6%). Whilst this non-significant between-group difference in physical activity levels is likely negligible on a day to day basis, it may have contributed to the different responses observed in the loss of peak exercise after follow-up between groups.

There is significant evidence to suggest that changes in peak cardiac output, specifically related to changes in blood volume and stroke volume, are a strong influencing factor on $\dot{V}O_{2peak}$ adaptation (2). Longitudinal studies have demonstrated that training-induced increases in $\dot{V}O_{2peak}$ result primarily from an increase in maximal cardiac output, rather than a widening of the arterio-venous oxygen difference, suggesting that $\dot{V}O_{2peak}$ training adaption is predominantly related to central adaptions rather than peripheral adaptation (2). Following SIT, there was a significant increase in $\dot{Q}_{peak}$ of +4.0% and +16.1% in the OC group and the MC group, respectively, with the MC group showing a significantly greater improvement (p=0.013). At follow-up, $\dot{Q}_{peak}$ returned towards pre-training levels in both groups, and there were no between-group differences at follow-up. When considered in conjunction with the significant positive relationship between change in $\dot{V}O_{2peak}$ and change in $\dot{Q}_{peak}$ with training (r=0.362, p=0.020), this, at least in part, explains the dampened $\dot{V}O_{2peak}$ response to training observed in OC users compared to naturally-menstruating women.

Reductions in blood volume and cardiac output have previously been found following de-training in men and women (13, 26), and have previously been suggested to explain the loss of $\dot{V}O_{2peak}$ during follow-up. However, changes in cardiac output only partially explain the observed changes in $\dot{V}O_{2peak}$ with training. The effects of oestrogen and progesterone on plasma volume expansion and fluid retention [through the potential mechanisms of capillary filtration/permeability and stimulation of nitric oxide production and subsequent effect on the renin-angiotensin aldosterone system (9)] may explain why the OC group showed a practically meaningful (though not statistically significant) lower decline in $\dot{Q}_{peak}$ (due to potential preservation of training-induced blood volume changes) four weeks following the completion of

SIT training (4.0% in the OC group vs. 7.7% in the MC group) (35). However, central adaptations including changes in cardiac output and stroke volume seen in the present study do not completely explain the change in $\dot{V}O_{2peak}$ with training, therefore further research should investigate peripheral adaptations, such as peripheral muscle deoxygenation or mitochondrial oxidative capacity, which may be associated with the discrepancy in physiological adaptations to exercise training between naturally-menstruating women and OC users.

It is a further interesting finding of this investigation that the luteal phase deficient women excluded from primary analyses demonstrated dampened $\dot{Q}_{peak}$ adaptations to training in a similar manner to the OC group (both compared to the group with normal menstrual function). This result (see Table, Supplemental Digital Content, Luteal phase deficient participant demographics, control parameters, body composition, serum hormone concentrations and peak exercise parameters at baseline, following training and after a four-week follow-up period, http://links.lww.com/MSS/A819) provides preliminary evidence that luteal phase deficiency significantly influences cardiovascular adaptations to training. This suggests that circulating endogenous ovarian hormone concentrations may be more influential on adaptation to training compared to exogenous ovarian hormones. Indeed, previous research has demonstrated that high oestrogen and progesterone levels are independently associated with plasma volume expansion (37), in comparison to primarily circulating exogenous hormones (35). In contrast to previous speculation that the cardiovascular limitation to exercise training adaptation in OC users is primarily due to the influence of exogenous oestradiol on the cardiovascular system, this finding suggests that it may instead be the low endogenous oestradiol concentrations that are implicated.

Both OC and MC groups improved PPO by 13.1% and 13.8%, respectively, with no difference between groups. After a four-week follow-up period, PPO returned towards baseline, with just over half of the PPO adaptation preserved in both groups. As PPO is strongly correlated with exercise performance (17), the present data suggest performance adaptations to SIT, and the preservation of these adaptations following SIT, are not influenced by OC use. Therefore, depending on the aims of an intervention, it may be more appropriate to use non-physiological measures of performance, such as PPO, when assessing adaptation to training in women who have varied hormone status, due to the apparent impact of OC use on physiological parameters following a training intervention. However, further research investigating performance in competitive athletic women using sport-specific contexts and/or time-trials is needed to confirm this. There were no significant changes in peak HR, RPE, R$\dot{Q}$ and $\dot{V}_E\dot{V}_{O2slope}$ follow training, and no differences between groups were observed. While there were no significant between-group differences in SV adaptations to training, the transient effect of both endogenous and exogenous progestogens on HR may have masked any potential changes in peak HR and SV following training.

We recognise several limitations of the present study. It is a notable limitation of the present study that no non-exercising control group was included, therefore the changes seen with training must be interpreted with caution. All participants were taking a monophasic OC; however, there were variations in androgenic (n=5), anti-androgenic (n=5) and non-androgenic (n=15) formulations. While no differences in training adaptations were present among the different OC formulations, the small sample size following sub-grouping may have limited our ability to detect differences; further investigation is necessary to confirm whether OC androgenicity

influences adaptation to training in women. Secondly, following training, the oestrogen and progesterone concentrations of women with normal menstrual function were significantly lower. Despite the challenges it posed, we chose to test within the mid-luteal phase within this study to ensure that endogenous ovarian hormones were as high as possible and compare to OC use when exogenous hormones were as high as possible and endogenous hormones were as low as possible to maximise the effect of ovarian hormones on outcome measures. While we are confident that the timing of testing, based on individual participant cycle lengths, was appropriate, it is possible that the sudden perturbation in energy balance caused by the sprint interval training program induced probably luteal phase deficiency within subsequent cycles in some women which is an important consideration for future training studies in women. Therefore, to minimise the influence of fluctuating ovarian hormones on study outcomes, it may be more practical to test in the early follicular phase during training interventions that could elicit luteal phase deficiency in at risk women.

Thirdly, although self-report physical activity during the four-week period following completion of SIT training was not different between groups, objective monitoring would have minimised any potential recall bias associated with self-report measures. Finally, following serum hormone analyses, six naturally-menstruating women who completed the training intervention were excluded from the primary analysis as they did not meet the minimum progesterone concentration criterion for normal mid-luteal menstrual phase. Therefore, we recommend oversampling by 30% in normally-menstruating participants in studies including women, to account for the required exclusion of potential luteal-phase deficient participants from analysis. We have presented supplementary data which suggests that luteal-phase deficiency may indeed

influence cardiovascular adaptation to training. Whether forms of menstrual dysfunction influence adaptation to training is an important consideration in the area of female athletic performance and adaptation to training in physically-active women, and requires further investigation.

In conclusion, this study suggests that compared to natural menstruation, OC use dampens $\dot{V}O_{2peak}$ and $\dot{Q}_{peak}$ adaptation to SIT in recreationally-active women, yet better preserves these adaptations following completion of SIT training, particularly for $\dot{V}O_{2peak}$. In contrast, PPO adaptation appeared unaffected by OC use. These findings demonstrate the clear need to consider exogenous hormone use in exercise training studies involving women of reproductive age. Further investigation is required to elucidate the influences of OC use compared to natural menstruation on the central and peripheral adaptations to exercise training, and how these may manifest in exercise performance.

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Conflicts of interest

None declared. The results of the present study do not constitute endorsement by ACSM and are presented clearly, honestly and without fabrication, falsification or inappropriate data manipulation.

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Figure 1: Participant flow diagram

Figure 2: Standardised between-group differences for within-group changes for the oral contraceptive group versus the menstrual cycle groups following training.

$\dot{V}O_{2peak}$: peak aerobic capacity; **PPO:** peak power output; **$\dot{Q}_{peak}$:** peak cardiac output; **HR_{peak}:** peak heart rate; **RPE_{peak}:** peak rating of perceived exertion; **SV_{peak}:** peak stroke volume; **RQ_{peak}:** peak respiratory quotient; **$\dot{V}e\dot{V}CO_{2slope}$:** minute ventilation**; min:** minute; **bpm:** beats per minute.

Supplemental Digital Content

Supplementary Table 1: Luteal phase deficient participant demographics, control parameters, body composition, serum hormone concentrations and peak exercise parameters at baseline, following training and after a four-week follow-up period (n=6).

Figure 1

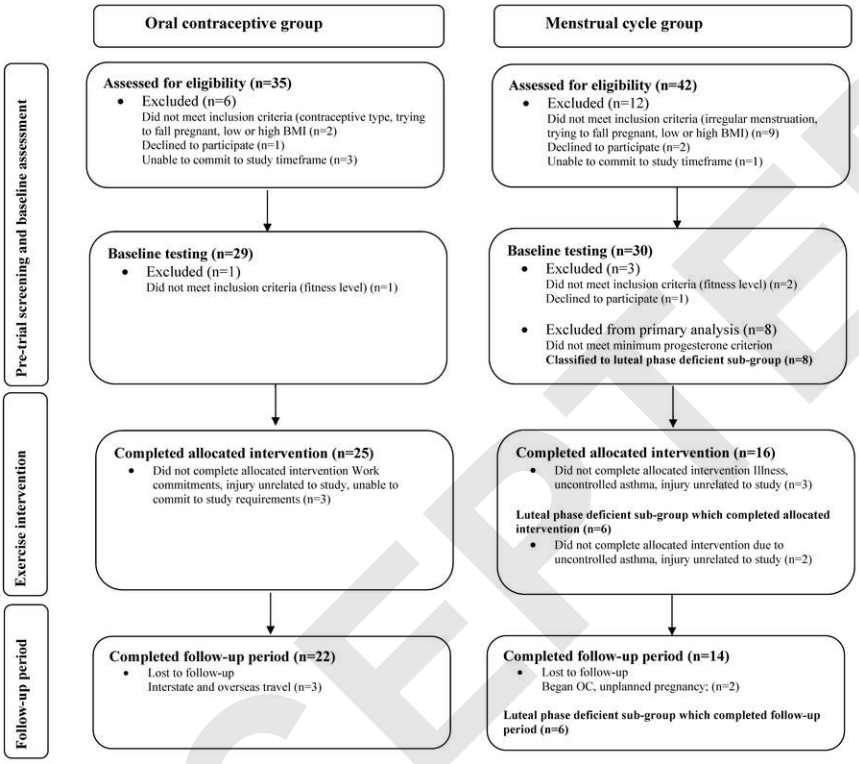

Figure 2

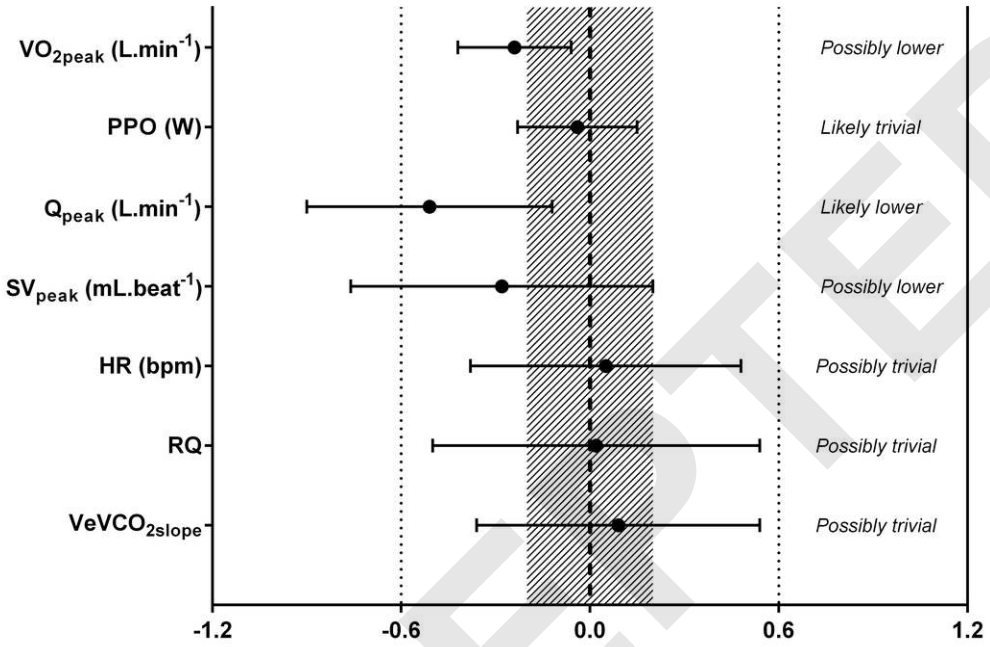

Table 1: Participant demographics, control parameters, body composition and serum hormone concentrations at baseline, following training and after a four-week follow-up period.

	Oral contraceptive group (n=25)			Menstrual cycle group (n=16)		
	Pre-training	**Post-training**	**De-training**	**Pre-training**	**Post-training**	**De-training**
Participant demographics and control measures						
Age	25.5±5.4 (23.1-27.8)	x	x	27.6±5.4 (24.5-30.8)	x	x
Menstrual cycle length (days)	28±0 (28-28)	x	x	30±2 (29-31)	x	x
Testing day (days)	17±4 (16-19)	18±6 (16-21)	19±5 (16-21)	23[20-24] (21-23)[#]	23±4 (21-26)	23±2 (21-24)
Physical activity (min.wk^{-1})	247±64 (222-272)	235±61 (211-258)	246±64 (221-271)	229±44 (210-247)	217±43 (199-234)	228±43 (210-246)
Energy intake (kJ.kg^{-1}.day^{-1})	8461± 3194 (6896-10026)	8490±2452 (7103-9877)	8385±2110 (7077-9692)	8373±2360 (7179-9567)	8490±1971 (7269-9712)	8267±2102 (7078-9692)
Body composition						
Body mass (kg)	63.6±7.8 (60.3-66.8)	63.4±7.2 (60.4-66.3)	62.5±7.1 (59.4-65.6)	66.1±8.7 (61.0-71.1)	66.4±8.7 (61.3-71.4)	66.6±8.5 (61.7-71.4)
Body mass index (kg.m^{-2})	22.6±2.1	22.6±2.1	22.6±2.1	23.0±2.1	23.0±2.1	23.0±2.1

	(21.7-23.4)	(21.7-23.4)	(21.7-23.4)	(21.8-24.2)	(21.8-24.2)	(21.8-24.2)
Fat mass (kg)	20.7±4.8	20.5±4.6	20.1±2.0	22.7±4.7	22.6±5.0	22.7±4.7
	(18.7-22.7)	(18.6-22.4)	(18.2-22.0)	(20.0-25.4)	(19.8-25.5)	(20.0-25.4)
Lean body mass (kg)	40.6±4.4	40.6±4.1	40.2±4.3	41.1±5.7	41.4±5.5	41.6±5.6
	(38.8-42.5)	(38.9-42.3)	(38.3-42.1)	(37.8-44.4)	(38.2-44.6)	(38.3-44.8)
Lean body mass – legs (kg)	13.6±1.8	13.8±1.9	13.5±1.8	13.8±2.5	14.0±2.5	14.1±2.6
	(12.9-14.4)	(13.0-14.5)	(12.7-14.3)	(12.4-15.3)	(12.5-15.4)	(12.6-15.6)
Body fat (%)	32.3±4.8	32.1±4.8	32.0±4.6	34.2±4.8	33.9±5.0	34.0±5.0
	(30.3-34.3)	(30.1-34.0)	(30.0-34.0)	(31.5-37.0)	(31.0-36.8)	(31.1-36.9)
Hormone and blood measures						
Oestradiol ($pg.mL^{-1}$)	5.6 [5.0-10.3]	5.1[5.0-10.2]	9.2[5.0-11.8]	136.4±61.9	107.8±73.1	68.8[40.8-123.6]
	(5.7-13.5)	(-11.0-63.8)	(6.7-16.2)	(102.1-170.7)	(67.3-148.3)	(47.4-121.7)
Progestogen ($ng.mL^{-1}$)	0.6±0.3	0.5±0.3	0.5±0.3	13.1±6.6	1.1[0.8-5.9]	1.0[0.6-4.0]
	(0.4-0.7)	(0.4-0.6)	(0.4-0.7)	(9.4-16.8)	(-0.1-9.6)	(0.5-6.1)
Total testosterone ($ng.mL^{-1}$)	0.20±0.10	0.13±0.07	0.13±0.06	0.21±0.16	0.26±0.16	0.23±0.16
	(0.10-0.20)	(0.10-0.16)	(0.10-0.15)	(0.12-0.30)	(0.17-0.35)	(0.13-0.32)
Sex-hormone-binding globulin ($pg.mL^{-1}$)	209.0±87.1	189.6±99.3	198.3±96.5	67.5±31.4	58.5[34.3-78.7]	71.0±49.3
	(170.3-247.6)	(145.6-233.7)	(155.6-241.1)	(50.0-84.9)	(45.4-74.9)	(42.5-99.4)
Free androgen index (%)	7.8[4.2-8.5]	8.6±5.6	3.1[6.5-12.7]	25.6[13.8-42.0]	59.1±53.6	30.2[13.7-76.5]

	(6.9-14.7)	(6.1-11.0)	(5.4-12.4)	(12.8-82.6)	(29.5-88.8)	(20.6-77.1)

Parametric data are presented as mean±SD (95% CI); non-parametric data are presented as median [IQR] (95% CI). *p<0.001 vs. oral contraceptive group; #p<0.01 vs. pre-training

Table 2: Summary of attendance, adherence, heart rate, power output and rating of perceived exertion responses for each interval over 12 sessions (120 intervals) of training.

	Oral contraceptive group (n=25)	Menstrual cycle group (n=16)	p-value
Adherence (no. of intervals at target power)	95±21 (86-104)	89±23 (77-102)	0.311
Adherence (% intervals at target power)	79±17 (72-86)	74±19 (64-85)	0.311
Mean heart rate (bpm)	179±8 (176-182)	178±6 (175-181)	0.591
Heart rate/peak heart rate (%)	98±3 (97-100)	97±3 (95-98)	0.329
Mean power output (Watts)	229±37 (213-245)	233±48 (207-260)	0.871
Power output/peak power output (%)	108±4 (107-110)	106±5 (103-108)	0.084
Mean rating of perceived exertion (Borg 6-20)	17±2 (16-18)	17±1 (16-17)	0.915

Data are presented as mean±SD (95%CI); independent t-test.

Table 3: Peak exercise parameters at baseline, following four weeks of training and after a four-week follow-up period.

	Oral contraceptive group (n=25)			Menstrual cycle group (n=16)		
	Baseline	**Post-training**	**Follow-up**	**Baseline**	**Post-training**	**Follow-up**
$\dot{V}O_{2peak}$ (L·min^{-1})	2.3±0.4	2.5±0.4	2.4±0.4	2.3±0.5	2.6±0.5	2.4±0.4
	(2.1-2.5)	(2.3-2.6)*	(2.3-2.6)*	(2.1-2.6)	(2.4-2.9)*	(2.1-2.7)*
PPO (Watts)	214.0±33.7	242.0±33.2	230.2±33.6	215.6±44.1	245.3±40.6	230.8±33.0
	(200.2-228.0)	(228.3-255.7)*	(215.7-244.7)*$^{+}$	(192.1-239.1)	(223.6-266.9)*	(211.7-249.8)*$^{\#}$
$\dot{Q}_{peak}$ (L·min^{-1})	19.6±3.3	20.4[19.1-22.4]	19.8±2.7	19.2±3.1	22.3±2.1	20.2±2.9
	(18.2-21.0)	(19.9-22.2)$^{\#}$	(18.7-21.0)	(17.5-20.8)	(21.2-23.4)*	(18.5-21.9)$^{\#}$
HR_{peak} (bpm)	182.0±9.0	184.8±8.5	183.3±9.5	182.3±7.1	184.8±6.2	183.6±7.0
	(178.1-185.9)	(181.3-188.3)$^{\#}$	(179.7-187.9)	(178.5-186.0)	(181.5-188.0)	(179.6-187.7)
RPE_{peak} (Borg)	19.0[17.0-20.0]	18.0[17.0-19.0]	18.0[17.0-19.0]	18.0[17.0-19.0]	18.3±1.3	17.9±1.4
	(17.9-19.0)	(17.6-18.7)	(17.7-18.9)	(17.3-18.4)	(17.6-18.9)	(17.1-18.6)
SV_{peak} (mL)	103.2±20.7	109.5±15.9	102.0±15.5	103.3±19.1	115.0±11.8*	112.0±20.7*
	(94.3-112.2)	(102.8-116.2)	(95.0-109.1)	(93.1-113.4)	(108.7-121.2)	(98.9-125.1)
$R\dot{Q}_{peak}$	1.26±0.06	1.29±0.05*	1.27±0.05	1.31±0.07	1.33±0.05	1.32±0.05

	(1.24-1.29)	(1.27-1.31)	(1.25-1.29)	(1.27-1.34)	(1.30-1.36)	(1.28-1.35)
$\dot{V}e\dot{V}O_{2slope}$	31.4±4.5	31.7±4.3	31.6±4.2	29.7±4.6	29.6±2.8	30.9±3.4^{+}
	(31.7-4.3)	(29.9-33.5)	(29.8-33.5)	(27.3-32.2)	(28.1-31.0)	(28.8-32.9)

$\dot{V}O_{2peak}$: peak aerobic capacity; **PPO:** peak power output; **$\dot{Q}_{peak}$:** peak cardiac output; **HR_{peak}:** peak heart rate; **RPE_{peak}:** peak rating of perceived exertion; **SV_{peak}:** peak stroke volume; **RQ_{peak}:** peak respiratory quotient; **$\dot{V}e\dot{V}CO_{2slope}$:** minute ventilation**; min:** minute; **bpm:** beats per minute. Parametric data are presented as mean±SD (95%CI); non-parametric data are presented as median [IQR] (95%CI). *p<0.01 vs. pre; #p<0.05 vs. pre; $^{+}$p<0.01 vs. post.

Supplementary Table 1: Luteal phase deficient participant demographics, control parameters, body composition, serum hormone concentrations and peak exercise parameters at baseline, following training and after a four-week follow-up period (n=6).

	Pre-training	Post-training	De-training
Participant demographics and control measures			
Age (years)	23.0±3.0 (20.1-25.4)*	x	x
Menstrual cycle length (days)	34±5 (31-38)*^	x	x
Testing day (days)	22±3 (19-24)[#]	24±3 (21-27)	23±2 (21-25)
Physical activity (min.wk^{-1})	252±89 (215-289)	235±72 (205-265)	233±92 (195-272)
Energy intake (kJ.kg^{-1}.day^{-1})	7924±1911 (6394-9455)	7972±2046 (6334-9609)	7954±1285 (6926-8982)
Body composition			
Body mass (kg)	58.0±7.8* (49.9-66.2)	58.6±7.4* (50.8-66.3)	58.8±7.7* (50.7-66.9)
Body mass index (kg.m^{-2})	21.5±2.2 (19.2-23.8)	21.5±2.2 (19.2-23.8)	21.5±2.2 (19.2-23.8)
Fat mass (kg)	16.7±4.7* (11.8-21.6)	16.4±5.2 (10.9-21.9)	16.7±5.3 (11.2-22.3)
Lean body mass (kg)	39.0±4.6	39.8±4.6	39.8±4.3

	(34.2-43.9)	(35.0-44.6)	(35.2-44.3)
Lean body mass - legs (kg)	12.8±1.8	13.0±1.7	13.0±2.0
	(10.9-14.6)	(11.2-14.8)	(10.9-15.2)
Body fat (%)	28.5±5.4*	27.8±6.4*	28.1±5.9*
	(22.8-34.2)	(21.1-34.5)	(21.9-34.3)
Hormone and blood measures			
Oestradiol ($pg.mL^{-1}$)	88.5±78.2	56.6±32.3	68.9±58.9
	(-8.5-185.6)	(22.7-90.4)	(7.1-130.7)
Progestogen ($ng.mL^{-1}$)	0.8±0.2*	0.8±0.2	0.8±0.3
	(0.5-1.0)	(0.6-1.0)	(0.5-1.2)
Total testosterone ($ng.mL^{-1}$)	0.37±0.27	0.37±0.23	0.31±0.17
	(0.04-0.70)	(0.13-0.60)	(0.13-0.49)
Sex-hormone-binding globulin ($pg.mL^{-1}$)	71.6±58.2^	67.5±46.3^	67.0±47.9^
	(-0.6-143.9)	(18.9-116.1)	(16.7-117.3)
Free androgen index (%)	57.5[32.2-145.1]*	99.3±121.3*	50.2[34.6-115.0]*
	(-54.9-256.0)	(-28.0-226.6)	(-13.7-172.0)
Peak exercise parameters			
$\dot{V}O_{2peak}$ ($L \cdot min^{-1}$)	2.2±0.5	2.5±0.5	2.3±0.5
	(1.6-2.7)	(1.9-3.1)*	(1.8-2.8)
PPO (W)	208.3±45.7	233.3±43.1	222.9±42.9
	(160.3-256.3)	(188.1-278.5)*	(177.9-267.9)
$\dot{Q}_{peak}$ ($L \cdot min^{-1}$)	17.6±4.0	18.7±3.0	18.4±2.4
	(13.4-21.8)	(15.6-21.9)^	(15.8-20.9)
HR_{peak} (bpm)	177.7±5.4	179.8±5.3	181.3±3.6
	(172.0-183.4)	(174.2-185.4)	(177.6-185.1)

RPE_{peak} (Borg)	17.7±1.5 (16.1-19.3)	18.0±1.3 (16.7-19.3)	17.5[16.0-18.0] (16.1-18.2)
SV_{peak} (mL)	92.8±18.8 (73.0-112.5)	92.2±14.2 (77.3-107.1)	91.4±16.6 (70.8-112.0)
$R\dot{Q}_{peak}$	1.30±0.07 (1.23-1.38)	1.33±0.07 (1.25-1.40)	1.36±0.12$^{\#}$ (1.21-1.51)
$\dot{V}e\dot{V}CO_{2slope}$	27.2±1.3 (25.8-28.6)	29.5±2.8$^{\#}$ (29.6-29.9)	28.8±3.0 (25.1-32.5)

$\dot{V}O_{2peak}$: peak aerobic capacity; **PPO:** peak power output; **$\dot{Q}_{peak}$:** peak cardiac output; **HR_{peak}:** peak heart rate; **RPE_{peak}:** peak rating of perceived exertion; **SV_{peak}:** peak stroke volume; **RQ_{peak}:** peak respiratory quotient; **$\dot{V}e\dot{V}CO_{2slope}$:** minute ventilation**; min:** minute; **bpm:** beats per minute. Parametric data are presented as mean±SD (95%CI); non-parametric data are presented as median [IQR] (95%CI). *p<0.05 vs. normal menstrual function group; ^p<0.05 vs. oral contraceptive group; $^{\#}$p<0.01 vs. pre-training