European Journal of Applied Physiology (2019) 119:2733–2744
https://doi.org/10.1007/s00421-019-04254-y

ORIGINAL ARTICLE

Effects of oral contraceptive use on female sexual salivary hormones and indirect markers of muscle damage following eccentric cycling in women

Karen Mackay[1] · Cristopher González[1] · Hermann Zbinden-Foncea[1] · Luis Peñailillo[1]

Received: 26 August 2019 / Accepted: 31 October 2019 / Published online: 4 November 2019

Abstract

Purpose To determine the effects of oral contraceptive (OC) use on salivary concentrations of testosterone, estrogen, progesterone, and its effects on the changes in indirect markers of muscle damage following eccentric cycling in women.

Methods 10 oral contraceptive users at follicular phase (OC-FOL), 10 non-oral contraceptives users at follicular phase (NOC-FOL), and 10 non-oral contraceptives users at ovulation phase (NOC-OV) participated. Subjects performed 30 min of eccentric cycling at 90% of their maximal concentric power output (PO). Maximal voluntary isometric contraction (MVC), creatine kinase activity (CK), muscle soreness (SOR), and pain pressure threshold of vastus lateralis (PPT-VL) was assessed before, immediately after, and 24–96 h after cycling. Salivary estrogen, progesterone and testosterone concentrations were measured before, 72 and 96 h after exercise.

Results No difference in estrogen levels between users and non-users was observed. Testosterone was 45% lower in OC-FOL than NOC-FOL at 96 h post-exercise ($P = 0.01$). Progesterone was 30.8-fold higher in NOC-OV than OC-FOL and 9.7-fold higher than NOC-FOL at 96 h post-exercise. The NOC-FOL recovered all indirect markers of muscle damage by 72 h post-exercise ($P > 0.05$). NOC-OV recovered MVC strength and muscle soreness (SOR and PPT-VL) by 96 h post-exercise ($P > 0.05$). OC-FOL did not recover baseline values of MVC, SOR, CK, and PPT-VL by 96 h.

Conclusion These results suggest that recovery after exercise-induced muscle damage took longer in OC-FOL, followed by NOC-OV and by NOC-FOL, respectively. Furthermore, testosterone and progesterone levels may affect recovery of indirect markers of muscle damage in women.

Keywords Exercise-induced muscle damage · Eccentric cycling · Muscle recovery · Oral contraception

Abbreviations

ANOVA	One-way measures of variance
BMI	Body mass index
CK	Creatine kinase activity
DOMS	Delayed onset of muscle soreness
DRSP	Drospirenone
EE	Ethynyl estradiol
EIMD	Exercise-induced muscle damage
ELISA	Enzyme-linked immunosorbent assays
FOL	Follicular phase
HR_{max}	Maximal heart rate
LSD	Fisher's least significant difference
MVC	Maximal voluntary contraction
OC	Oral contraceptives
OV	Ovulation
PO_{max}	Maximal concentric power output
PPT	Pain pressure threshold
PPT-VL	Pain pressure threshold for vastus lateralis muscle
SD	Standard deviation
SOR	Muscle soreness
VAS	Visual analogue scale
VO_{2peak}	Peak oxygen consumption
W	Watts

Communicated by Michalis G. Nikolaidis.

✉ Luis Peñailillo
lpenailillo@uft.cl

[1] Exercise Science Laboratory, School of Kinesiology, Finis Terrae University, 1509 Pedro de Valdivia Av., Providencia, Santiago, Chile

Introduction

Women's participation in competitive sports has increased in an exponential manner in the last 30 years. Competition and high-training volume have shown to induce muscle fatigue and symptoms of muscle damage in women (Wiewelhove et al. 2015). Thus, both prevention and optimal recovery after competition and training may play a key role in achieving high-level performance (Borish 1996; Lallana 2005). Exercise-induced muscle damage (EIMD) is typically evidenced after unaccustomed eccentric exercise by increases in indirect markers of muscle damage such as strength loss, muscle soreness and creatine kinase in the blood stream (CK) (Clarkson and Sayers 1999; Nosaka et al. 2002; Margaritelis et al. 2015; Peñailillo et al. 2018). Interestingly, it has been suggested that women develop less muscle damage after eccentric exercise when compared to men (Minahan et al. 2015). Moreover, a protective effect of estrogen hormone against muscle damage has been suggested in women (Sewright et al. 2008; Joyce et al. 2014; Minahan et al. 2015), but conclusions from human studies are still equivocal.

In animal models, high estrogen concentrations have shown to prevent muscle damage (Tiidus et al. 2001). For example, lower concentrations of CK activity, minimal myofibrillar disruption and inflammation have been reported in female versus males rats after performing eccentric exercise (Komulainen et al. 1999). Moreover, male rats treated with estrogen showed lesser muscle damage than those without estrogen treatment. Thus, the authors speculated that estrogen plays an important role in protecting skeletal muscle against injury in female animals (Bär et al. 1988). However, changes in symptoms of EIMD between men and women are not as clear. Some studies have shown that women have lesser (Sewright et al. 2008; Joyce et al. 2014; Minahan et al. 2015), higher (Rinard et al. 2000), or even no differences (MacIntyre et al. 2000; Stupka et al. 2000) in markers of muscle damage when compared to men. The equivocal results of muscle damage after eccentric exercise in women may be partially explained due to the lack of control of the menstrual cycle phase, hormonal contraception, and age (Thompson et al. 1997; Clarkson and Hubal 2001; Savage and Clarkson 2002; Minahan et al. 2015; Nkechinyere Chidi-Ogbolu 2018). All variables that influence hormonal concentrations of estrogen, but also of testosterone and progesterone in women. However, the effect of all sexual hormones on EIMD and recovery after eccentric exercise is still lacking.

The manipulation of circulating hormones by hormone replacement therapy or by the use of oral contraceptives (OC) have been used as methods to understand the effects of sexual hormones over EIMD. It has been shown that OC consumption decreases endogenous concentrations of estrogen (~ 50% less), attenuates regular hormonal fluctuations (Bell et al. 2011; Bryant et al. 2008), and inhibits the increase of estrogen at ovulation phase during the menstrual cycle (Hicks et al. 2017). Furthermore, OC use has also shown to decrease testosterone concentration in 41.6% at the follicular phase and 30% at the luteal phase compared to Non-OC users (Timmons et al. 2005). The use of OC has shown to suppress the ovarian production of androgens, increase the levels of sex hormone-binding globulin (SHBG) and suppress adrenal androgen synthesis, negatively affecting blood levels of testosterone in women (Zimmerman et al. 2014; van Lunsen et al. 2018). Since testosterone has important anabolic effects on muscle biogenesis (Sinha-Hikim et al. 2003; Velders and Diel 2013a), it has been proposed a suppressive effect of OC on testosterone which could contribute to higher levels of EIMD and slower muscle recovery (Zimmerman et al. 2014). Moreover, the use of OC has shown to reduce progesterone concentrations, avoiding its increase at luteal phase (Timmons et al. 2005; Bell et al. 2011). Taken together, the use of OC seems to modify all female hormones and not only estrogen, which could influence the response to eccentric exercise on EIMD.

It seems that female sexual hormonal concentrations affect the response of indirect markers of muscle damage after eccentric exercise. Minahan et al. (2015) reported that OC users presented ~ 100% higher CK concentration 48 h after exercise in comparison to Non-OC users. In this same study, OC users presented a prolonged strength recovery after EIMD when compared to Non-OC users, evidenced by a continued decrease in peak torque (~ 10%) 24 and 48 h after exercise (Minahan et al. 2015). Hence, it is possible that the use of OC could influence the magnitude of muscle damage and recovery after eccentric exercise, due to changes in the physiological fluctuations of testosterone, progesterone and estrogen throughout the menstrual cycle. Only few studies (Elliott et al. 2003; Montgomery and Shultz 2010; Bell et al. 2011, 2012) have analysed the combination of testosterone, progesterone and estrogen concentrations, within the menstrual cycle phase and its effects on parameters of muscle function. Nevertheless, these studies only involve the analysis of muscle–tendon mechanics and hormonal concentrations. Therefore, research involving all hormones, menstrual cycle phase, and markers of muscle damage is still lacking. Thus, the aim of this study was to determine the effects of OC use on the concentrations of salivary testosterone, estrogen and progesterone, and to investigate the effects of OC on changes of indirect markers of muscle damage following eccentric cycling in women. We hypothesized that the use of OC will decrease estrogen, testosterone, and progesterone concentrations, promoting greater magnitude of muscle damage and a slower recovery after eccentric

cycling. Additionally, Non-OC users at ovulation will present lesser magnitude of muscle damage and a faster recovery due to increased estrogen concentrations.

Methods

Participants

Thirty healthy young women volunteered for this study. Ten of the women used third- and fourth-generation monophasic OC type (ethynyl estradiol: 0.02 mg; drospirenone: 3 mg). The remaining participants were all Non-OC users with regular menstrual cycle (< 24 days and/or > 36 days) and did not use any other form of hormone-based contraception methods for at least 6 months prior to the commence of this study (Hicks et al. 2017). Participants were divided into three groups: (1) OC-FOL ($N = 10$): oral contraceptive users tested at their follicular phase (days 1–2 of their cycle); (2) NOC-FOL ($N = 10$): non-oral contraceptives users tested at their follicular phase (days 1–2 of their cycle); and (3) NOC-OV ($N = 10$): non-oral contraceptive users tested at their ovulation phase (14 ± 2 day of their menstrual cycle). Ovulation in the OV group was confirmed using an ovulation kit (Clear blue, Ovulation test, SPD Swiss Precision Diagnostics). As shown in Table 1, no statistical differences were observed between groups for anthropometric measurements for weight, height, body mass index (BMI), age and peak oxygen consumption. Women who were actively participating in any resistance or flexibility training in the last 6 months, were pregnant or lactating, were excluded from the study. Participants were instructed to not perform any exercise, not take anti-inflammatory medication, or to undergo any treatments (e.g. massage, stretching) 2 days before and throughout the 5 days of the study. All participants signed written informed consent before participating in the study. The study was approved by the Institutional Ethic Committee, which adhered to the Declaration of Helsinki.

Study design

Overview

Prior to the commencement of the testing period, an incremental cycling test was performed to measure peak oxygen consumption (VO_{2peak}) and maximal concentric power output (PO_{max}). At least 96 h afterwards, and according to their menstrual cycle phase, all participants returned to the laboratory to perform 30 min of eccentric cycling at 90% of their PO_{max}. Maximal voluntary isometric contraction of the quadriceps (MVC) was measured before (Pre-), immediately after (Post-) and 24, 48, 72, and 96 h after exercise. Capillary creatine kinase activity (CK), and muscle soreness (SOR) using a visual analogue scale (VAS) and pain pressure threshold (PPT) were measured before (Pre-), 24, 48, 72, and 96 h after exercise. All testing and experimental trials were done at the same time of the day (07.00–10.00 a.m.) to avoid any circadian hormonal variations.

Incremental cycling protocol

Participants performed an incremental cycling test to exhaustion on an electromagnetically braked upright cycling ergometer (Matrix U1x-U, USA). The test started at 50 W for 4 min after which intensity was increased by 25 W every minute until volitional exhaustion or until participants were unable to maintain the required cadence of 60 rpm. Participants received verbal encouragement during the test. Throughout the incremental cycling tests, gas exchanges were assessed using respiratory gas analyser (Ergocard, Medisoft, Belgium). Prior to the tests, the gas analyser was calibrated using alpha gases of known concentrations, and the airflow was calibrated using a 3-l syringe (Hans Rudolph, Kansas, MO, USA). The determination of maximal concentric power output and VO_{2peak} was as previously described (Penailillo et al. 2013). Following the incremental cycling test, participants were familiarized with the eccentric cycling ergometer by performing 5 min of eccentric cycling at ~ 50 W. This familiarization procedure has shown to be sufficient in other similar studies (Peñailillo et al. 2017).

Table 1 Participants' physical and fitness characteristics

	OC-FOL ($N = 10$)	NOC-FOL ($N = 10$)	NOC-OV ($N = 10$)	P value
Age (years)	26 ± 3.59	29.4 ± 4.79	29.3 ± 5.98	0.23
Weight (kg)	60.9 ± 6.73	67.4 ± 7.8	62.6 ± 8.01	0.15
Height (cm)	160.7 ± 3.97	163.3 ± 3.57	163.4 ± 5.02	0.27
BMI (kg m^{-2})	23.7 ± 3.18	25.3 ± 2.88	23.5 ± 2.96	0.34
VO_{2peak} (ml kg^{-1} min^{-1})	28.9 ± 6.95	28 ± 5.14	30.7 ± 5.74	0.63
MVC strength (N)	837.7 ± 94.67	1010.96 ± 80.13	887.5 ± 84.1	0.09

Values are means ± SD

N number of subjects, *OC* oral contraceptive, *FOL* follicular, *OV* ovulation

Eccentric cycling exercise

The eccentric cycling was performed on a recumbent ergometer with a motor moving the cranks of the ergometer backwards at a selected cadence (Eccentric Trainer, Metitur, Finland). The backward movement of the cranks primarily induces eccentric contractions of the knee extensor muscles. Participants were instructed to resist the backward movement of the cranks and maintain 90% PO_{max} displayed on a screen for 30 min at 60 rpm. Heart rate was measured throughout the whole session.

Saliva collection and storage

Hormones were assessed by saliva sampling due to its non-invasive procedure and its correlation with plasma active compounds (Gatti and De Palo 2011). Participants were asked to rinse their mouth with distilled water and accumulate as much saliva as possible within 2 min each day they had to visit the laboratory. They were given a sterile polypropylene vial for storage and had to freeze the samples (− 20 °C) immediately after completing the 2 min of collection. All samples were taken at the same time of the day (morning) and in a fasted state. For storage, samples were brought to room temperature (22 °C) and centrifuged at 5000 rpm during 5 min at 18 °C (Walsh et al. 2002), the resulting supernatants were stored frozen in Eppendorf tubes at − 80 °C until samples were assayed.

Salivary hormones

Enzyme-linked immunosorbent assays (ELISA) were used to measure salivary estradiol, testosterone and progesterone concentrations according to the manufacturer's protocol (DRG, Germany). All the analyses were performed in duplicate according to the kit's manufacturer procedures. The intra-assay coefficient of variation for the present study was 4.3 ± 4.4% for estradiol, 3.3 ± 2.8% for testosterone and 4.1 ± 3.3% for progesterone.

Saliva flow rate

Flow rate was estimated by dividing the total saliva sample by the time taken to produce it (2 min). It was assumed that saliva density was 1.00 g/ml (Walsh et al. 2002).

Indirect markers of muscle damage

Maximal voluntary contraction (MVC)

MVC of knee extensors at 90° of knee flexion of the dominant leg was measured in a leg press machine (R2 sport, Forest Park, Illinois, USA) implemented with a force plate (Twin plates, Globus, Italia). After a 3-min warm-up in a cycle ergometer at 50 W and 60 rpm, participants were asked to perform three submaximal warm up contractions at 50%, 70%, 80% of their perceived MVC with 1-min rest between contractions. Participants then performed three maximal contractions for 3 s each with 1-min rest between contractions. They were given verbal encouragement, and the highest value was recorded for further analysis.

Creatine kinase activity (CK)

CK activity was obtained by the analysis of 35 µl of blood obtained by a capillary sample (Reflotron Plus, Roche, Switzerland).

Muscle soreness

Visual analogue scale (VAS) Pain of thigh muscles was quantified using a 100-mm visual analogue scale in which 0 indicates no pain and 100 represents the worst pain imaginable (Nosaka and Clarkson 1995). The participants were asked to mark on the visual analogue scale the level of perceived pain of the quadriceps femoris muscles while sitting down and up from a chair three times (Penailillo et al. 2015).

Pain pressure threshold (PPT) PPT was measured using a digital algometer (Force One FDIX, Wagner, USA) on the vastus lateralis (PPT-VL) portion of the quadriceps muscle. Participants had to refer the first sensation of pain in muscle. The algometer was applied gradually and perpendicularly to each muscle at a point (1 cm^2 area of stimulation) with a pressure of 50 k Pa s^{-1}. The average of three measurements was used for further analyses.

Statistical analysis

The distribution of each variable was verified by the Shapiro–Wilk normality test, from which all variables resulted normally distributed. One-way measures of variance (ANOVA) were used to compare physical characteristics between the groups. Two-way repeated measures ANOVA was used to compare changes in dependent variables (hormones concentrations, MVC, CK activity, PPT, and muscle soreness) between groups at the 4 days after performing the exercise. If a significant group, time or interaction effect was found, pairwise comparisons were performed using a Fisher's Least Significant Difference (LSD) test. The significance level was set at $P \leq 0.05$. All statistical analyses were performed with PASW Statistics 19 software for Mac (SPSS Inc, IBM company, USA). Data are presented as mean ± standard deviation (SD).

Results

Eccentric cycling

All participants completed the eccentric cycling session. There were no differences in average power output between groups ($P=0.58$). The OC-FOL group performed an average power output of 131.3 ± 17.7 W (87.2% ± 13.6 of PO_{max}), similar to the NOC-FOL group (143.2 ± 35.1 W; 90.9% ± 8.7 of PO_{max}) and the NOC-OV group (141.6 ± 26.6 W; 84.6% ± 4.6 of PO_{max}). Average heart rate was also similar between groups ($P=0.34$). The OC-FOL group presented and average heart rate of 141.6 ± 22.4 bpm (72.9% ± 11.3 of HR_{max}), similar to the NOC-FOL group (126 ± 23.2 bpm; 66.1% ± 12.2 of HR_{max}) and the NOC-OV group (139.8 ± 30.0 bpm; 73.5% ± 16.5 of HR_{max}).

Salivary hormonal concentrations

Total estradiol

As shown in Fig. 1a, no interaction effect was observed (group × time: $P=0.41$) for salivary estradiol concentrations. Additionally, no differences were observed for estradiol concentrations across time after performing the exercise. Salivary estradiol concentrations showed no correlations at 72 h and 96 h after exercise ($P>0.05$) with MVC, CK, VAS or PPT-VL in any group (Table 3).

Total testosterone

As shown in Fig. 1b, no interaction effect was observed for salivary testosterone concentrations (group × time: $P=0.09$). The OC-FOL group showed a tendency to decrease total testosterone concentrations, from baseline to 96 h post-exercise (from 50.2 pg/ml to 27.4 pg/ml; $P=0.07$). These variances were not observed in the NOC-FOL group (from 39.1 pg/ml to 52.4 pg/ml; $P=0.27$) or the NOC-OV group (from 40.2 pg/ml to 38.3 pg/ml; $P=0.85$). When comparing NOC-FOL with OC-FOL, testosterone concentrations were 91.2% higher in the NOC-FOL group at 96 h post-exercise (52.4 ± 26.7 pg/ml for NOC-FOL and 27.4 ± 16.6 pg/ml for OC-FOL; $P=0.04$). Salivary testosterone concentrations showed no correlations at 72 h and 96 h after exercise ($P>0.05$) with MVC, CK, VAS or PPT-VL in any of the groups (Table 3).

Progesterone

As shown in Fig. 1c, no interaction effect was observed for salivary progesterone (group × time: $P=0.07$). Progesterone

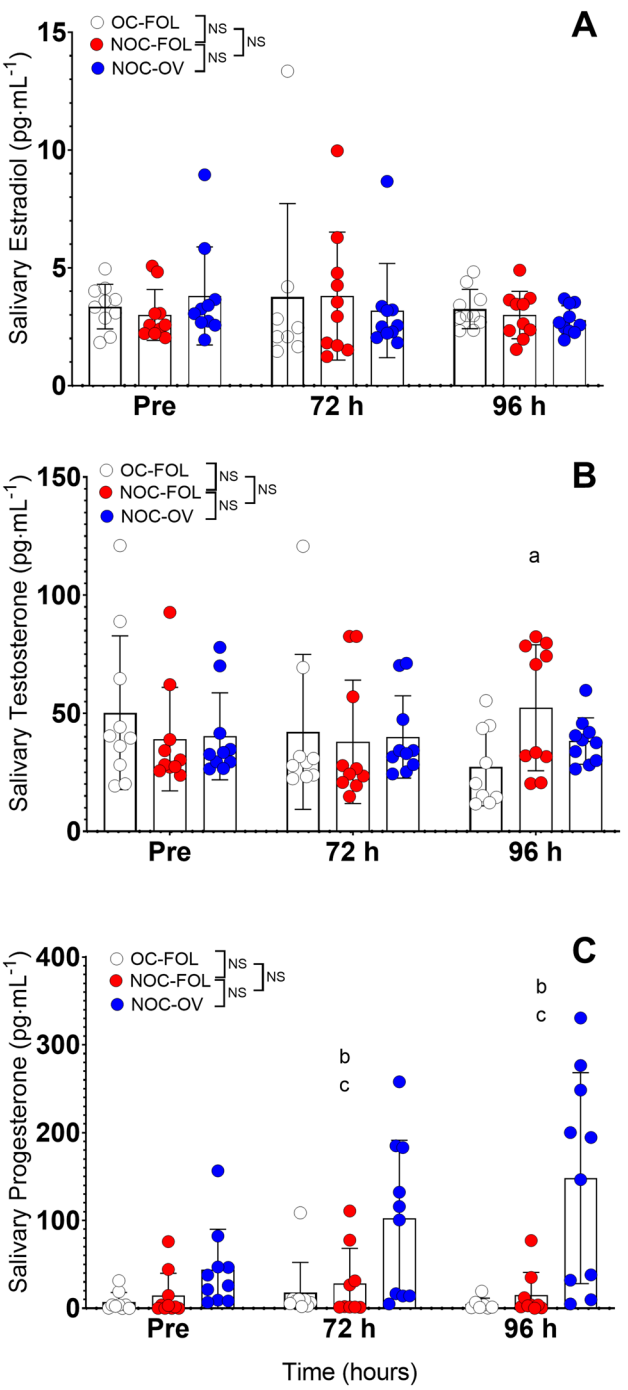

Fig. 1 Individual (circles) and average group (bars) changes in salivary estradiol (**a**), testosterone (**b**), and progesterone (**c**) at baseline (Pre), 72 and 96 h after performing eccentric cycling. White circles, OC-FOL. Red circles, NOC-FOL. Blue circles, NOC-OV. a: statistical group difference between OC-FOL and NOC-FOL. b: statistical group difference between NOC-OV and NOC-FOL. c: statistical group difference between NOC-OV and OC-FOL. *: statistical difference from baseline in OC-FOL. #: statistical difference from baseline in NOC-FOL. §: statistical difference from baseline in NOC-OV. Statistical difference set at $P<0.05$

concentrations increased 235% from baseline to 96 h post-exercise in the NOC-OV group (from 44.2 pg/ml to 148.1 pg/ml; $P=0.001$). These differences in progesterone concentrations were not observed in the OC-FOL group (from 7.2 pg/ml to 4.8 pg/ml; $P=0.95$) or the NOC-FOL group (from 14.4 pg/ml to 12.2 pg/ml; $P=0.97$). The post hoc analysis showed that progesterone concentrations are 5.6- and 30.8-fold higher for the NOC-OV compared to OC-FOL at 72 h and 96 h post-exercise ($P<0.01$), respectively. Similarly, NOC-OV showed 3.6-fold higher progesterone concentrations than NOC-FOL at 72 h post-exercise, and 9.7-fold higher at 96 h ($P<0.01$). Salivary progesterone concentrations showed no correlations at 72 h and 96 h after exercise ($P>0.05$) with MVC, CK, VAS or PPT-VL in any of the groups (Table 3).

Salivary flow

As shown in Table 2, no statistical differences in time for salivary flow were observed between the groups.

Indirect markers of muscle damage

Maximal voluntary contraction (MVC)

Baseline MVC strength (Table 1) and immediately after exercise MVC strength loss (Fig. 2a) were similar in all groups (OC-FOL = − 15.9 ± 7.7%, NOC-FOL = − 20.4 ± 9.2% and NOC-OV = − 17.1 ± 9.4%; $P=0.64$). The two-way ANOVA analysis showed no interaction effect between OC-FOL and NOC-FOL ($P=0.06$), NOC-FOL and NOC-OV ($P=0.77$), or OC-FOL and NOC-OV ($P=0.88$). A group effect was observed between OC-FOL and NOC-FOL ($P=0.02$) showing that MVC strength was 15.0 ± 21.6% and 18.4 ± 21.9% greater in the NOC-FOL compared to OC-FOL at 72 h ($P=0.02$) and 96 h ($P=0.01$) after cycling, respectively. A time effect was observed in all groups ($P<0.01$), where the NOC-FOL group was able to recover their baseline MVC strength values at 72 h (90.9 ± 6.9%; $P=0.08$) and at 96 h (64.5 ± 8.2; $P=0.29$) post-exercise. The NOC-OV group recovered their MVC strength by 96 h (86.0 ± 14.0%; $P=0.08$), and the OC-FOL group was not able to fully recovery baseline MVC strength values after cycling at 96 h post-exercise ($P<0.01$).

Creatine kinase (CK) activity

CK activity concentrations can be observed in Fig. 2b. A group × time effect can be observed between the NOC-FOL and the NOC-OV group ($P=0.04$), CK activity increased at 72 h (from 29.7 ± 11.6 UI/L at baseline to 155.9 ± 172.1 UI/L, $P=0.03$) and at 96 h (299.6 ± 307.9 UI/L; $P<0.01$). A time effect ($P<0.01$) was observed in the OC-FOL group for CK activity, from 46.3 ± 35.5 UI/L at baseline to 250.2 ± 276.5 UI/L at 96 h post-exercise. No increases of CK activity were observed within the NOC-FOL group after eccentric exercise.

Muscle soreness (VAS)

As shown in Fig. 2c, two-way ANOVA showed no interaction effect between OC-FOL and NOC-FOL group ($P=0.59$), OC-FOL and NOC-OV ($P=0.64$), or between NOC-FOL and NOC-OV group ($P=0.94$). Peak muscle soreness was registered at 48 h for all groups (49.6 ± 26.8 mm for OC-FOL; 40.5 ± 25.8 mm for NOC-FOL; and 33.6 ± 20.3 mm for NOC-OV; $P<0.05$). A group effect was observed between OC-FOL and NOC-FOL ($P<0.01$) and between OC-FOL and NOC-OV ($P<0.01$) at 72 h and 96 h, respectively. OC-FOL presented 39.0 ± 47.6% higher muscle soreness than NOC-FOL and 34.7 ± 88.1% higher than NOC-OV at 72 h. Furthermore, at 96 h, OC-FOL presented 18.5 ± 96.2% higher muscle soreness than NOC-FOL and 31.0 ± 59.4% higher than NOC-OV at 96 h. Most importantly, time analysis show that when compared to baseline values, the OC-FOL group was the only group which did not fully recover from muscle soreness, rising from 1.6 ± 2.8 mm to 39.6 ± 22.1 mm ($P<0.01$) at 24 h to 27.5 ± 24.7 mm ($P<0.01$) at 96 h. On the other hand, NOC-FOL and NOC-OV fully recovered at 96 h (9.0 ± 8.0; $P=0.40$ and 11.8 ± 14.8 mm; $P=0.14$, respectively).

Pain pressure threshold of vastus lateralis (PPT-VL)

As shown in Fig. 2d, no interaction effect was observed between OC-FOL and NOC-FOL group ($P=0.28$), OC-FOL and NOC-OV ($P=0.86$), or between NOC-FOL and NOC-OV group ($P=0.83$). Peak decrease in PPT-VL was observed for all groups at 48 h post-exercise (− 39.6 ± 26.0%

Table 2 Salivary flow rate (mL/min). Baseline: before eccentric exercise, 72 h: 72 h post-exercise; 96 h: 96 h post-exercise

	OC-FOL ($N=10$)	NOC-FOL ($N=10$)	NOC-OV ($N=10$)	P value
Baseline	1.1 ± 0.61	1.0 ± 0.65	1.0 ± 0.30	0.68
72 h	0.9 ± 0.40	1.0 ± 0.52	0.8 ± 0.26	0.65
96 h	0.8 ± 0.50	0.9 ± 0.40	0.8 ± 0.31	0.99

Values are means ± SD

N number of subjects, *OC* oral contraceptive, *FOL* follicular, *OV* ovulation

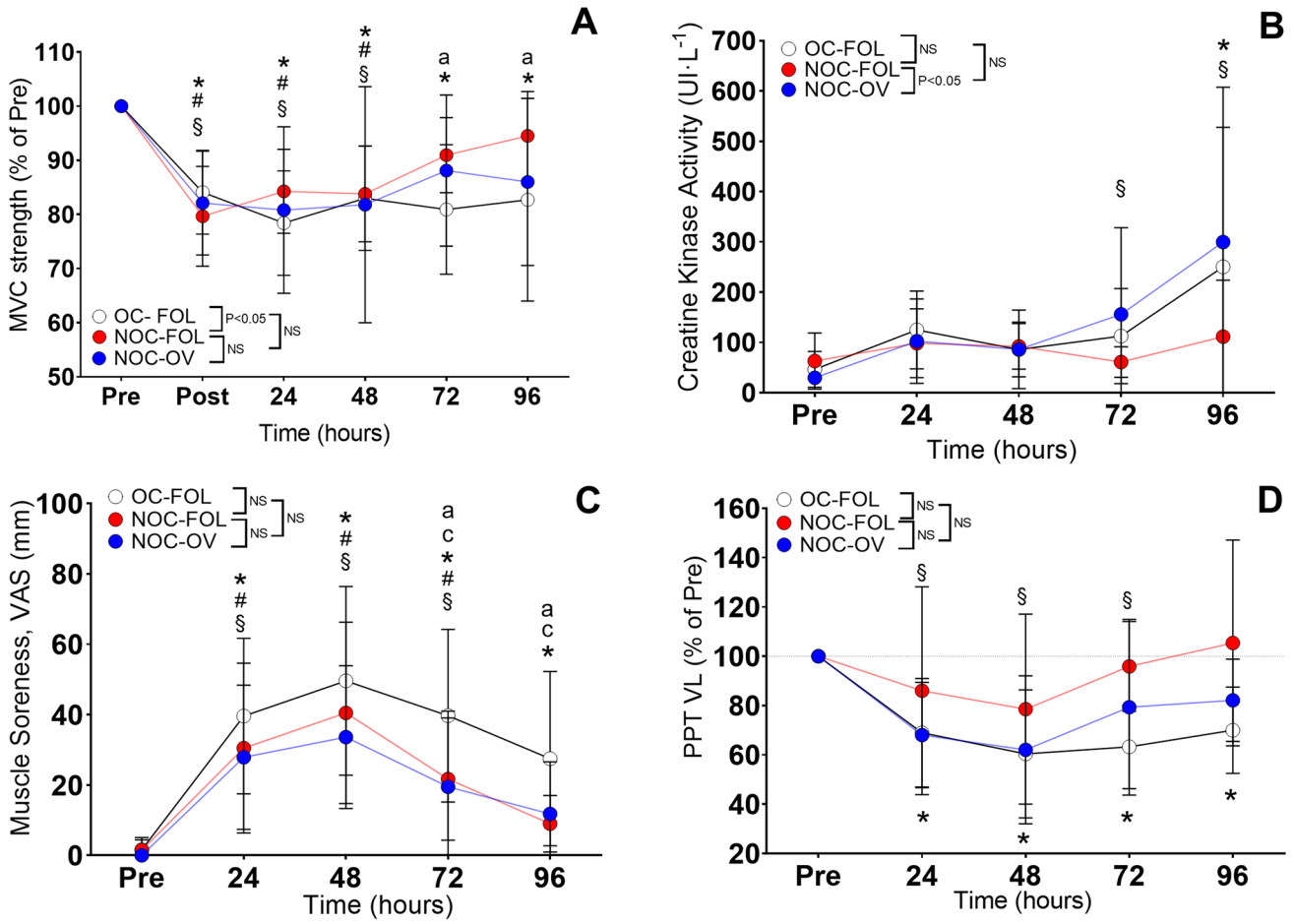

Fig. 2 Markers of muscle damage at baseline and at 24, 48, 72, and 96 h after eccentric cycling. Percentage of maximal voluntary contraction (**a**), creatine kinase activity (**b**), muscle soreness from visual analogue scale (**c**) and pain pressure threshold at vastus lateralis (**d**). White circles, OC-FOL. Red circles, NOC-FOL. Blue circles, NOC-OV. a: statistical difference between OC-FOL and NOC-FOL. b: statistical difference between NOC-OV and NOC-FOL. c: statistical difference between NOC-OV and OC-FOL. *: statistical difference from baseline in OC-FOL. #: statistical difference from baseline in NOC-FOL. §: statistical difference from baseline in NOC-OV. Statistical difference set at $P < 0.0$

for OC-FOL, $P < 0.01$; $-21.5 \pm 38.6\%$ for NOC-FOL, $P = 0.08$; and $-380.3 \pm 30.0\%$ for NOC-OV, $P < 0.01$). No PPT-VL differences can be observed in time within the NOC-FOL group from baseline to 96 h post-exercise ($P > 0.05$). On the other hand, the OC-FOL group decreased $31.1 \pm 22.1\%$ their PPT at 24 h ($P < 0.01$) and was not able to recover by 96 h ($-30.0 \pm 17.5\%$, $P < 0.01$) post-exercise, whereas the NOC-OV group decreased $-43.7 \pm 32.2\%$ their PPT from baseline ($P = 0.01$) at 24 h and was able to recover by 96 h ($-18.0 \pm 16.7\%$, $P = 0.15$).

Discussion

The aim of this study was to determine the effect of oral contraceptives (OC) use in women on indirect markers of muscle damage following eccentric cycling. Furthermore, it was tested if the phases of the menstrual cycle and the concentrations of salivary testosterone, estrogen, or progesterone could influence the magnitude of muscle damage. The main finding of this study showed that women consuming oral contraceptives (OC-FOL group) presented a slower recovery of symptoms of exercise-induced muscle damage (EIMD) following eccentric cycling compared to Non-OC users at follicular (NOC-FOL group) or ovulation phase (NOC-OV group). Therefore, our results suggest a negative effect of OC use on muscle recovery following a single bout of eccentric cycling. However, even though differences in recovery of indirect markers of muscle damage between groups were observed, no correlations of these markers with salivary concentrations of estrogen, progesterone or testosterone were found. Therefore, our hypothesis was only partially supported.

Table 3 Correlations between salivary hormonal concentrations and indirect markers of muscle damage in OC-FOL, NOC-FOL and NOC-OV groups

	MVC	CK	VAS	PPT-VL
Estrogen				
OC-FOL				
Pearson *r* value	0.04	− 0.09	− 0.29	− 0.15
P value	0.88	0.74	0.25	0.55
NOC-FOL				
Pearson *r* value	− 0.19	− 0.22	0.26	− 0.28
P value	0.43	0.38	0.28	0.23
NOC-OV				
Pearson *r* value	0.08	− 0.01	0.25	− 0.18
P value	0.74	0.96	0.30	0.44
Testosterone				
OC-FOL				
Pearson *r* value	0.04	− 0.16	− 0.38	− 0.08
P value	0.88	0.54	0.12	0.75
NOC-FOL				
Pearson *r* value	− 0.3	− 0.04	0.09	− 0.38
P value	0.19	0.86	0.70	0.10
NOC-OV				
Pearson *r* value	0.05	− 0.25	− 0.27	− 0.24
P value	0.83	0.30	0.24	0.31
Progesterone				
OC-FOL				
Pearson *r* value	0.26	− 0.34	− 0.33	− 0.07
P value	0.34	0.24	0.22	0.81
NOC-FOL				
Pearson *r* value	− 0.1	− 26	0.28	− 0.23
P value	0.69	0.32	0.26	0.36
NOC-OV				
Pearson *r* value	− 0.21	− 0.18	0.44*	− 0.14
P value	0.38	0.46	0.05	0.56

OC oral contraceptive, *OV* ovulation, *MVC* maximal voluntary contraction, *CK* creatine kinase activity, *VAS* visual analogue scale, pain pressure threshold in vastus lateralis

Eccentric cycling is a multijoint eccentric exercise that allows performing a high volume of submaximal eccentric contractions (e.g. 60 rpm × 30 min = 1800 eccentric contractions) while controlling exercise intensity. Eccentric cycling has shown to be distinctive to previously described eccentric exercise modalities (Coratella et al. 2019). Previous studies have shown that eccentric cycling induced moderate decreases in muscle strength after similar eccentric cycling protocols (Peñailillo et al. 2013, 2015, 2017). For instance, Peñailillo et al. (2013) reported moderate muscle damage in young men following 30 min of eccentric cycling at 60% of their maximal concentric power output (PO_{max}.). They observed ~ 15% decrease in their maximal voluntary contraction (MVC) strength 24 h after eccentric cycling. In our study, a similar exercise was performed, but intensity was greater (90% of PO_{max}). All participants were able to complete the exercise protocol and we found a similar decrease in MVC strength after eccentric cycling between groups, with an overall MVC strength decrease of 20.8 ± 14.0% 24 h after exercise (Fig. 2). Thus, it seems like our eccentric cycling protocol induced moderate muscle damage, evidenced by similar increases in indirect markers of muscle damage to previous studies.

Interestingly, our results showed that the recovery of baseline values of indirect markers of EIMD was different between OC and Non-OC users. Even though all groups had similar baseline measurements of MVC strength (Table 1) and performed similar workload during cycling, Non-OC users (NOC-FOL and NOC-OV) showed a full recovery, whereas OC users (OC-FOL group) were not able to recover by 96 h (Fig. 2). These results suggest a negative effect of OC on EIMD and recovery, which agree (Savage and Clarkson 2002; Minahan et al. 2015) and also contradict (Hicks et al. 2017) previous research. Discrepancies might be due to participants in Hicks et al. (2017) being at their ovulation phase, showing a 51% difference in estrogen concentrations between OC and Non-OC users. Whereas in our study, participants were at their follicular phase, and we observed no differences in estrogen concentrations (3.0 ± 1.1 pg/ml for NOC-FOL vs. 3.4 ± 0.9 pg/ml for OC-FOL) between the groups (Fig. 1a). Although unexpected, similar salivary estrogen concentrations between OC and Non-OC users at the follicular phase have been previously reported (Griksiene and Ruksenas 2011; Petersen et al. 2014). This finding could be partially explained because the use of chronic oral contraceptives could induce a steady low concentration of estrogen during the menstrual cycle, even after the consumption of the inactive pills (i.e. hormone free, placebo pills) (Savage and Clarkson 2002; Oosthuyse and Bosch 2017). Furthermore, it was expected that the NOC-OV group showed greater concentrations of estrogen at baseline due to ovulation in comparison to NOC-FOL or OC-FOL. Nevertheless, salivary estrogen concentrations were similar between groups. We speculate that this unexpected finding was probably due to baseline measurements being performed during the physiological drop of estrogen after ovulation, i.e. in the luteal phase. The low estrogen (~ 3.8 pg/ml), but high progesterone (~ 44.2 pg/ml) concentrations in the NOC-OV group suggests that our participants were not at their ovulation phase. Instead, those values match values observed of women at their luteal phase (Schultheiss et al. 2003; Gandara et al. 2007). Chidi-Ogbolu and Baar (2019) explain that after estrogen increases (i.e. near ovulation), there is an immediate decrease in estrogen concentrations related to higher concentrations of luteinizing hormone, indicating the beginning of the luteal phase (Chidi-Ogbolu and Baar 2019). Thus, a possibility exists that our participants were

not in their ovulation phase as expected, but more into their luteal phase. Taken together, due to similarities in estrogen concentrations between groups, our results suggest that the differences in recovery observed between OC and Non-OC users (both in follicular and luteal phases) could be associated to other hormones rather than estrogen, such as testosterone or progesterone.

It has been reported that the use of OC could negatively affect total testosterone concentrations in females due to the presence of synthetic progestins (Timmons et al. 2005; Hansen et al. 2011; Coelingh Bennink et al. 2017). The use of OC suppresses the production of ovarian and adrenal androgen production, and stimulates the production of sex hormone-binding globulin (SHBG) in the liver (Zimmerman et al. 2014), reducing total testosterone levels. The magnitude of decrease in testosterone depends on the type of progestin in each OC, where the combination of ethynyl estradiol (EE) and drospirenone (DRSP) has shown to decrease concentrations of testosterone in 54.5% (Coelingh Bennink et al. 2017). In our study, the OC-FOL group showed a decrease in salivary testosterone concentration at 96 h post-exercise when compared to baseline (− 45.4%; $P = 0.07$), whereas the NOC-FOL group showed a slight yet non-significant increase in testosterone at 96 h (+ 34%; $P = 0.16$) (Fig. 1b). All of our participants in the OC-FOL group used EE and DRSP combined OC, thus, the 45.4% decrease in testosterone concentrations is in accordance with previous reports. It is known that testosterone has important anabolic effects over satellite cell proliferation, promoting muscle growth and repair (Sinha-Hikim et al. 2003; Serra et al. 2013; Velders and Diel 2013b). Therefore, considering that there was a 91% difference in testosterone concentrations between the NOC-FOL and OC-FOL groups at 96 h post-exercise ($P = 0.04$), we speculate that the slower muscle function recovery after eccentric cycling observed in the OC-FOL group could be due to negative effects over muscle satellite cell proliferation caused by lower testosterone concentrations.

Fluctuations in progesterone have been related to EIMD in previous studies (Oosthuyse and Bosch 2010). Women at their luteal phase have showed greater MVC loss and higher CK activity than women at their follicular phase after eccentric exercise (Thompson et al. 1997; Markofski and Braun 2014; Oosthuyse and Bosch 2017; Chidi-Ogbolu and Baar 2019). The NOC-OV group had similar concentrations of estrogen and testosterone than the other groups, but a 141.7% ($P = 0.06$) and 235% ($P = 0.001$) greater concentrations of progesterone at 72 h and 96 h after exercise compared to baseline, respectively (Fig. 1c). This suggests that participants in the NOC-OV group were at the beginning of their luteal phase and not at ovulation as expected. Our results show that the NOC-FOL group recovered baseline values of MVC, VAS and PPT-VL at 72 h after eccentric cycling, whereas NOC-OV group recovered baseline values at 96 h (Fig. 2). This slower, yet effective recovery could be explained by higher progesterone concentrations in the NOC-OV group compared to NOC-FOL. High progesterone concentrations have been related to higher concentrations of amino acid catabolism (Oosthuyse and Bosch 2010) and to decrease ovarian estrogen production, which may also limit muscle recovery (Thompson et al. 2006). Therefore, we speculate that the slower recovery observed in NOC-OV could be partially explained by the effect of high progesterone levels on muscle repair after eccentric exercise.

Our results demonstrate that the chronic long-term consumption of exogenous hormones (OC-FOL group) have the slowest muscle function recovery and repair after eccentric exercise, whereas non-users (at either their follicular or luteal phase) can fully recover (by 96 h for NOC-OV and by 72 h for NOC-FOL) after eccentric cycling. Nevertheless, no correlations between indirect markers of muscle damage and hormone concentrations were found (Table 3), probably due to small sample size. We speculate that the differences between the magnitude of EIMD and recovery between groups were related to variances in sexual hormonal concentrations, specifically testosterone and progesterone concentrations. Similar estrogen concentrations were observed between all the groups, whereas variances of testosterone and progesterone were observed for the NOC-FOL and NOC-OV. The use of OC has previously shown to decrease myofibrillar protein synthesis and have an inhibiting effect over anabolic growth hormones (i.e. testosterone) (Hansen et al. 2011). In addition, progesterone has been related to cause a catabolic state promoting insulin resistance (Oosthuyse and Bosch 2010). Therefore, variances in these hormones may play a key role in protein turnover and translate into slower muscle recovery. Furthermore, it has been suggested that OC use can alter the concentrations of other anabolic and catabolic hormones (e.g., IGF-I, cortisol), which could also contribute to slower muscle recovery (Hansen et al. 2011; Smith et al. 2014). The chronic use of synthetic hormones (e.g. OC) may have a stronger deleterious effect over muscle recovery than high levels of progesterone, as evidenced by our OC group. The OC group was not able to fully recover by 96 h after exercise, whereas the NOC-OV group did. Further research in the topic is warranted, with analysis regarding the effect of oral contraceptives on other parameters that may influence muscle damage, such as body temperature, inflammation or muscle–tendon stiffness.

This study is not except of limitations, which should be considered in future studies in this field. Although the use of saliva collection is a valid alternative due to its non-invasive, simple and stress-free methodology (Lu et al. 1999; Liening et al. 2010; Gatti and De Palo 2011; Griksiene and Ruksenas 2011; Hayes et al. 2015), blood samples could have allowed a more precise assessment of specific hormones, and could

have contributed to better correlations with EIMD. Previous research shows that correlations determined using saliva as a substitute for serum are likely to underestimate the true relationship with the matter in question. Nevertheless, this can be addressed by increasing the sample size (Shirtcliff et al. 2000). Therefore, a limitation to our results might be related to a small sample size. Moreover, we observed high within-group variability on salivary hormones. The use of standardized protocols of saliva sampling such as testing within a similar time period throughout the day, similar menstrual phase, and collection of samples at fasting state, can decrease levels of variability. However, variations can still exist between and within women (Gandara et al. 2007). The occurrence of nonovulatory cycles, advanced or belated ovulatory window, onset of menarche, age, body mass, and body composition, are all factors that contribute to hormonal variability and could explain our results (Gandara et al. 2007; Smith et al. 2014). Nonetheless, since all our hormonal concentrations are within physiological range (Shirtcliff et al. 2000; Schultheiss et al. 2003; Gandara et al. 2007), we are confident that our results are valid and representative of the physiological hormonal status of our participants.

Conclusions

Our findings support that the use of OC negatively influenced muscle recovery and repair after eccentric cycling exercise in women. Although we were not able to fully relate the changes in the indirect markers of muscle damage with variations in female hormone concentrations, our results suggest that low levels of testosterone negatively affect muscle repair in women and that high progesterone levels could also slow down recovery after muscle damage. Most importantly, the use of OC showed the slowest recovery rates from EIMD when compared to Non-OC users at either their follicular or luteal phase.

Acknowledgements This work was funded by the Institutional Research Grant awarded to K.M and L.P. by Universidad Finis Terrae (Grant no. CAI 2015).

Author contributions LP and KM conceived and designed the research. CG, KM and LP conducted the data collection. KM and HZ performed the experiments and biochemical analyses. KM wrote the manuscript. All the authors read and approved the manuscript.

Compliance with ethical standards

Conflict of interest There were no conflicts of interest.

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