Oral contraceptive pill phase does not influence muscle protein synthesis or myofibrillar proteolysis at rest or in response to resistance exercise

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Running title: Oral Contraceptives and Muscle Protein Turnover

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ABSTRACT

There is speculation that oral contraceptive pill (OCP) use affects skeletal muscle biology and protein turnover in response to resistance exercise; however, research in this area is scarce. We aimed to assess, using stable isotope tracers and skeletal muscle biopsies, how second-generation OCP phase affected muscle protein synthesis and whole-body proteolysis. Participants (n=12) completed two 6-day study phases in a randomized order: an active pill phase (Active; week two of a monthly active OCP cycle) and an inactive pill phase (Inactive; final week of a monthly OCP cycle). Participants performed unilateral resistance exercise in each study phase, exercising the contralateral leg in the opposite phase in a randomized, counterbalanced order. The Active phase myofibrillar protein synthesis (MPS) rates were 1.44 ± 0.14 %•d^{-1} in the control leg and 1.64 ± 0.15 %•d^{-1} in the exercise leg ($p < 0.001$). The Inactive phase MPS rates were 1.49 ± 0.12 %•d-1 %/d in the control leg and 1.71 ± 0.16 %•d^{-1} in the exercise leg ($p < 0.001$), with no interaction between phases ($p = 0.63$). There was no significant effect of OCP phase on whole-body myofibrillar proteolytic rate (active phase $k = 0.018 \pm 0.01$; inactive phase $k = 0.018 \pm 0.006$; $p = 0.55$). Skeletal muscle remains equally as responsive, in terms of stimulation of MPS, during Active and Inactive OCP phases; hence, our data does not support a pro-anabolic or catabolic, based on myofibrillar proteolysis, effect of OCP phase on skeletal muscle in females.

NEW AND NOTEWORTHY

We discovered that women taking a second-generation oral contraceptive pill (OCP) showed no difference in integrated daily muscle protein synthesis or whole-body myofibrillar proteolysis in the active or placebo pill phases of the pill cycle. Our data show that OCP phase does not influence skeletal muscle protein turnover in females and does not support a marked pro-catabolic or anabolic effect.

INTRODUCTION

Premenopausal females are frequently excluded from exercise physiology research, with an often-cited reason being the potential for the effects of menstrual cycle (MC) hormones or oral contraceptive pills (OCP) on the outcomes (1). Although the primary purpose of ovarian hormones (estradiol [E2] and progesterone [P4]) is for reproductive function, it has been proposed that E2 may be pro-anabolic and involved in pathways and processes that influence muscular adaptations to exercise (2). In contrast, the presence of P4 has been proposed to antagonize the action of E2 (3). Despite this speculation, we recently showed that MC phase does not affect rates of MPS or whole-body myofibrillar proteolysis (4). We have also pointed out that P4 is more androgenic than E2, which conflicts with the notion that high P4 in the luteal phase creates a catabolic environment (3, 5, 6).

Hansen et al. studied the effects of OCP on myofibrillar protein synthesis (MPS) (7) and tendon and muscle connective tissue synthesis (8). These authors reported lower MPS and tendon as well as muscle (and possibly bone based on indirect biomarkers) connective tissue protein synthesis in OCP users (8). When data were split into those taking second- versus third-generation OCP, Hansen et al. concluded that the lower MPS was predominantly due to third-generation OCP users (n=7) who showed a marked lowering of MPS versus those taking second-generation OCP (n=4). Notably, rates of myofibrillar proteolysis were also determined using microdialysis and reported to be no different between OCP users and controls and were unaffected by OCP generation (7). These were short-term studies lasting several hours, and the exercise used was a high-intensity single-leg kicking (7, 8); thus, the effects over longer periods and with a more anabolic exercise stimulus, such as resistance exercise training (RET), are less well known. Given that the net balance of MPS and muscle protein breakdown (MPB), along

with other processes (9), contribute to RET-induced hypertrophy, there is an important research gap.

It is unknown how OCP use may affect RET outcomes such as hypertrophy and strength; however, speculation is that since OCP use downregulates the production of endogenous E2 and P4, this suppression may affect molecular mechanisms that are important in hypertrophy and somehow suppress normal adaptation (2, 10). RET studies involving females taking OCP have produced mixed results (11-15); however, generally, no significant differences were observed between OCP users and non-users regarding RET-induced muscle hypertrophy. Nolan et al. (10) systematically reviewed how OCP affects RET outcomes and found no consistent effect on hypertrophy, power, or strength.

The purpose of this study was to investigate MPS and myofibrillar proteolysis in response to resistance exercise in females on OCP. Subjects were assessed during their active pill phase and the inactive phase of second-generation OCP. We aimed to test the hypothesis that muscle protein synthesis would increase in response to resistance exercise in both phases but with no differences between phases.

METHODS

The study was approved by the Hamilton Integrated Research Ethics Board (project number: 14067) and conformed to the standards for the use of human subjects in research as outlined by the Canadian Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans – TCPS 2, 2022 (https://ethics.gc.ca/eng/policy-politique_tcps2-eptc2_2022.html) and the Declaration of Helsinki (https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/). Each participant was informed of

the purpose of the study, experimental procedures, and potential risks before written informed consent was obtained. The trial was registered with the National Institutes of Health at http://www.clinicaltrials.gov repository as NCT05347667.

Participants. Healthy young females (n=12) were recruited for the study. Eligible participants were between the ages of 18 and 30 and in good health (as determined by a medical screening questionnaire). All participants reported taking second-generation OCP (Allese or Alysena) for at least 6 mo prior to taking part in the study. Participants were excluded if they: i) suffered from an orthopedic, cardiovascular, pulmonary, renal, liver, infectious disease, immune, metabolic or gastrointestinal disorder likely to impact study outcomes; ii) took medications known to affect protein metabolism (i.e., corticosteroids, non-steroidal anti-inflammatory drugs, or high strength acne medication, testosterone replacement); iii) used tobacco or cannabis or tobacco/cannabis-related products (smoking or vaping); iv) had been previously diagnosed with a menstrual cycle disorder, polycystic ovarian syndrome, or endometriosis. Participants' characteristics are shown in Table 1.

A sample size of 9 subjects, using a crossover design, was determined based on an *a priori* power analysis calculated using G*power (Version 3.1.9.6, Franz Faul, Kiel University, Germany) based on our previous trial (4) (target alpha of 0.05 and power of 0.80) with a small effect size of 0.2 to be sufficient to detect a change of ~25% in muscle protein synthesis, which we deemed to be physiologically relevant. To protect power and account for any dropouts, we included 12 subjects.

Study Overview. Participants completed two 6-day study phases in a randomized order during each phase of their OCP cycle: Active pill phase (days 9-14) and Inactive pill phase (days 23-28). A schematic of the study protocol is shown in Figure 1.

Participants completed a general health questionnaire to indicate their current health status and medication use to ensure eligibility for the study. Height and body mass were assessed using a calibrated stadiometer and scale. Participants underwent a dual X-ray absorptiometry (DXA; GE-Lunar iDXA; Aymes Medical, Toronto, ON) scan to assess body composition. DXA-derived lean mass was used to determine D_2O dosing. Unilateral knee extension 10 repetition maximum (RM) was assessed for each leg to determine the starting load for subsequent study visits.

Much of the details of the protocol and methods we employed, such as RET protocol and the calculation of the rates of MPS and whole-body myofibrillar proteolysis, were reported in our previous paper (4); thus, we provide only relevant details here. The salient difference between our previous protocol (4) and this study was the timing of the study protocols, which was completed within one OCP cycle and took place during the Active and Inactive pill phases. Participants arrived for the first study visit after an overnight fast. Following a pregnancy test, subjects provided a baseline saliva sample (to obtain baseline body water enrichment), a baseline urine sample (to measure D_3-creatinine enrichment for muscle mass; see below), a blood sample (to assess serum hormones), and a baseline muscle biopsy from the *vastus lateralis* of the control leg. The control (non-exercised) leg was randomly determined for phase one, and the contralateral leg served as the control for phase two. On the priming dose day, participants were given three (1.25 ml•kg^{-1} lean mass) aliquots of 70 atom % D_2O to consume 30 min apart. An oral dose of 30 mg D_3-Cr was included in the third aliquot of D_2O to assess skeletal muscle mass as previously described in detail (6, 16). Participants performed three sets of 10 unilateral knee extensions to volitional fatigue, defined as an inability to complete a repetition through the full

range of motion. If the participant completed more than 12 or less than eight repetitions, the load was adjusted, up or down, accordingly.

Participants returned to the laboratory 48h after Visit 1 to provide a urine sample and perform three additional sets of unilateral knee extensions as outlined above. Participants returned to the laboratory 72h after Visit 1 to provide a urine sample and 18h prior to the scheduled Visit 5 to consume 10mg of D_3-3-methyl-histidine (3MH) dissolved in water. After an overnight fast, participants reported to the laboratory for the final visit. Muscle biopsies were taken from the exercise and control legs. Blood samples were collected hourly for five hours to assess plasma D_3-3MH and measure whole-body myofibrillar proteolysis as described (4, 6, 16, 17).

Blood Analysis. Blood samples were analyzed using the Ortho Vitros MicroWell, using the VITROS 5600 Integrated System that provides enhanced chemiluminescence detection for serum estradiol (E2; pmol/L; by competitive immunoassay; inter-assay CV <4%), progesterone (P4; nmol/L; by competitive immunoassay; inter-assay CV <6%), and luteinizing hormone (LH; IU/L; by non-competitive immunometric assay; inter-assay CV <5%) by Hamilton Regional Laboratory Medicine and D_3-3-methyl-histidine enrichment as described previously (4, 6, 16, 17).

Deuterium Oxide. The incorporation of deuterium (as D_2O) into muscle protein-bound alanine was assessed to quantify MPS rates (6, 16-18). The protocol consisted of one loading day and four maintenance days with the goal of enriching and maintaining the body water pool.

Muscle Biopsies. Muscle biopsy samples were obtained on 7 occasions using a 5mm Bergstrom needle modified for manual suction under 1% xylocaine local anesthesia. The first biopsy site was approximately 15 cm above the patella, and subsequent biopsy sites were spaced ~3-5cm

apart. Biopsies were taken from the control limb pre-exercise (phase [randomized to Active or Inactive] 1, visit 1) and the control and exercise limbs (phase 1, visit 5; phase 2, visit 1; and phase 2, visit 5). Visible connective and adipose tissue were dissected from each specimen prior to being snap-frozen in liquid nitrogen and stored at −80°C.

Saliva Analysis. Saliva samples were obtained by gently chewing on a cotton swab for 2–3 min until completely saturated with saliva. Salivettes were centrifuged at 1500g for 10 min and diluted in doubly distilled water. Saliva samples were analyzed for ^{2}H (D) enrichment by cavity ringdown spectroscopy (L2130-i, Picarro Inc., Santa Clara, CA). Measurements were corrected for machine drift and background enrichment, and the ^{2}H (D) isotopic enrichments for saliva were converted to atom % excess using standard equations as reported previously (4, 6, 16, 17).

Myofibrillar Extraction. Snap-frozen muscle samples were homogenized using 5mm stainless steel beads in a 2mL Eppendorf (2 x 40 s at 20 Hz, TissueLyser, Hilden, Germany) with 500µl fresh, ice-cold homogenization buffer (25mM Tris buffer [Tris-HCl, Trizma Base, doubly distilled H_2O (ddH_2O) pH 7.2], 1 PhosStop Tablet (Roche, Switzerland), 1 complete (Roche) mini protease inhibitor tab, 100µl TritonX-100). Samples were then processed as described in our previous work (4, 17).

Integrated myofibrillar protein synthesis. Ingestion of D_2O was used to label newly synthesized myofibrillar proteins (18). Myofibrillar protein synthesis rates were determined using the standard precursor-product method (19, 20). Total body water (saliva) deuterium (^{2}H) enrichment (converted to its natural log) was used as a surrogate for plasma alanine labeling (precursor). The change in ^{2}H enrichment (relative to ^{1}H) of muscle alanine (product) over time was used to calculate the myofibrillar fractional synthesis rates (4, 17).

184 *Creatinine Enrichment.* Samples were thawed at room temperature and had 250 μL of ice-cold
185 acetonitrile added, then were vortexed, mixed, and cooled on ice for 30 min. Samples were then
186 centrifuged at 17000g for 20 min. The supernatant was filtered through a 0.2 μm filter and
187 transferred to vials ready for mass spectrometry analysis as previously described (4, 6, 16, 17).
188 The estimated creatine pool size was divided by 4.3 g/kg, which reflects the average
189 concentration of creatine found in whole wet muscle tissue (21). Details of the analyses are
190 provided in our previous papers (4, 6, 16, 17).
191 *Plasma D_3-3-methyl-histidine.* We have previously described the methods used for this analysis
192 (4, 6, 16, 17). Briefly, plasma samples were defrosted and centrifuged at 1200g for 3 min. A 0–
193 10% D_3-3-methyl-histidine enrichment curve was prepared as a serial dilution. 100 μL of plasma
194 was de-proteinized using 1 mL of MeCN: MeOH (1:1). Samples were vortex mixed and
195 incubated at − 20°C for 1 h. Samples were centrifuged at 20,800g for 5 min at 4°C. The
196 supernatant was dried down in a TechneBlock at < 40°C using nitrogen gas. Samples were re-
197 suspended using 100 μL MeCN: ddH2O (65:35) and ready to be analyzed using High-
198 Performance Liquid Chromatography (HPLC; Dionex Ultimate3000, Thermo Scientific) mass
199 spectrometry (MS; Q-Exactive, Thermo Scientific) with a Sequant ZIC-HILIC column (150 mm
200 Å~ 2.1 Å~ 5um; Merck Millipore) using previously described methods (6, 16). The enrichment
201 ratios were log-transformed to determine the decay rates (k), representative of the rate of whole-
202 body MPB (22).
203 *Statistical Analysis.* Data were analyzed using SPSS (IBM Corp. Released 2023. IBM SPSS
204 Statistics for Windows, Version 29.0.2.0 Armonk, NY: IBM Corp) using a 2-way ANOVA with
205 repeated measures with OCP phase (Active or Inactive) and leg (exercise or control) as within-
206 subject factors; all factors nested within-subject. Significance was set at $P < 0.05$. Data are

presented as means ± standard deviation (SD) in tables and as box and whisker plots showing interquartile range, median, and upper and lower bounds in figures unless otherwise indicated.

RESULTS

Participants. Participants used daily monophasic pills (Alesse and Alysena) containing 0.02 mg ethinylestradiol and 0.1 mg levonorgestrel (days 1-21), with a 7-day inactive week (days 22-28). Participant characteristics are shown in Table 1.

Hormones. Serum E2, P4, and LH were assessed in both phases. Data are presented in Table 1. There were no differences across any phase in any hormone measured (all $P > 0.4$).

Myofibrillar Protein Synthesis. The mean MPS in the Active pill phase were 1.44 ± 0.14 %•d^{-1} in the control leg and 1.64 ± 0.15 %•d^{-1} in the exercise leg ($p < 0.001$). The Inactive phase MPS rates were 1.49 ± 0.12 %•d-1 %/d in the control leg and 1.71 ± 0.16 %•d^{-1} in the exercise leg ($p < 0.001$). The two-way ANOVA showed a main effect of condition (leg; $p < 0.001$) but no significant effects of phase ($p = 0.48$; effect size 0.16) or interaction between phases ($p = 0.63$; effect size 0.12). The results are presented in Figure 2.

Myofibrillar Protein Breakdown. The mean rate (k) of whole-body myofibrillar proteolysis was 0.018 ± 0.01 in the Active phase and 0.018 ± 0.006 in the Inactive phase ($p = 0.55$). The results are presented in Figure 3.

DISCUSSION

While there was a strong and consistent effect of RET on MPS in both phases, the response did not differ between the active and inactive OCP phases (Figure 2). We also observed no difference in whole-body myofibrillar proteolysis (Figure 3), which would include and may

be predominantly skeletal muscle-derived (22). Our work shows that females taking OCP derive no specific anabolic advantage, nor are they in a pro-catabolic state in any particular phase. We also note that compared to our recent data (using an identical design) from naturally cycling women (4), we observed no marked differences between resting or post-exercise anabolic and catabolic rates. In fact, combining datasets and running statistical analyses, including a between factor for OCP use and either phase of the MC, showed no pill-by-phase interaction and only main effects for exercise (exercise > rest; data not shown). Thus, our data show, in accordance with meta-analytic analyses of cross-sectional OCP effects (10) and phase-specific effects across the MC (5, 23), that there are no specific differences in terms of muscle anabolism or catabolism in response to RET related to OCP use.

As expected, endogenous ovarian hormones were downregulated, compared with normally cycling females (4), in the OCP cohort across all time points. Our data indicate that a week of inactive pills was insufficient to restore the endogenous hormone profile of a naturally menstruating individual. As a result it is perhaps unsurprising that we did not see a change in the muscle protein synthetic or whole-body myofibrillar proteolysis responses.

We acknowledge that our data are short-term (days) but propose that they offer new and deeper insight than acute infusions of isotopes (hours) (7, 8). Longer-term trials and the use of third- and fourth-generation OCP, as well as intrauterine progesterone-emitting contraception, would be interesting avenues in which to apply similar methods. We note, however, that these methods of contraception have been compared in vascular and cellular physiology and show statistically significant but physiologically trivial differences (24). We hypothesize that since third- and fourth-generation OCPs have even less androgenic forms of progesterone (25) than

second-generation OCPs, it is unlikely there would be marked anabolic or catabolic effects of these OCPs compared to earlier generations (5).

Our results are largely in line with those of Hansen and colleagues, who assessed MPS in a group of naturally cycling participants compared to a group of habitual OCP users, reporting that MPS and MPB did not differ between groups (7). However, MPS was significantly lower in a sub-group (n=7) of third-generation but not second-generation (n=4) OCP users compared to the naturally cycling group (n=9). Further investigations into other forms of contraception, including intrauterine devices, would be an interesting avenue to pursue.

We conclude that second-generation OCP phase does not alter muscle anabolic capacity nor influence myofibrillar proteolysis in response to RET. Our results concur with reviews and meta-analyses showing no influence of OCP or MC-related changes in sex hormones on muscle propensity for anabolism (3, 5, 10, 23). Longer-term trials and studies of other contraceptive methods will be needed to confirm whether our findings of day-to-day protein turnover are generalizable and align with RET phenotypes.

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REFERENCES

1. **Lew LA, Williams JS, Stone JC, Au AKW, Pyke KE, and MacDonald MJ**. Examination of Sex-Specific Participant Inclusion in Exercise Physiology Endothelial Function Research: A Systematic Review. *Front Sports Act Living* 4: 860356, 2022. doi: 10.3389/fspor.2022.860356
2. **Oosthuyse T, Strauss JA, and Hackney AC**. Understanding the female athlete: molecular mechanisms underpinning menstrual phase differences in exercise metabolism. *Eur J Appl Physiol* 123: 423-450, 2023. doi: 10.1007/s00421-022-05090-3
3. **Van Every DW, D'Souza AC, and Phillips SM**. Hormones, Hypertrophy, and Hype: An Evidence-Guided Primer on Endogenous Endocrine Influences on Exercise-Induced Muscle Hypertrophy. *Exerc Sport Sci Rev* 52: 117-125, 2024. doi: 10.1249/jes.0000000000000346
4. **Colenso-Semple LM, McKendry J, Lim C, Atherton PJ, Wilkinson DJ, Smith K, and Phillips SM**. Menstrual cycle phase does not influence muscle protein synthesis or whole-body myofibrillar proteolysis in response to resistance exercise. *J Physiol* 2024. doi: 10.1113/jp287342
5. **D'Souza AC, Wageh M, Williams JS, Colenso-Semple LM, McCarthy DG, McKay AKA, Elliott-Sale KJ, Burke LM, Parise G, MacDonald MJ, Tarnopolsky MA, and Phillips SM**. Menstrual cycle hormones and oral contraceptives: A multi-method systems physiology-based review of their impact on key aspects of female physiology. *J Appl Physiol (1985)* 2023. doi: 10.1152/japplphysiol.00346.2023
6. **Cegielski J, Brook MS, Phillips BE, Boereboom C, Gates A, Gladman JFR, Smith K, Wilkinson DJ, and Atherton PJ**. The Combined Oral Stable Isotope Assessment of Muscle (COSIAM) reveals D-3 creatine derived muscle mass as a standout cross-sectional biomarker of muscle physiology vitality in older age. *Geroscience* 44: 2129-2138, 2022. doi: 10.1007/s11357-022-00541-3
7. **Hansen M, Langberg H, Holm L, Miller BF, Petersen SG, Doessing S, Skovgaard D, Trappe T, and Kjaer M**. Effect of administration of oral contraceptives on the synthesis and breakdown of myofibrillar proteins in young women. *ScandJMedSciSports* 2009. doi:
8. **Hansen M, Miller BF, Holm L, Doessing S, Petersen SG, Skovgaard D, Frystyk J, Flyvbjerg A, Koskinen S, Pingel J, Kjaer M, and Langberg H**. Effect of administration of oral contraceptives in vivo on collagen synthesis in tendon and muscle connective tissue in young women. *J ApplPhysiol* 106: 1435-1443, 2009. doi:
9. **Roberts MD, McCarthy JJ, Hornberger TA, Phillips SM, Mackey AL, Nader GA, Boppart MD, Kavazis AN, Reidy PT, Ogasawara R, Libardi CA, Ugrinowitsch C, Booth FW, and Esser KA**. Mechanisms of mechanical overload-induced skeletal muscle hypertrophy: current understanding and future directions. *Physiol Rev* 103: 2679-2757, 2023. doi: 10.1152/physrev.00039.2022
10. **Nolan D, McNulty KL, Manninen M, and Egan B**. The Effect of Hormonal Contraceptive Use on Skeletal Muscle Hypertrophy, Power and Strength Adaptations to Resistance Exercise Training: A Systematic Review and Multilevel Meta-analysis. *Sports Med* 54: 105-125, 2024. doi: 10.1007/s40279-023-01911-3
11. **Dalgaard LB, Jørgensen EB, Oxfeldt M, Dalgaard EB, Johansen FT, Karlsson M, Ringgaard S, and Hansen M**. Influence of Second Generation Oral Contraceptive Use on Adaptations to Resistance Training in Young Untrained Women. *J Strength Cond Res* 36: 1801-1809, 2022. doi: 10.1519/jsc.0000000000003735

12. **Dalgaard LB, Dalgas U, Andersen JL, Rossen NB, Møller AB, Stødkilde-Jørgensen H, Jørgensen JO, Kovanen V, Couppé C, Langberg H, Kjær M, and Hansen M**. Influence of Oral Contraceptive Use on Adaptations to Resistance Training. *Front Physiol* 10: 824, 2019. doi: 10.3389/fphys.2019.00824
13. **Wikström-Frisén L, Boraxbekk CJ, and Henriksson-Larsén K**. Effects on power, strength and lean body mass of menstrual/oral contraceptive cycle based resistance training. *J Sports Med Phys Fitness* 57: 43-52, 2017. doi: 10.23736/s0022-4707.16.05848-5
14. **Sung ES, Han A, Hinrichs T, Vorgerd M, and Platen P**. Effects of oral contraceptive use on muscle strength, muscle thickness, and fiber size and composition in young women undergoing 12 weeks of strength training: a cohort study. *BMC Womens Health* 22: 150, 2022. doi: 10.1186/s12905-022-01740-y
15. **Riechman SE, and Lee CW**. Oral Contraceptive Use Impairs Muscle Gains in Young Women. *J Strength Cond Res* 36: 3074-3080, 2022. doi: 10.1519/jsc.0000000000004059
16. **Cegielski J, Wilkinson DJ, Brook MS, Boereboom C, Phillips BE, Gladman JFR, Smith K, and Atherton PJ**. Combined in vivo muscle mass, muscle protein synthesis and muscle protein breakdown measurement: a 'Combined Oral Stable Isotope Assessment of Muscle (COSIAM)' approach. *Geroscience* 43: 2653-2665, 2021. doi: 10.1007/s11357-021-00386-2
17. **D'Souza AC, Rajmohan S, Younes R, McKendry J, Lim C, Wilkinson DJ, Smith K, Atherton PJ, and Phillips SM**. Application of the COSIAM method for muscle protein turnover and skeletal muscle mass in young females: A comparison of methods for body composition assessment. *Advanced Exercise and Health Science* 1: 248-259, 2024. doi: https://doi.org/10.1016/j.aehs.2024.11.003
18. **Wilkinson DJ, Franchi MV, Brook MS, Narici MV, Williams JP, Mitchell WK, Szewczyk NJ, Greenhaff PL, Atherton PJ, and Smith K**. A validation of the application of D2O stable isotope tracer techniques for monitoring day-to-day changes in muscle protein sub-fraction synthesis in humans. *AmJ Physiol EndocrinolMetab* 306: E571-E579, 2013. doi:
19. **Stokes T, Timmons JA, Crossland H, Tripp TR, Murphy K, McGlory C, Mitchell CJ, Oikawa SY, Morton RW, Phillips BE, Baker SK, Atherton PJ, Wahlestedt C, and Phillips SM**. Molecular Transducers of Human Skeletal Muscle Remodeling under Different Loading States. *Cell Rep* 32: 107980, 2020. doi: 10.1016/j.celrep.2020.107980
20. **McGlory C, von Allmen MT, Stokes T, Morton RW, Hector AJ, Lago BA, Raphenya AR, Smith BK, McArthur AG, Steinberg GR, Baker SK, and Phillips SM**. Failed Recovery of Glycemic Control and Myofibrillar Protein Synthesis With 2 wk of Physical Inactivity in Overweight, Prediabetic Older Adults. *J Gerontol A Biol Sci Med Sci* 73: 1070-1077, 2018. doi: 10.1093/gerona/glx203
21. **Clark RV, Walker AC, O'Connor-Semmes RL, Leonard MS, Miller RR, Stimpson SA, Turner SM, Ravussin E, Cefalu WT, Hellerstein MK, and Evans WJ**. Total body skeletal muscle mass: estimation by creatine (methyl-d3) dilution in humans. *J Appl Physiol (1985)* 116: 1605-1613, 2014. doi: 10.1152/japplphysiol.00045.2014
22. **Sheffield-Moore M, Dillon EL, Randolph KM, Casperson SL, White GR, Jennings K, Rathmacher J, Schuette S, Janghorbani M, Urban RJ, Hoang V, Willis M, and Durham WJ**. Isotopic decay of urinary or plasma 3-methylhistidine as a potential biomarker of pathologic skeletal muscle loss. *J CachexiaSarcopeniaMuscle* 2013. doi:
23. **Colenso-Semple LM, D'Souza AC, Elliott-Sale KJ, and Phillips SM**. Current evidence shows no influence of women's menstrual cycle phase on acute strength performance or

adaptations to resistance exercise training. *Front Sports Act Living* 5: 1054542, 2023. doi: 10.3389/fspor.2023.1054542

24. **Williams JS, Cheng JL, Stone JC, Kamal MJ, Cherubini JM, Parise G, and MacDonald MJ**. Menstrual and oral contraceptive pill cycles minimally influence vascular function and associated cellular regulation in premenopausal females. *Am J Physiol Heart Circ Physiol* 327: H1019-h1036, 2024. doi: 10.1152/ajpheart.00672.2023

25. **Torgrimson BN, Meendering JR, Kaplan PF, and Minson CT**. Endothelial function across an oral contraceptive cycle in women using levonorgestrel and ethinyl estradiol. *Am J Physiol Heart Circ Physiol* 292: H2874-2880, 2007. doi: 10.1152/ajpheart.00762.2006

Table 1. Participant characteristics and serum hormone levels

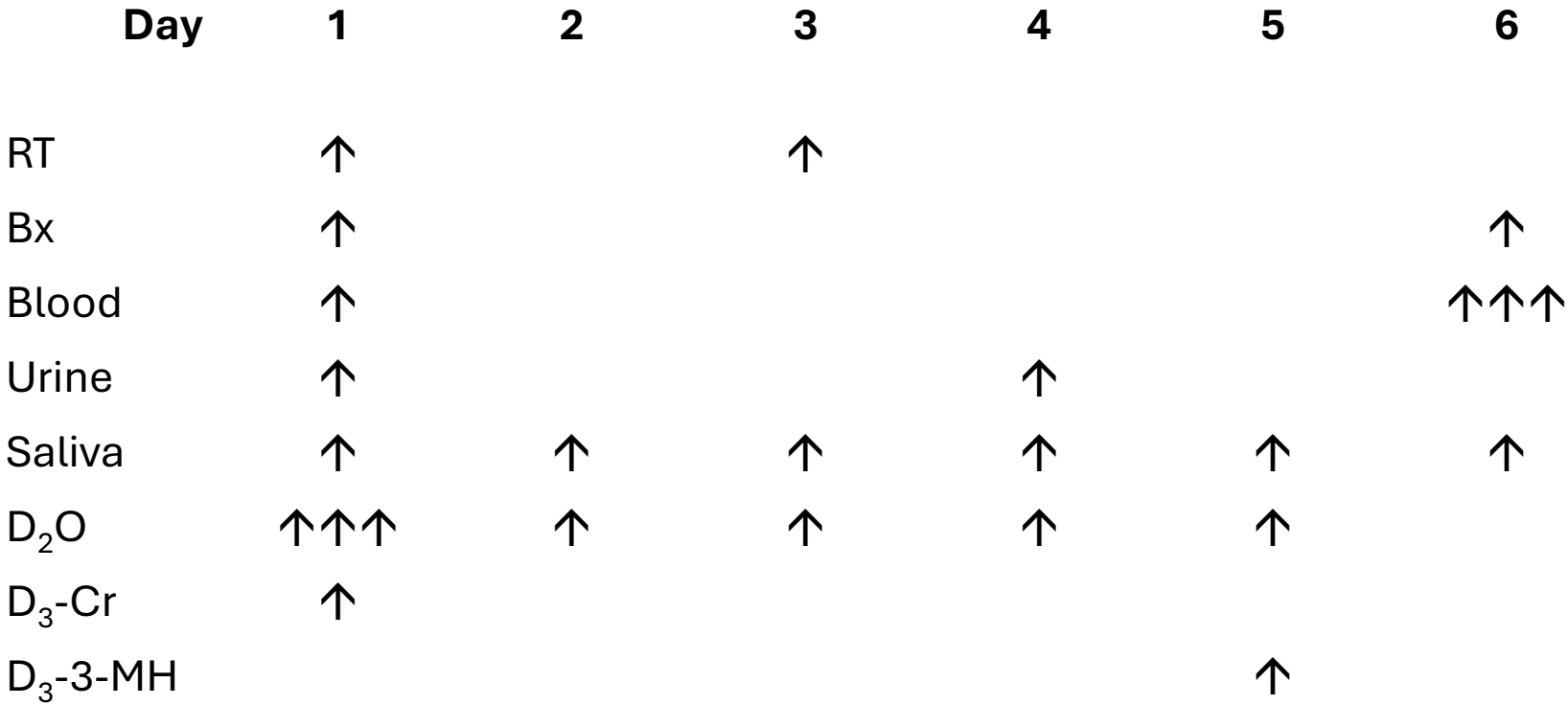
Age (y)	20±2			
Height (cm)	164±2			
Body mass (kg)	61.9±8.3			
BMI (kg/m^2)	22.9±2.3			
Lean mass (kg)*	40.8±4.9			
Muscle mass (kg)**	22.3±1.2			
	Inactive Phase (OC Cycle Days 23-28)		Active Phase (OC Cycle Days 9-14)	
	Visit 1	Visit 6	Visit 1	Visit 6
E2 (pM)	97±26	153±47	108±50	101±29
P4 (nM)	9±3	7±2	8±3	7±3
LH (IU/L)	3±3	4±3	3±2	2±3

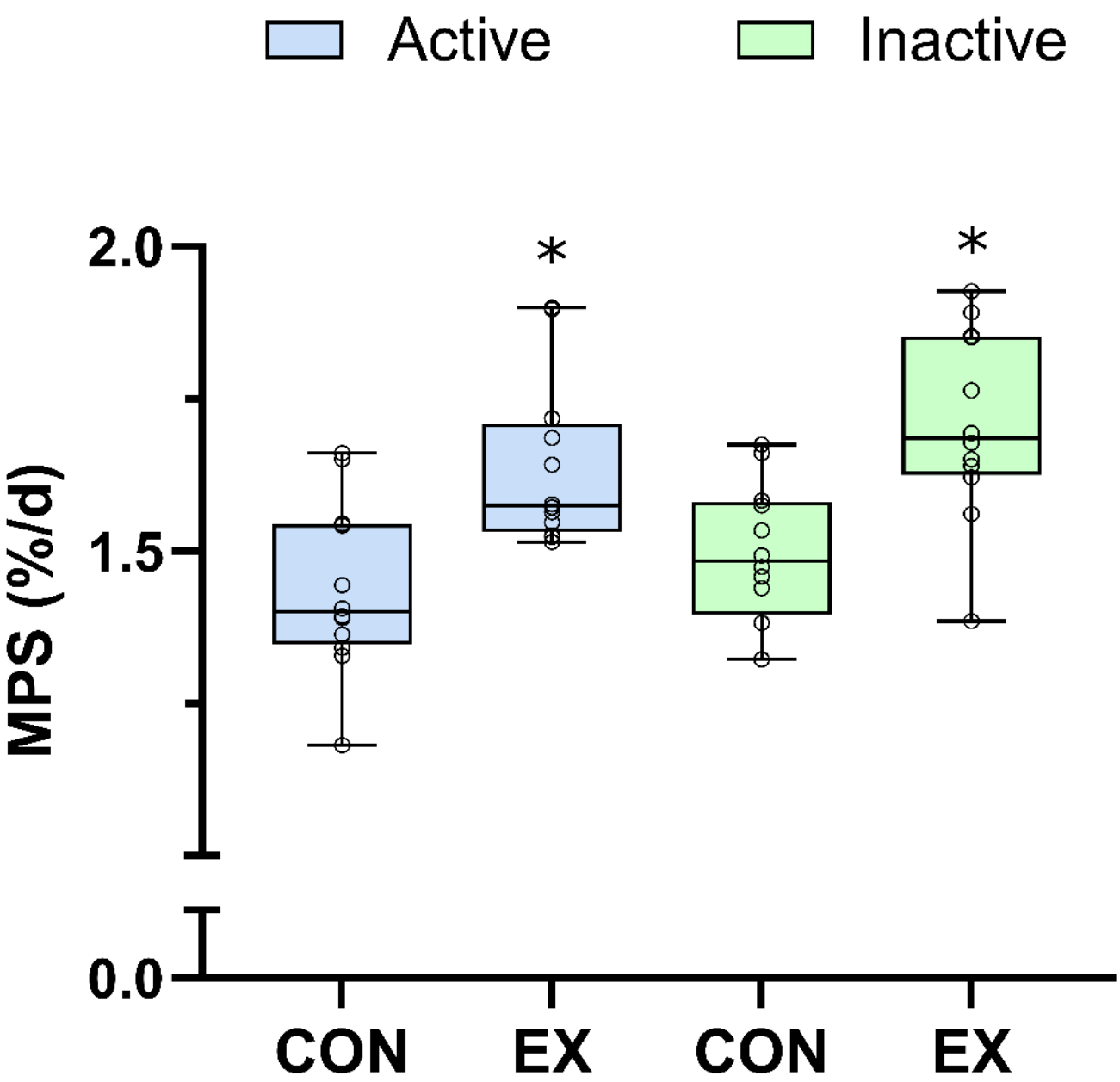
Values are means±SD. * Derived from DXA. ** Derived from D_3-creatine. E2 – estradiol; P4 – progesterone; and LH – luteinizing hormone.

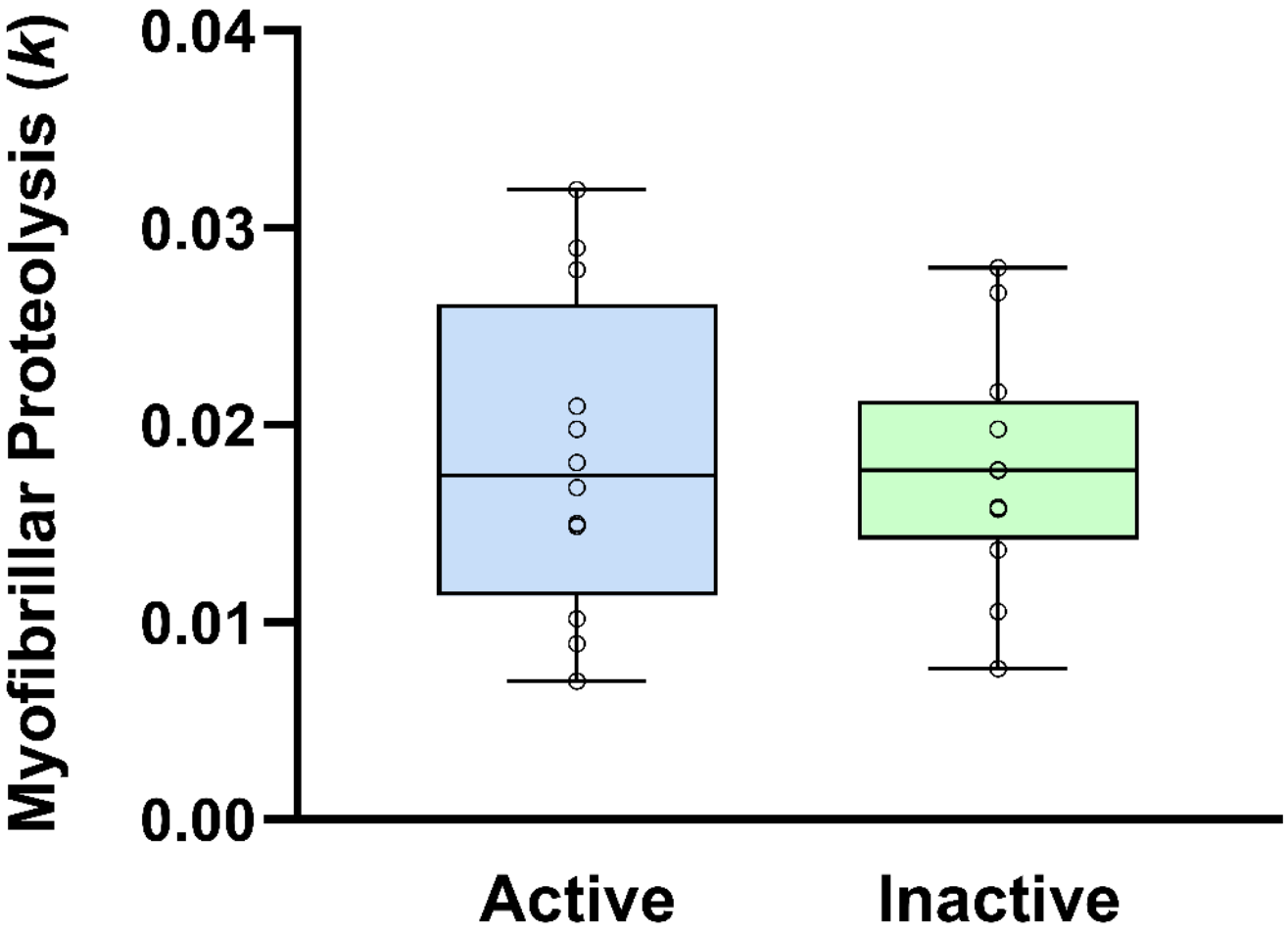
Figure 1. Schematic depiction of the protocol that was repeated in each OCP phase. The exercised limb was randomly selected and was switched in a counterbalanced manner, as was the phase in which each participant began the protocol. RT – resistance training; Bx – muscle biopsy; D_2O – oral dose of deuterated water; D_3Cr – oral dose of deuterated creatine; D_3-3-MH – oral dose of deuterated 3-methylhistidine.

Figure 2. Integrated muscle protein synthesis in active and inactive phases. *Significant ($P < 0.001$) difference (main effect) between EX and CON. There was no significant effect of OCP phase nor an interaction between phases and conditions (all $P > 0.5$).

Figure 3. Whole body myofibrillar protein breakdown rate (k) in active and inactive OCP phases.

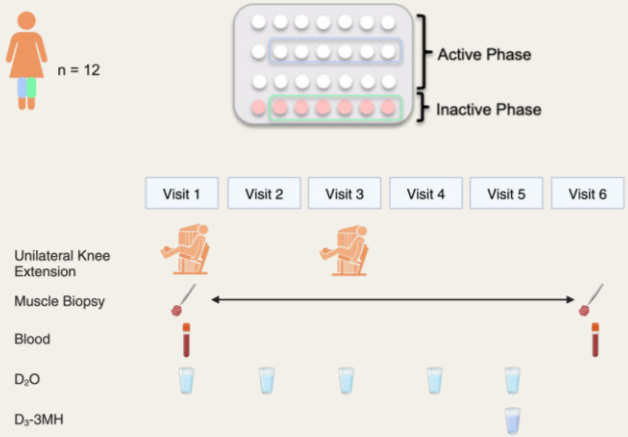






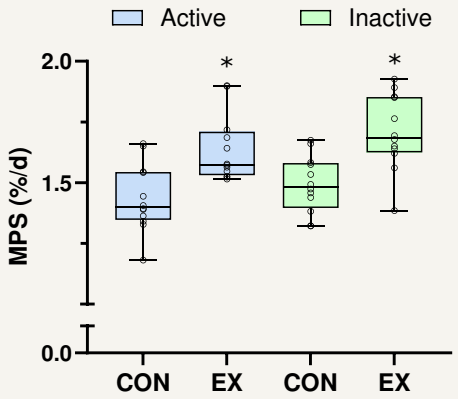
Muscle Protein Turnover is Unaffected by Oral Contraceptive Pill Phase

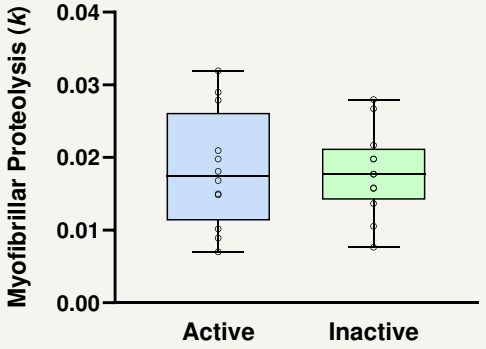
Methods



n = 12 females studied in Active and Inactive OCP phases. Unilateral resistance exercise in each phase: control (CON) and exercised (EX) legs.

No Effect of OCP phase on Muscle Protein Synthesis (MPS) or Whole-body Myofibrillar Protein Breakdown





Conclusion: Resistance exercise, but not OCP phase, increased MPS. Myofibrillar proteolysis was unaffected by OCP phase.