Hormonal contraceptive use and physical performance, body composition, and musculoskeletal injuries during military training

Thomas J O'Leary[1,2], Hope A Evans[1], Marie-Elise O Close[1], Rachel M Izard[3], Neil P Walsh[4], Charlotte V Coombs[1], Alexander T Carswell[5], Samuel J Oliver[6], Jonathan C Y Tang[5,7], William D Fraser[5,7], Julie P Greeves[1,2,5]

[1]Army Health and Performance Research, Army Headquarters, Andover, United Kingdom; [2]Division of Surgery and Interventional Science, UCL, London, UK; [3] Defence Science and Technology, Ministry of Defence, Porton Down, United Kingdom; [4]Faculty of Science, Liverpool John Moores University, Liverpool, United Kingdom; [5]Norwich Medical School, University of East Anglia, Norwich, United Kingdom; [6]College of Medicine and Health, Bangor University, Bangor, United Kingdom; [7]Departments of Clinical Biochemistry, Diabetes and Endocrinology, Norfolk and Norwich University Hospital, Norwich, United Kingdom

Corresponding author: Thomas J O'Leary PhD, Army Health and Performance Research, Army Headquarters, Andover, Hampshire, SP11 8HT, United Kingdom. Email: thomas.oleary100@mod.gov.uk

Running title: Hormonal contraceptives in military training.

Abstract

Purpose: To investigate associations between hormonal contraceptive use and physical performance, body composition, and musculoskeletal injuries in basic military training. **Methods:** Female British Army recruits (n = 450) were grouped as non-users (n = 182), combined oral contraceptive users (COCP; n = 184), or progestin-only users (POC; n = 144). Physical performance (2.4 km run, lift strength, leg power), body composition, iron and vitamin D status, and bone metabolism were measured at the start (week 1) and end (week 13) of training. Lower body musculoskeletal injuries were recorded from medical records. **Results:** Training decreased 2.4 km run time (–3.7%) and fat mass (–9.6%), and increased lift strength (4.5%), leg power (1.5%), lean mass (5.4%), and whole-body (0.9%), arms (1.8%), and legs (1.4%) aBMD ($p \leq 0.015$); the training response was not different between groups ($p \geq 0.173$). Lift strength was lower in COCP users than non-users ($p = 0.044$). Whole-body, trunk, and legs aBMD were lower in POC users than non-users and/or COCP users ($p \leq 0.041$). There were no associations between hormonal contraceptive use and musculoskeletal or bone stress injury ($p \geq 0.429$). Training did not change ferritin ($p = 0.968$), but decreased haemoglobin and total 25(OH)D, and increased PTH, βCTX, and PINP ($p \leq 0.005$); the training response was not different between groups ($p \geq 0.368$). Total 25(OH)D was higher, and βCTX and PINP were lower, in COCP users than non-users and POC users; PTH was lower in COCP users than non-users, and; βCTX and PINP were higher in POC users than non-users ($p \leq 0.017$). **Conclusions:** Hormonal contraceptive use was not associated with performance or injury outcomes in military training.

Key Words: Bone; Endurance; Muscle, Musculoskeletal Injury; Oestrogens; Progestogens

Introduction

Hormonal contraceptive use is common in military personnel and athletes; 58% of British Servicewomen (unpublished) and 50% of elite female UK athletes (1) use hormonal contraceptives compared with 34% of the UK general population (2). Hormonal contraceptives contain synthetic oestradiol (ethinyl oestradiol) and / or progestogens (progestins), which can suppress the hypothalamic-pituitary-ovarian axis and lower endogenous 17β-oestradiol (3, 4). Whilst both the combined oral contraceptive pill (COCP) and progestin-only contraceptives (POC) lower endogenous oestradiol, COCPs provide exogenous ethinyl oestradiol whereas POCs do not (3, 4). Oestradiol has wide spread effects on energy metabolism and nervous system, cardiovascular, muscle, ligament, tendon, and bone function (5–8). Therefore, hormonal contraceptive type may differentially influence physical performance, body composition, and musculoskeletal injury risk in active women (3, 9–12). Basic military training prepares recruits for the demands of military service and is physically arduous, more so for female recruits than male recruits (13–15). Basic military training improves physical performance (16) and body composition (17), but also results in a high incidence of musculoskeletal injuries and bone stress injuries (18). On average, female recruits have poorer physical performance (16), lower lean mass and higher body fat (15), and higher risk of musculoskeletal injuries and bone stress injuries (18) than male recruits in military training.

Longitudinal studies show the COCP can decrease maximal oxygen uptake ($\dot{V}O_{2max}$) (19–21) and increase body mass and body fat (21), and cross-sectional studies show COCP users have slightly lower muscle strength (3) and similar muscle adaptations (strength, power, or hypertrophy) to resistance training (10) than non-users. These potential negative effects of the COCP on performance and body composition may result in increased risk of musculoskeletal injuries in military training (22) in COCP users. Conversely, COCP users can have higher total

25-hydroxyvitamin-D (25(OH)D) than non-users and depot medroxyprogesterone (DMPA, a long acting POC) users at the start of military training (23); higher baseline total 25(OH)D is associated with better baseline physical performance (24) and a lower risk of stress fractures (25) in military training, indicating a potential mechanism by which COCPs might reduce musculoskeletal injury risk. Generally, hormonal contraceptives might also reduce risk of musculoskeletal injury and increase physical performance in military training by reducing or suppressing menstrual bleeding to preserve iron status (26). Depot medroxyprogesterone users have been shown to have higher circulating concentrations of bone formation and resorption markers (27) and higher bone stress injury risk (28) in military training than non-users and / or COCP users. Several studies also show a loss of areal bone mineral density (aBMD) with long-term DMPA use but the effect of other POCs on bone are poorly understood (4, 29, 30). Conversely, some studies show that COCP users have lower circulating concentrations of markers of bone formation and bone resorption (27) and higher fracture risk (11) compared with non-users. Both COCP (23) and POC (23, 31) users experience blunted adaptation of distal tibial trabeculae in response to military training, compared with non-users, which may contribute to increased bone stress injury risk (32).

Better understanding of the effects of hormonal contraceptives on physical performance, body composition, and musculoskeletal injury risk in military training will help inform hormonal contraceptive choices for female recruits (33). Most studies investigating the effect of hormonal contraceptives on physical performance and body composition have small sample sizes, are typically cross-sectional and not longitudinal, and exclude POC use. There are also little data in Servicewomen, where long-acting POCs are a common choice of hormonal contraceptive (34). The primary aims of this study were to examine associations between hormonal contraceptive use (non-users *vs* COCP users *vs* POC users) and physical performance, body

composition, and musculoskeletal injury incidence during military training. Secondary aims were to explore the effect of hormonal contraceptive use on circulating markers of iron status, vitamin D status, and bone metabolism. We hypothesised that COCP use would be associated with poorer physical performance and higher body fat and total 25(OH)D at baseline and follow-up, but COCP users, POC users, and non-users would have similar performance and body composition adaptations to training. We also hypothesised that POC use will be associated with lower aBMD and higher markers of bone turnover at baseline and follow-up, and increased risk of musculoskeletal and bone stress injury risk.

Methods

Participants

The study was advertised to new female British Army recruits from January 2014 to July 2017 during week one of their standard entry basic training course (non-infantry, non-officer) at the Army Training Centre, Pirbright, UK. All participants passed an initial military medical assessment and were confirmed to be injury free and not have any medical condition that precluded military service. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by the Ministry of Defence Research Ethics Committee (ref: 165/Gen/10). Each participant had the study procedures and risks fully explained verbally and in writing. Written informed consent was obtained from all participants.

Experimental Design

This study was an observational prospective cohort study. These data were secondary analyses as part of a larger study exploring micronutrient deficiencies and health and performance outcomes (24, 26, 35, 36). Participants had measures of physical performance and body

composition taken at the start (week 1) and end (week 13) of their basic military training course, and venous blood samples were taken for the analysis of biochemical markers of iron status, vitamin D status, and bone metabolism. Participants had height and body mass measured, and self-reported their hormonal contraceptive use, menstrual cycle status, smoking habits, and bone stress injury history using bespoke questionnaires at week 1. Participants were asked to select their current hormonal contraceptive by type (*e.g.,* COCP, progestin-only pill, implant, injection) and all questionnaires were completed with a researcher to ensure correct coding of hormonal contraceptive type. Participants were grouped according to current hormonal contraceptive use in training as follows: i) no hormonal contraception (non-users, n = 182); ii) COCP users (*e.g.,* Microgynon 30) (n = 124); iii) POC users (progestin-only pill [*e.g.,* Micronor], implant [*e.g.,* Nexplanon], and injection [DMPA]) (n = 144) (31, 37). Progestin-only methods were grouped together based on their similar endocrine effects (37). Participants using another hormonal contraceptive (*e.g.,* intrauterine device), multiple hormonal contraceptives, or did not know their hormonal contraceptive were excluded from analyses. Week 1 measurements were made following the initial medical assessment and before military training commenced. Participants completed the 14-week British Army soldier basic military training course, which is intended to develop basic military skills and physical fitness. The 14-week training programme contains periods of aerobic endurance training, strength and conditioning, military-specific fitness training (obstacle course, circuit training), military drill, progressive loaded marching, and basic military skills (field exercise, weapon handling). Week 14 involves a decrease in typical military activities and an increase in the administrative burden as trainees prepare to complete the basic training component of their course, so post-training measurements were taken in week 13. Participants' medical records were accessed to obtain a record of clinician-diagnosed lower body (including hip / pelvis and lower limb) overuse musculoskeletal injuries and bone stress injuries during the first 14 weeks of training; lower

body bone stress injuries were recorded separately from overuse musculoskeletal injuries. Overuse musculoskeletal injuries were defined as pain, inflammation, or a functional disorder that involved joints, muscles, tendons, ligaments, and associated connective tissues, with the mechanism of injury a result of use over time, rather than a traumatic event (*e.g.,* plantar fasciitis, Achilles tendinopathy, patellar tendinitis, iliotibial band syndrome) (38). Bone stress injuries encompassed the spectrum of overuse injuries caused by microdamage accumulation in bone, frequently referred to as stress reactions and stress fractures (e.g., medial tibial stress syndrome, femoral, tibial, calcaneal, and metatarsal stress fractures) (38).

Physical Performance

Endurance performance was assessed as the time to complete a maximal effort 2.4 km run on a standardised running course. Participants completed an 800 m warm-up and completion time was recorded to the nearest second. The time to complete a 2.4 km run is indicative of maximal aerobic capacity (39) and is a military field test assessed during selection, training, and throughout a military career. All participants were accustomed to performing this test from selection, before commencing military training. Maximal lift strength was determined as the maximal weight lifted using an incremental lift machine that simulates a power clean weightlifting movement (24). The device consisted of a vertically moving carriage with handgrips positioned 0.30 m above the ground. Participants lifted the weight (20 kg starting mass) to a height of 1.45 m, the height of a British Army four tonne truck. The weight was increased by 5 kg after each successful lift and the test continued until participants failed to lift a weight to 1.45 m after two successive attempts. This measure of maximal lift strength correlates with, and predicts performance in military tasks (40). Vertical jump peak power output was assessed by countermovement vertical jump (24) using a jump mat (Takei Scientific Instruments, Tokyo, Japan) and calculated as: peak power (W) = (51.9 × maximal vertical jump

height (cm)) + (48.9 × body mass (kg)) – 2007 (41). Lower body power is important for the performance of military specific tasks (40). A belt was fitted around the waist of each participant and secured to a rubber mat. Participants were instructed to jump as high as possible three times, with their hands placed on their hips. A fourth jump was performed if jump height increased across the three attempts, indicative of a learning effect. Maximal jump height was recorded as the highest score achieved.

Body Composition

Whole-body lean mass, fat mass, and aBMD were assessed by DXA (Lunar iDXA, GE Healthcare, Buckinghamshire, UK) whilst wearing light clothing. Arms, legs, and trunk aBMD were derived from regional analysis of the whole-body scan. The CV and least significant change for whole-body aBMD is 0.5% and 1.5% with regional aBMD ≤ 1.5% and ≤ 4.2% (31).

Blood Collection and Biochemical Analyses

A venous blood sample was collected either in the morning (~0900 to 1100 h) after breakfast (0600 to 0700 h), or early afternoon (~1300 to 1500 h) after lunch (1200 to 1300 h). Follow-up measurements at week 13 were taken at the same time of day. Venous blood was withdrawn from a vein in the antecubital fossa and collected in serum and EDTA BD Vacutainer® tubes (Becton Dickinson, New Jersey, USA). Serum samples were left to clot for 1 h at room temperature. Haemoglobin was measured in EDTA whole blood within 30 min of collection using the COULTER A^{C}·T diff 2 Analyzer (Beckman Coulter, California, USA). Blood samples were centrifuged at 1500 *g* and 4°C for 10 min before serum and plasma were separated into universal tubes and stored at −80°C until analysis. Plasma procollagen type 1 N-terminal propeptide (PINP), c-telopeptide cross-links of type 1 collagen (βCTX), intact parathyroid hormone (PTH), and serum ferritin were analysed by electro-chemiluminescence

174 immunoassays (ECLIA) on the COBAS c601 (Roche Diagnostics, Mannheim, Germany)
175 platform. PINP inter-assay coefficient of variation (CV) was < 3% between 20 and 600 $\mu g \cdot L^{-1}$
176 with a sensitivity of 8 $\mu g \cdot L^{-1}$. βCTX inter-assay CV was < 3% between 0.02 and 1.50 $\mu g \cdot L^{-1}$
177 with a sensitivity of 0.01 $\mu g \cdot L^{-1}$. PTH inter-assay CV was < 3.8% between 1.2 and 5000.0
178 $pg \cdot mL^{-1}$. Ferritin inter-assay CV was < 4.2% between 0.5 and 2000.0 $\mu g \cdot L^{-1}$. Serum samples
179 were analysed for total 25(OH)D by liquid chromatography tandem mass spectrometry (42).
180 The $25(OH)D_3$ and $25(OH)D_2$ assays were calibrated using the National Institute of Science
181 and Technology standard reference material SRM972a. Total 25(OH)D was calculated from
182 the sum of $25(OH)D_3$ and 25(OH)D2. Total 25(OH)D inter-assay CV was < 8.5% between 0.1
183 and 200.0 $nmol \cdot L^{-1}$. All biochemical analyses (excluding haemoglobin) were undertaken
184 following Good Clinical Laboratory Practice and assays met the certification requirements of
185 the Vitamin D External Quality Assessment Scheme (DEQAS) certified Bioanalytical Facility,
186 University of East Anglia, Norwich, UK.
187
188 *Statistical Analyses*
189 These data were secondary analyses (24, 26, 35, 36) so no *a priori* sample size was calculated.
190 The smallest hormonal contraceptive group was COCP users (n = 124). A three-group sample
191 size of 372 could detect effect sizes of $f^2 \geq 0.04$ (small effect) with an $\alpha = 0.05$ and a $1 - \beta =$
192 0.80 (*pwr package v*1.3-0). All data were analysed using the R programming language
193 (*v.*4.3.3). Distribution of the demographic and anthropometric data were checked using
194 frequency distribution histograms. Participant demographics and anthropometrics at week 1
195 were compared between groups using a one-way ANOVA. Linear mixed effect models with
196 restricted maximum likelihood estimation were used to compare changes in physical
197 performance (2.4 km run time, maximal lift strength, and peak power output), body
198 composition (fat mass, lean mass, and aBMD), and markers of iron status (haemoglobin and

ferritin), vitamin D status (total 25(OH)D), and bone metabolism (βCTX, PINP, and PTH) between different groups (*lme4 package v*.1.1-35.3). Hormonal contraceptive use (non-users *vs* COCP *vs* POC), time (week 1 *vs* week 13), and their interaction were included as fixed effects to examine differences between hormonal contraceptive groups. Each model controlled for age, height, and body mass by including these outcomes as fixed effects. Random intercepts were assigned to each participant to account for within-participant correlation for repeated measures. Participants were also nested within different training platoons, but a random effect was not assigned to platoon due to the low number of different platoons (n = 18). Significance of the fixed effects from each model were determined with Sattherwaite degrees of freedom (*lmerTest package v*.3.1-3). Variance and normality of the residuals for each model were checked visually by plotting the residuals against the fitted values and from Q-Q plots. Data were log transformed for models where the residuals seriously violated these assumptions. In the event of a significant interaction, pairwise comparisons with Holm-Bonferroni corrections and Kerward-Roger degrees of freedom were used on the linear mixed effects model to identify differences between time points within each hormonal contraceptive group and differences between hormonal contraceptive groups within each time point (*emmeans package v*.1.10.1). Pooled data were used for main effects when there was no significant interaction with Holm-Bonferroni corrections. Effect sizes are presented as η_p^2 for main and interaction effects, Hedges' g for between-group comparisons, and paired Hedges' g for within-group paired comparisons (*effectsize package v*.0.8.7). Binary logistic regression was performed to assess the association between injury (lower body overuse musculoskeletal injury or bone stress injury) and hormonal contraceptive use controlling for height, body mass, age, 2.4 km run time, smoking, and bone stress injury history (26). The bone stress injury model produced implausibly large estimates and standard errors likely due to the low incidence of bone stress injuries leading to sparse data bias; therefore a bias reduction method was used (*brglm package*

*v.*0.7.2) (43–45). Figures were drawn in the *ggplot2 package* (*v.*3.5.0). Significance was accepted as $p < 0.05$.

Results

Participants

A total of 475 female recruits volunteered; 25 were excluded due to missing hormonal contraceptive data or using multiple hormonal contraceptives resulting in 450 recruits included in this study (Table 1). Most participants were non-users (40%) with 28% using the COCP and 32% using a POC (progestin-only pill, n = 27 [19%]; DMPA, n = 32 [22%]; implant, n = 85 [59%]). Most non-users reported having a monthly menstrual cycle on entry (89%) with 11% reporting having a menstrual cycle every 2 months or less frequently. A total of 260 participants (non-users, n = 110 [42%]; COCP users, n = 73 [28%]; POC users, n = 77 [30%]) completed week 13 testing (Figure 1). Loss to follow-up was due to voluntary discharge from military service, involuntary discharge from military service (poor course performance or for medical reasons), voluntary withdrawal from the study, and being unavailable at time of follow-up. The POC users were younger and had a lower body mass and BMI than non-users and COCP users (all $p < 0.033$) (Table 1).

Hormonal Contraceptives and Physical Performance

Mean and individual physical performance data are shown in Figure 2 with mean absolute changes shown in Table 2. Training decreased 2.4 km run time (–3.7%, main effect of time, $p < 0.001$, $\eta_p^2 = 0.421$) but there was no difference between hormonal contraceptive groups (main effect of group, $p = 0.291$, $\eta_p^2 = 0.008$; group × time interaction, $p = 0.451$, $\eta_p^2 = 0.011$). Training increased maximal lift strength (4.5%, $p < 0.001$, $\eta_p^2 = 0.077$) but the training response was not different between hormonal contraceptive groups (group × time interaction, $p = 0.685$,

$\eta_p^2 = 0.005$). There was a main effect of group for lift strength ($p = 0.036$, $\eta_p^2 = 0.020$), where maximal lift strength was lower in COCP users than non-users irrespective of week ($p = 0.044$, $g = 0.36$). Training increased peak power output (1.5%, main effect of time, $p = 0.015$, $\eta_p^2 = 0.026$) but there was no difference between hormonal contraceptive groups (main effect of group, $p = 0.845$, $\eta_p^2 = 0.001$; group × time interaction, $p = 0.228$, $\eta_p^2 = 0.013$).

Hormonal Contraceptives, Body Composition, and Areal Bone Mineral Density

Mean and individual body composition data are shown in Figure 3 with mean absolute changes shown in Table 2. Training decreased fat mass (–9.6%, main effect of time, $p < 0.001$, $\eta_p^2 = 0.584$) but there was no difference between hormonal contraceptive groups (main effect of group, $p = 0.181$, $\eta_p^2 = 0.009$; group × time interaction, $p = 0.847$, $\eta_p^2 = 0.002$). Training increased lean mass (5.4%, main effect of time, $p < 0.001$, $\eta_p^2 = 0.701$) but there was no difference between hormonal contraceptive groups (main effect of group, $p = 0.173$, $\eta_p^2 = 0.009$; group × time interaction, $p = 0.801$, $\eta_p^2 = 0.002$). Training increased whole-body aBMD (0.9%, main effect of time, $p < 0.001$, $\eta_p^2 = 0.224$) but the training response was not different between hormonal contraceptive groups (group × time interaction, $p = 0.648$, $\eta_p^2 = 0.004$). There was a main effect of group for whole-body aBMD ($p = 0.027$, $\eta_p^2 = 0.019$). Whole-body aBMD was lower in POC users than COCP users ($p = 0.041$, $g = 0.54$) but not different between POC users and non-users ($p = 0.053$, $g = 0.37$) irrespective of week. Training had no effect on trunk aBMD (main effect of time, $p = 0.963$, $\eta_p^2 = 0.004$, group × time interaction, $p = 0.959$, $\eta_p^2 < 0.001$) but there was a main effect of group ($p = 0.010$, $\eta_p^2 = 0.024$), where trunk aBMD was lower in POC users than non-users ($p = 0.041$, $g = 0.36$) and COCP users ($p = 0.012$, $g = 0.58$) irrespective of week. Training increased arm aBMD (1.8%, main effect of time, $p < 0.001$, $\eta_p^2 = 0.070$) but there was no difference between hormonal contraceptive groups (main effect of group, $p = 0.065$, $\eta_p^2 = 0.013$; group × time interaction, $p = 0.766$, $\eta_p^2 = 0.002$).

Training increased legs aBMD (1.4%, main effect of time, $p < 0.001$, $\eta_p^2 = 0.369$) but the training response was not different between hormonal contraceptive groups (group × time interaction, $p = 0.334$, $\eta_p^2 = 0.012$). There was a main effect of group for legs aBMD ($p = 0.017$, $\eta_p^2 = 0.022$), where legs aBMD was lower in POC users than non-users irrespective of week ($p = 0.015$, $g = 0.39$).

Hormonal Contraceptives, Musculoskeletal Injuries, and Bone Stress Injuries

Associations between hormonal contraceptive use and injury incidence controlling for height, body mass, age, 2.4 km run time, smoking status, and previous bone stress injury are shown in Table 3. Data with POCs broken down by type are shown in Supplementary Table 1. The incidence of at least one lower body overuse musculoskeletal injury was 33.7% and for lower body bone stress injury was 3.5% across the entire study sample. There was no evidence of associations between hormonal contraceptive use and developing a lower body musculoskeletal injury or bone stress injury. A slower run time and being a smoker were associated with greater risk of lower body overuse musculoskeletal injury.

Hormonal Contraceptives and Iron Status, Vitamin D Status, and Bone Metabolism

Biochemical markers of iron status, vitamin D status, and bone metabolism are presented in Figure 4 with mean absolute changes presented in Table 2. There was no effect of training (main effect of time, $p = 0.968$, $\eta_p^2 < 0.001$) or hormonal contraceptive use (main effect of group, $p = 0.523$, $\eta_p^2 = 0.003$; group × time interaction, $p = 0.408$, $\eta_p^2 = 0.006$) on ferritin. Training decreased haemoglobin (main effect of time, $p < 0.001$, $\eta_p^2 = 0.458$) but there was no difference between hormonal contraceptive groups (main effect of group, $p = 0.627$, $\eta_p^2 = 0.003$; group × time interaction, $p = 0.368$, $\eta_p^2 = 0.012$). Training decreased total 25(OH)D (main effect of time, $p < 0.001$, $\eta_p^2 = 0.060$) but the training response was not different between

hormonal contraceptive groups (group × time interaction, $p = 0.576$, $\eta_p^2 = 0.003$). There was a main effect of group for total 25(OH)D ($p < 0.001$, $\eta_p^2 = 0.039$). Total 25(OH)D was higher in COCP users than non-users ($p < 0.001$, $g = 0.45$) and POC users ($p < 0.001$, $g = 0.49$). Training increased PTH (main effect of time, $p = 0.004$, $\eta_p^2 = 0.026$) but the training response was not different between hormonal contraceptive groups (group × time interaction, $p = 0.770$, $\eta_p^2 = 0.002$). There was a main effect of group for PTH ($p = 0.005$, $\eta_p^2 = 0.023$). PTH was lower in COCP users than non-users ($p = 0.005$, $g = 0.38$). Training increased βCTX (main effect of time, $p < 0.001$, $\eta_p^2 = 0.077$) but the training response was not different between hormonal contraceptive groups (group × time interaction, $p = 0.442$, $\eta_p^2 = 0.005$). There was a main effect of group for βCTX ($p < 0.001$, $\eta_p^2 = 0.084$). βCTX was lower in COCP users than non-users and POC users (both $p < 0.001$, $g \geq 0.43$). βCTX was higher in POC users than non-users ($p = 0.012$, $g = 0.36$). There was a group × time interaction for PINP ($p = 0.004$, $\eta_p^2 = 0.035$). PINP increased from week 1 to week 13 in all hormonal contraceptive groups with the increase larger in POC *vs* COCP *vs* non-users (all $p \leq 0.005$, $g \geq 0.30$). At week 1 and week 13, PINP was lower in COCP than non-users and POC users, and higher in POC users than non-users (all $p \leq 0.017$, $g \geq 0.27$).

Discussion

Military training improved physical performance and body composition, but hormonal contraceptive use was not associated with adaptations in aerobic endurance, muscle strength or power, lean mass, fat mass, or aBMD. The use of POCs was associated with lower aBMD and higher circulating markers of bone resorption and formation than non-users and / or COCP users (at both time-points). The use of COCPs was associated with lower circulating markers of bone resorption and formation and higher total 25(OH)D than non-users and POC users (at both time-points). These differences between hormonal contraceptive groups did not influence

lower body overuse musculoskeletal or bone stress injury risk. The data from this study do not support making hormonal contraceptive choices based on adaptations to training or injury risk in basic military training, but there may be longer term implications for skeletal health. The large sample of female recruits completing the same training provides novel insight into the effects of hormonal contraceptives on physical performance, body composition, injury risk, and musculoskeletal adaptations to exercise in young physically active women.

Endurance Performance

Training decreased 2.4 km run time (–3.7%) (improved aerobic fitness) but there was no difference between hormonal contraceptive groups. The 2.4 km run is used as a field test of aerobic fitness on entry and in-service in the British Army and is related to $\dot{V}O_{2max}$ (39). Several studies have explored the effect of COCP use on $\dot{V}O_{2max}$, but to our knowledge, no study has explored associations with POC use. Several small longitudinal studies report that COCP use for 1 to 6 months reduced absolute and relative $\dot{V}O_{2max}$ (19–21), although several other studies have found that COCP did not affect $\dot{V}O_{2max}$ (46, 47). Differences in findings between studies could be due to small samples, differences in COCP preparation and length of use, and differences in the training status of participants. One randomised controlled trial showed that a decrease in $\dot{V}O_{2max}$ with COCP use did not translate to a decrease in endurance performance (21), consistent with cross-sectional data (48). A systematic review reported that COCP users had poorer endurance exercise performance than non-users (3), although the sample size (n = 5 to 25) and effect size in the included studies were small, and most studies were acute cross-sectional comparisons of different groups of hormonal contraceptive users. Our findings are based on a large sample size of female recruits completing three months of identical and unfamiliar training, and included a POC group, providing evidence that different types of hormonal contraceptives are not associated with training adaptations. The COCP users had the

heaviest body mass — consistent with the effects of the COCP on increasing body mass (21) — but this higher body mass did not result in differences in endurance performance between groups. Training decreased fat mass (–9.6%), but there was no difference in fat mass loss between hormonal contraceptive groups. A study of oligomenorrheic / amenorrhoeic endurance athletes showed that the first 10 months of COCP use increased fat mass (~18% relative increase in body fat %), and decreased multi-stage fitness test performance by 6% (49), but increases in body mass and fat mass with COCP use is not supported by all studies (9, 50) and are more consistently reported with POC use (9). Hormonal contraceptives are often used by Servicewomen (unpublished) and athletes (1) to control heavy menstrual bleeding, which is a cause of iron deficiency (51, 52); better iron stores (ferritin) are associated with better 2.4 km run performance in male and female British Army recruits (26), however, hormonal contraceptive use was not associated with iron status (ferritin or haemoglobin) at any time.

Muscle Strength and Power

Training increased lift strength (4.5%), leg power (1.5%), and lean mass (5.4%), but the training response was not different between hormonal contraceptive groups. Lift strength was lower in COCP users than non-users at both time-points suggesting that COCP use may impair muscle strength, but not strength adaptations to training. Basic military training is not a specific resistance training intervention — but resistance training forms a component of military training — and is not optimal for promoting the adaptive response to exercise because of poor sleep, high endurance exercise volumes, and sub-optimal nutrition (53). Nevertheless, the data from this study are supported by recent systematic reviews showing that COCP users have slightly lower baseline muscle strength (3), but similar muscle strength, power, or hypertrophy responses to resistance training (10), compared with non-users. Oestradiol promotes sensitivity of muscle to anabolic signals (5) and acts as a neurosteroid in promoting neuromuscular

performance (8). Poorer muscle strength with COCP use might be due to low endogenous oestradiol (3) but it is not clear why we observed no difference in muscle strength between POC users and COCP users or non-users at either time-point. Ethinyl oestradiol also reduces free testosterone by increasing sex hormone binding globulin (37) and can interfere with muscle protein synthesis, muscle repair, and the insulin-like growth like factor-I and growth hormone axis (9). Progestins in hormonal contraceptives may have an anabolic effect on muscle — given their androgenicity — but even though most progestins bind to androgen receptors, some progestins have an antiandrogenic effect (4, 54). Higher potency androgenic progestins may also inhibit improvements in strength by reducing the ability of testosterone to bind to androgen receptors (4, 9). We did not separate groups by progestin type, but amongst COCP users, androgenicity of progestins has been associated with the lean mass and / or strength responses to strength training; greater increases have been seen in those using high compared with low (55) and low compared with high (56) androgenicity progestins.

Bone Mineral Density and Bone Metabolism

Training increased whole-body (0.9%), arms (1.8%), and legs (1.4%) aBMD, but the training response was not different between hormonal contraceptive groups. Although these increases are close to or below the least significant change for DXA, they are similar in magnitude to those previously reported in response to military training using a range of imaging modalities and could be clinically important for improving resistance to stress fractures (32). An increase in aBMD is consistent with training-induced adaptive bone formation by increased remodeling / modeling (57). We have previously observed inhibited adaptation of trabecular bone in the distal tibia in response to military training in COCP and / or POC users compared with non-users (23, 31) — consistent with the effects of low oestradiol on increasing remodelling of

trabecular bone (6) — but it is likely we lacked sensitivity with DXA to detect differences between hormonal contraceptive groups in adaptation to training.

Whole-body, trunk, and legs aBMD were lower in POC users than non-users and / or COCP users at both time-points. These data are consistent with the lower tibial speed of sound in DMPA users than non-users in military recruits (27) and longitudinal studies showing decreased aBMD with DMPA use (4, 29, 30); however, data on other POCs suggests they have little effect on aBMD (4, 29, 30). Different progestins can have different effects on bone through different actions on the progesterone and androgen receptor (4), but we were unable to detect progestin-specific effects in this study. The lower aBMD in the POC users compared with COCP users and non-users may be due to the independent effects of DMPA, however, data from other military populations also show lower spinal aBMD with the use of hormonal contraceptives (all types grouped) compared with non-users (60). The higher βCTX and PINP in the POC users compared with the non-users and COCP users at both time-points is consistent with the effects of low oestradiol on increasing bone turnover (6); higher bone turnover in the POC users may also explain why aBMD was lower in POC users than non-users and / or COCP users (6). Higher bone turnover and lower aBMD with long-term POC use may have long-term impacts on bone health and stress fracture risk. Conversely, βCTX and PINP were lowest in COCP users, consistent with the effects of high oestradiol on suppressing bone turnover (4). The mechanism for the greater training-induced increase in PINP in POC users compared with non-users and COCP users is unclear, but could be due to lower oestradiol in POC users increasing bone turnover. These data are in contrast to a previous study showing similar increases in PINP (and decreases in βCTX) in response to military training in non-users, COCP users, and DMPA users (23), but changes in bone metabolic markers in response to training likely depend on the time between measurements and other sources of variability. The decrease

in total 25(OH)D and increase in PTH with training in all groups may have contributed to the increase in bone resorption (61). Total 25(OH)D was higher and PTH lower in COCP users than non-users and POC users at both time-points, likely due to the effects of ethinyl oestradiol on increasing total 25(OH)D and vitamin D binding protein (62) that, in turn, suppresses PTH secretion through actions of the metabolite 1,25 dihydroxyvitamin D (61).

Musculoskeletal Injuries and Bone Stress Injuries

There was no evidence of associations between hormonal contraceptive use and developing a lower body overuse musculoskeletal injury in training. Beyond the role of maintenance of bone, oestradiol plays an important role in muscle, tendon, and ligament function, resulting in decreased tendon and ligament laxity, which may impact musculoskeletal injury risk (5). The higher total 25(OH)D in COCP users compared with non-users and POC users, could protect against musculoskeletal injuries in basic military training (25, 38), although we did not measure vitamin D metabolites, the vitamin D binding protein, and free vitamin D. We grouped all types of lower body overuse musculoskeletal injuries so differences in injury aetiology will have masked any associations between hormonal contraceptive use and specific injury types. There is some evidence that oral contraceptive pills (both COCP and progestin-only pills) reduce the risk of anterior cruciate ligament injuries compared with non-users (12), but this finding is not supported by all studies and the COCP may increase the risk of fracture compared with non-users (11). We separated lower body bone stress injuries due to the essential role of oestradiol in regulating bone metabolism (6) and determined that hormonal contraceptive use was not associated with bone stress injury incidence. The higher total 25(OH)D in COCP users (25) and lower aBMD in POC users (63) than the other groups could reduce bone stress injury risk, however, the relatively low number of bone stress injury cases in our study will have reduced our ability to detect any small effects and our bone stress injury data must be interpreted with

448 caution. Data from military studies show that DMPA might be associated with increased bone
449 stress injury risk (28), but contrasting data also show hormonal contraceptive use is not
450 associated with musculoskeletal injury or bone stress injury risk in military training (64–66)
451 compared with non-users. Inconsistent findings between studies could be due to the short
452 nature of studies, small sample sizes, inconsistency in methodologies, and inconsistencies in
453 definitions of hormonal contraceptive user groups.
454
455 *Limitations*
456 We were unable to subgroup COCP users and POC users by ethinyl oestradiol dose or by
457 progestin type, and our groups included multiple different types of hormonal contraceptive
458 preparations within each group. Therefore, the heterogenous hormonal contraceptive type
459 within each group might mask any effects of specific hormonal contraceptives. Due to the
460 nature of military training, our non-user group was tested at various points of the menstrual
461 cycle, but there are unlikely to be any effects on performance (67). We also did not include a
462 non-exercising control group to determine natural variability in our measures. We did not
463 measure oestrogens, progestogens, androgens, or sex hormone binding globulin, which could
464 help explain some of our findings. We also did not have more detailed measures of
465 appendicular bone geometry or microstructure, but have recently published data on hormonal
466 contraceptives and peripheral quantitative computed tomography (23) and high-resolution
467 peripheral quantitative computed tomography (31) outcomes. Measures of bone formation and
468 resorption have circadian patterns and can be influenced by feeding and exercise. Due to
469 training course demands, we were unable to obtain morning fasted samples on all participants,
470 which will increase the variability of this measure, but we did match the time of day for week
471 1 and week 13 measures. Our study only included the basic training component of a military
472 career, and it is not clear whether a longer follow-up time would detect other differences

between hormonal contraceptives groups. Hormonal contraceptive use was self-reported and we did not record length of use of each hormonal contraceptive, and each participant likely had a varied length of use at the start of training and may have had a history of using different hormonal contraceptives, but the inclusion of a 13-week follow-up period of standardised military training and feeding provides a methodological strength. Our physical performance and body composition data are subject to survivor bias; we only followed up those who completed training and not adapting to training may be a risk factor for failing to complete the training course, but the proportion of non-users, COCP users, and POC users was similar at baseline and follow-up. Our non-user groups were also not all eumenorrheic, which may have impacted outcomes. Finally, our sample number of bone stress injuries meant a larger study would likely be required to detect an effect of hormonal contraceptive use on bone stress injury risk.

Conclusions

Military training improved physical performance and body composition, but hormonal contraceptive use was not associated with adaptations in endurance performance, muscle strength and power, fat mass, lean mass, or aBMD. Progestin-only contraceptive use was associated with lower aBMD and higher markers of bone resorption and formation than COCP users and / or non-users, and COCP use was associated with lower markers of bone resorption and formation, lower PTH, and higher total 25(OH)D than POC users and / or non-users; these differences between hormonal contraceptive groups did not manifest in differences in lower body overuse musculoskeletal injury or bone stress injury risk in the short-term.

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Conflicts of Interest

The authors have no conflicts of interests to declare. The results of the present study do not constitute endorsement by ACSM. The results of this study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

Author Contributions

RMI, NPW, and JPG designed the study. TJO, SJ, NPW, ATC, SJO, and JPG collected the data. JCYT and WDF analysed the biochemical samples. TJO, HAE, MOC, and CVC produced the manuscript. TJO performed the data analysis. All authors edited the manuscript and approved the final version.

Data Availability

All data and code are available from the corresponding author pending approval of public release from UK Ministry of Defence.

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697

Tables

Table 1. Participant demographics. Data are mean ± SD.

	Non-users (n = 182)	COCP (n = 124)	POC (n = 144)
Age (y)	22.9 ± 3.7	22.3 ± 3.3	21.5 ± 3.1[a,b]
Height (m)	1.65 ± 0.06	1.66 ± 0.06	1.65 ± 0.06
Body Mass (kg)	64.8 ± 8.0	65.8 ± 8.1	63.6 ± 8.3[a,b]
Body Mass Index ($kg \cdot m^2$)	23.8 ± 2.4	23.9 ± 2.3	23.4 ± 2.5[a,b]

COCP, combined oral contraceptive pill; POC, progestin-only contraceptive.

[a]$p < 0.05$ vs non-users; [b]$p < 0.05$ vs COCP

703 **Table 2.** Mean absolute unadjusted change and 95% confidence intervals (95% CI) in physical performance, body composition, and circulating
704 markers of iron status, vitamin D status, and bone metabolism in response to 13 weeks basic military training in non-users, combined oral
705 contraceptive pill (COCP) users, and progestin-only contraceptive (POC) users.

	Non-user		COCP		POC	
	Change	95% CI	Change	95% CI	Change	95% CI
2.4 km Run Time (s)	**–25**	**–33, –17**	**–30**	**–42, –19**	**–27**	**–39, –15**
Maximal Lift Strength (kg)	1.5	–0.2, 3.1	1.2	–0.8, 3.1	1.9	–0.5, 4.2
Peak Power Output (W)	55	–5, 114	27	–65, 119	2	–74, 77
Fat Mass (kg)	**–2.1**	**–2.6, –1.5**	**–2.2**	**–3.0, –1.4**	**–2.0**	**–2.6, –1.5**
Lean Mass (kg)	**2.1**	**1.8, 2.5**	**2.1**	**1,7, 2.6**	**2.4**	**2.1, 2.8**
Whole-body aBMD ($mg \cdot cm^{-2}$)	**0.01**	**0.01, 0.01**	0.01	–0.00, 0.02	0.01	–0.00, 0.01
Trunk aBMD ($mg \cdot cm^{-2}$)	0.00	–0.01, 0.00	0.00	–0.01, 0.00	0.00	–0.01, 0.01
Arms aBMD ($mg \cdot cm^{-2}$)	**0.01**	**0.00, 0.02**	**0.01**	**0.00, 0.03**	**0.01**	**0.00, 0.03**
Legs aBMD ($mg \cdot cm^{-2}$)	**0.02**	**0.01, 0.02**	**0.02**	**0.01, 0.03**	**0.01**	**0.01, 0.02**
Ferritin ($\mu g \cdot L^{-1}$)	–4.2	–12.0, 3.4	1.8	–5.6, 9.3	–1.2	–6.3, 3.9
Haemoglobin ($g \cdot dL^{-1}$)	–0.9	–1.2, 0.6	–0.8	–1.2, 0.5	**–1.1**	**–1.3, –0.8**
Total 25(OH)D ($nmol \cdot L^{-1}$)	**–9.6**	**–15.0, –4.1**	**–15.0**	**–22.0, –7.2**	**–8.6**	**–14.0, –2.7**
Parathyroid Hormone ($pmol \cdot L^{-1}$)	–0.21	–0.43, 0.01	–0.03	–0.24, 0.19	–0.16	–0.37, 0.05
βCTX ($\mu g \cdot L^{-1}$)	0.02	–0.01, 0.05	**0.05**	**0.02, 0.08**	**0.06**	**0.02, 0.09**
PINP ($\mu g \cdot L^{-1}$)	**6.1**	**2.3, 9.8**	**13.0**	**8.2, 19.0**	**17.0**	**12, 23**

706 aBMD, areal bone mineral density; βCTX, c-telopeptide cross-links of type 1 collagen; COCP, combined oral contraceptive pill; PINP, procollagen type 1 N-terminal
707 propeptide; POC, progestin-only contraceptives; PTH, parathyroid hormone. Bold values indicate the 95% confidence interval does not contain 0.

708 **Table 3.** Associations between hormonal contraceptives and lower body overuse
709 musculoskeletal injury incidence.

	Lower Body Overuse Musculoskeletal Injury (n = 268)		Lower Body Bone Stress Injury (n = 268)	
	Odds Ratio (95% CI)	p	Odds Ratio (95% CI)	p
Height (m)	1.015 (0.965, 1.067)	0.554	0.999 (0.886, 1.125)	0.981
Body Mass (kg)	0.971 (0.931, 1.011)	0.153	1.034 (0.942, 1.134)	0.483
Age (y)	0.981 (0.908, 1.058)	0.616	1.058 (0.903, 1.239)	0.485
2.4 km Run Time (s)	1.010 (1.005, 1.014)	<0.001	1.007 (0.998, 1.016)	0.134
Smoking Status				
No	—		—	
Yes	2.105 (1.132, 3.930)	0.019	1.438 (0.328, 6.307)	0.630
Previous	1.270 (0.657, 2.421)	0.472	1.890 (0.438, 8.159)	0.394
Previous Bone Stress Injury				
No	—		—	
Yes	0.973 (0.273, 3.147)	0.965	0.937 (0.062, 14.20)	0.962
Unknown	1.877 (0.193, 17.46)	0.560	5.846 (0.233, 146.6)	0.283
Hormonal Contraceptives				
Non-users	—		—	
COCP	1.272 (0.682, 2.369)	0.448	1.632 (0.383, 6.953)	0.508
POC	0.964 (0.507, 1.820)	0.910	1.479 (0.305, 7.167)	0.627

COCP, combined oral contraceptive pill; POC, progestin-only contraceptives.

Odds ratios are controlling for all other factors in the table.

710

Figures

Figure 1. Participant flow through the study.

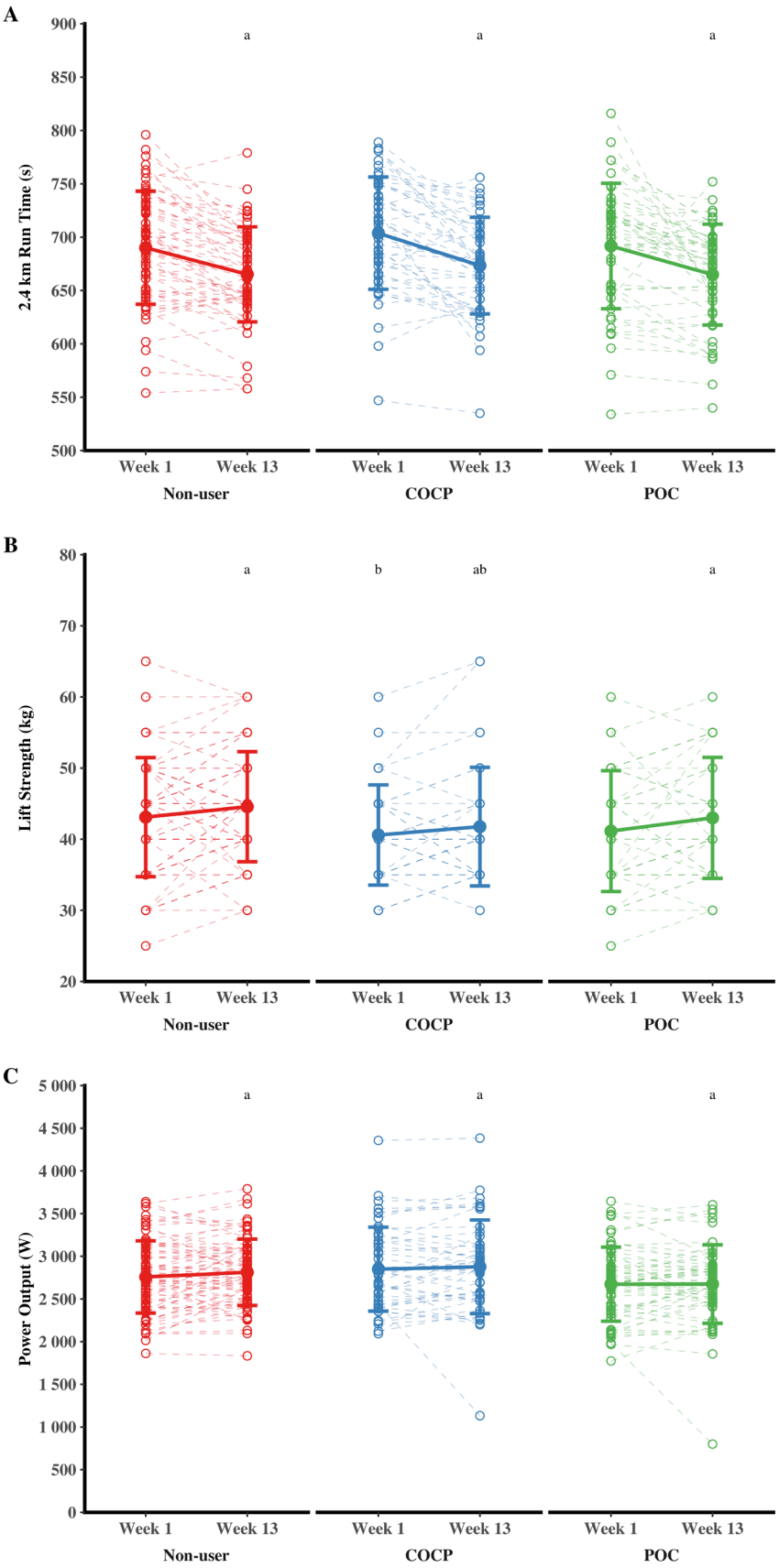

Figure 2. The effect of military training on physical performance in non-users, combined oral contraceptive pill (COCP) users, and progestin-only contraceptive (POC) users.

[a]$p < 0.05$ vs week 1 (main effect of time); [b]$p < 0.05$ vs non-users (main effect of group).

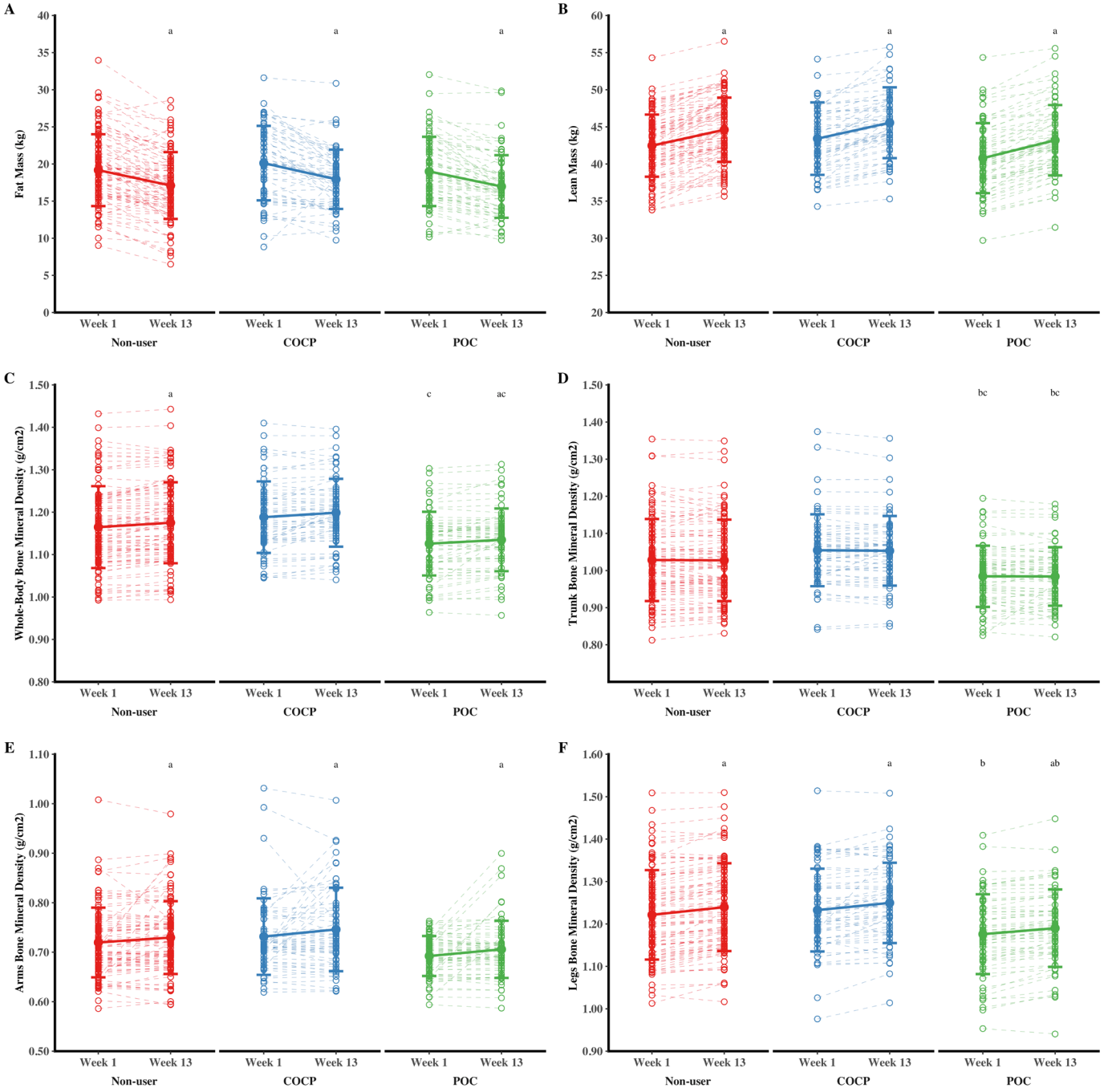

Figure 3. The effect of military training on body composition in non-users, combined oral contraceptive pill (COCP) users, and progestin-only contraceptive (POC) users.

[a]$p < 0.05$ vs week 1 (main effect of time); [b]$p < 0.05$ vs non-users (main effect of group); [c]$p < 0.05$ vs COCP users (main effect of group).

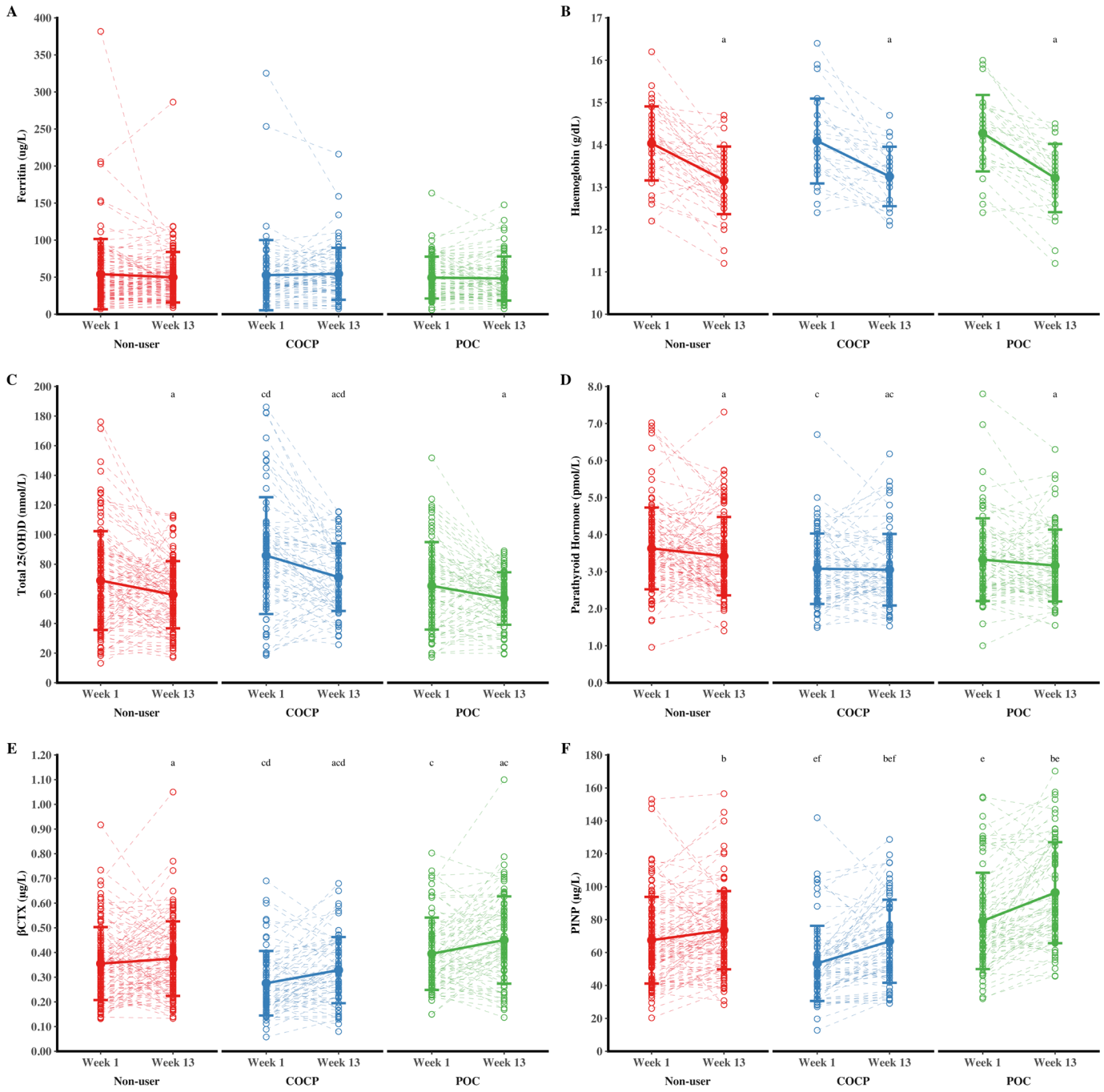

Figure 4. The effect of military training on iron status, vitamin D status, and bone metabolism in non-users, combined oral contraceptive pill (COCP) users, and progestin-only contraceptive (POC) users.

[a]$p < 0.05$ vs week 1 (main effect of time); [b]$p < 0.05$ vs week 1 (post-hoc after group × time interaction); [c]$p < 0.05$ vs non-users (main effect of group). [d]$p < 0.05$ vs POC (main effect of group); [e]$p < 0.05$ vs non-users (post-hoc after group × time interaction); [f]$p < 0.05$ vs POC (post-hoc after group × time interaction).

βCTX, c-telopeptide cross-links of type 1 collagen; PINP, procollagen type 1 N-terminal propeptide.

730 **Supplemental Digital Content**

731

732 **Supplementary Table 1.** Associations between hormonal contraceptive use and lower body
733 overuse musculoskeletal injury incidence with progestin only contraceptives separated into
734 contraceptive type.

	Lower Body Overuse Musculoskeletal Injury (n = 268)		Lower Body Bone Stress Injury (n = 268)	
	Odds Ratio (95% CI)	p	Odds Ratio (95% CI)	p
Height (m)	1.015 (0.965, 1.068)	0.560	0.999 (0.889, 1.122)	0.982
Body Mass (kg)	0.971 (0.931, 1.011)	0.156	1.029 (0.940, 1.125)	0.538
Age (y)	0.981 (0.908, 1.058)	0.614	1.059 (0.906, 1.237)	0.472
2.4 km Run Time (s)	1.010 (1.005, 1.014)	<0.001	1.007 (0.998, 1.015)	0.138
Smoker				
No	—		—	
Yes	2.100 (1.124, 3.941)	0.020	1.439 (0.346, 5.993)	0.617
Previous	1.273 (0.645, 2.437)	0.470	1.785 (0.426, 7.483)	0.428
Previous Bone Stress Injury				
No	—		—	
Yes	0.976 (0.273, 3.174)	0.969	0.870 (0.061, 12.30)	0.918
Don't Know	1.889 (0.193, 17.68)	0.557	5.138 (0.209, 126.0)	0.317
Hormonal Contraceptives				
Non-user	—		—	
COCP	1.272 (0.682, 2.370)	0.448	1.646 (0.384, 7.063)	0.502
POP	0.946 (0.299, 2.711)	0.921	1.310 (0.085, 20.30)	0.847
DMPA	0.993 (0.328, 2.872)	0.990	1.101 (0.056, 21.55)	0.949
Implant	0.959 (0.440, 2.048)	0.915	2.520 (0.496, 12.79)	0.265

735 COCP, combined oral contraceptive pill; DMPA, depot medroxyprogesterone acetate; POP, progestin only pill.
736 Odds ratios are controlling for all other factors in the table.