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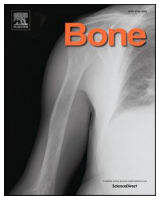

Full Length Article

Hormonal contraceptive use is associated with altered bone structural and metabolic responses to military training in women: An observational cohort study

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ABSTRACT

Military training increases tibial density and size. Female sex hormones may influence the adaption of bone to loading, but it is unknown if women using different hormonal contraceptives adapt similarly to military training. One hundred and sixteen women (57 women not using hormonal contraceptives [non-users], 38 combined oral contraceptive pill [COCP] users, 21 depot medroxyprogesterone acetate [DMPA] users) completed this study. Tibial volumetric bone mineral density (vBMD) and geometry were measured by peripheral quantitative computed tomography (4 %, 14 %, 38 %, and 66 % sites) at the start (week 1) and end (week 14) of British Army basic training. Circulating markers of bone and calcium metabolism were measured at weeks 1, 2, 4, 6, 10, and 14. Training increased trabecular vBMD at the 4 % site, periosteal perimeter at the 14 % and 66 % sites, and total area, cortical area, cortical thickness, and bone strength at all sites (0.1 to 1.6 %, $p \leq 0.009$), with no differences between hormonal contraceptive groups ($p \geq 0.127$). Trabecular vBMD increased at the 14 % site in non-users (0.8 %, $p = 0.005$), but not in COCP or DMPA users ($p \geq 0.205$). Periosteal perimeter increased at the 38 % site in COCP (0.4 %, $p < 0.001$) and DMPA (0.5 %, $p < 0.001$) users, but not in non-users ($p = 0.058$). Training had no effect on periosteal perimeter at the 4 % site or cortical vBMD or endosteal perimeter at any site ($p \geq 0.168$). βCTX decreased and PINP increased during training with no difference between hormonal contraceptive groups. Training increased iPTH in non-users, but not COCP or DMPA users. Hormonal contraceptives may exert site-specific effects on the mechanobiology of bone, with higher endogenous oestradiol promoting trabecularisation and inhibiting periosteal expansion in non-users compared with hormonal contraceptive users.

1. Introduction

Oestrogens play an important role in the development and maintenance of bone through actions on osteoblasts, osteoclasts, and osteocytes via the oestrogen receptor alpha (ERα) [1]. An increase in oestrogens during puberty limits periosteal and endosteal bone formation in women [1–3]. In adulthood, oestrogens protect bone by suppressing bone remodelling and may also influence the sensitivity of bone to mechanical loading [1]. Hormonal contraceptives contain synthetic oestradiol (ethinyl oestradiol) and/or progestogen (progestins), which can suppress the hypothalamic pituitary ovarian axis, lower endogenous oestradiol, and affect bone metabolism [4,5]. Data from female athletes with amenorrhoea or oligomenorrhoea suggest that low endogenous oestradiol can inhibit some of the skeletal adaptations at the tibia with loading [6,7], although weight-bearing exercise may provide some protection to the deleterious effects of low oestradiol [6–9].

Basic military training is characterised by a sudden increase in unaccustomed and repetitive mechanical loading, which causes a remodelling of fatigue damage and can—in some cases—eventually lead to a stress fracture [10]. Women typically have a three-fold higher risk of stress fracture than men in basic military training [11] and so interventions that promote adaptive bone formation in women may

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protect from stress fractures [12]. Several studies have observed marked changes in tibial geometry and density within 14 weeks of military training in men and women [13–20], providing evidence of adaptive bone formation and high mechanical stresses at the tibia [21]. Long-acting progestin-only contraceptives—excluding the intrauterine device—commonly used by women in the military [22], increase markers of bone formation and resorption [23] and may increase stress fracture risk for servicewomen [24]. A previous study of basic military training found that progestin-only contraceptive users had blunted adaptation of the trabecular microarchitecture, but the study sample size was small [19]. The effects of hormonal contraceptive use on skeletal adaptions to unaccustomed mechanical loading may have important clinical implications for exercising women of reproductive age.

This study compared changes in tibial density and geometry, and biochemical markers of bone and calcium metabolism, between women using different types of hormonal contraceptives during 14 weeks British Army basic training. We hypothesised that women using hormonal contraceptives would have suppressed adaptations in trabecular bone and increased periosteal expansion because of suppressed endogenous oestradiol. We also hypothesised that biochemical markers of bone formation and resorption would be lower in combined oral contraceptive users and lower in progestin-only injections users compared with non-users.

2. Materials and methods

2.1. Participants

A total of 147 female British Army recruits volunteered to participate in this study during week one of their British Army basic military training at the Army Training Centre, Pirbright. Participants were recruited throughout the year and started training across all seasons. Exclusion criteria were: pregnancy; history of adrenal, ovarian, or gonadotropin releasing hormone insufficiency; pituitary disease; thyroid disease in the past year; diabetes; hyperparathyroidism; osteopenia; glucocorticoid use; hormonal contraceptive use other than a combined oral contraceptive pill (COCP) or depot medroxyprogesterone acetate (DMPA); primary amenorrhoea, secondary amenorrhoea, or oligomenorrhoea not associated with hormonal contraceptive use; or current musculoskeletal injury. Each participant had the study procedures and risks fully explained verbally and in writing before providing written informed consent. All participants passed an initial medical assessment and were declared injury free and medically fit to train. This study was approved by the Ministry of Defence Research Ethics Committee (MODREC 0807/162).

2.2. Experimental protocol

All participants were undergoing the British Army 14-week basic military training course for soldiers (non-infantry, non-officers). Basic military training aims to improve physical fitness and teach basic military skills, is physically arduous, and involves completing high daily walking and running distances [25]. Recruits undertook 43 periods of military drill, 79 physical training periods including 21 loaded marches with increasing mass and distance over time (between 4.8 and 9.7 km carrying between a 10 kg and 20 kg backpack), 10 swimming sessions, 16 periods of strength and conditioning, 12 periods of self-selected sport, and 20 periods of military specific fitness (obstacle course, circuit training, and steeplechase run). Baseline demographic data were recorded during week 1 of training: maximal effort 2.4 km run time was recorded as part of the standard military aerobic fitness test; height (Seca 225, Seca Ltd., UK) and body mass (Seca 770, Seca Ltd., UK) were measured in standard issue military clothing without boots, and; hormonal contraceptive use and age of menarche were determined from a lifestyle questionnaire. Tibial density, geometry, and estimated bone strength were measured by pQCT at the start (week 1) and end (week 14) of training. Blood samples were taken at weeks 1, 2, 4, 6, 10, and 14 for the assessment of biochemical markers of bone and calcium metabolism. All week 1 measurements were made immediately following the initial medical assessment and before any military training had commenced. Participants were grouped based on current hormonal contraceptive use into those not using any hormonal contraceptive (non-users), COCP users, and DMPA users.

2.3. Peripheral quantitative computed tomography

Tibial volumetric bone mineral density (vBMD), geometry, and *estimated* strength of the dominant leg were measured by peripheral quantitative computed tomography (pQCT; XCT2000L, Stratec Germany) at the 4 %, 14 %, 38 % and 66 % sites [13]. Dominant leg was self-reported and described as the preferred leg for kicking a football. Tibial length was measured as the distance from the distal aspect of the medial malleolus to the medial joint line. Participants were seated comfortably with their lower leg extended through the scanning cylinder and were asked to remain still for the duration of the scan ($\leq$ 15 min). Initial scout scans were conducted at a speed of 40 mm·s^{-1} to identify the distal end plate of the tibia. Scans of single axial slices (2.2 mm thickness, voxel size 0.5 mm, measure diameter 140 mm) were taken at a translation speed of 20 mm·s^{-1} at 4, 14, 38, and 66 % distances of the approximate segment length, proximal to the distal endplate of the tibia. A daily quality assurance calibration check was undertaken by scanning phantoms with densities of 168.5, 317.4, and 462.5 mg·cm^3. The following outcomes were determined using the Bone Alignment and Measurement Package (BAMPack) (L-3 ATI, USA) as described previously [13]: total area, trabecular vBMD, trabecular area, cortical vBMD, cortical area, cortical thickness, periosteal perimeter (PPm), endosteal perimeter (EPm), and bone strength index (BSI). Polar stress-strain index (SSI) was calculated [15,17,26]. Density thresholds above 800 mg·mm^3 and below 600 mg·mm^3 were used to define cortical and trabecular bone with voxels with density values between 600 and 800 mg·mm^3 removed from analysis to delineate between cortical and trabecular regions. Only images with minimal motion artefacts (image quality >2) and alignment error (Root Mean Square of difference in the outer boundaries <0.4 mm) were included in the analyses. The CV of our measures were $\leq$ 1 % for trabecular and cortical vBMD and geometry using BAMPack.

2.4. Biochemical markers of bone metabolism

A venous blood sample was taken between 0500 and 0545 h after an overnight fast from 2200 h. Venous blood was withdrawn from a vein in the antecubital fossa and collected in serum and EDTA vacutainers (Becton Dickinson, USA). Serum samples were left at room temperature for 60 min to clot whilst EDTA samples were spun immediately. Blood samples were centrifuged at 2000 rpm (751 *g*) at 4 °C for 10 min before serum and plasma were separated and stored at −80 °C until analysis. EDTA plasma samples were analysed for procollagen type I N-terminal propeptide (PINP), c-terminal cross-links telopeptide of type 1 collagen (βCTX), intact parathyroid hormone (iPTH), and osteocalcin by electrochemiluminescence immunoassay (ECLIA) on Cobas e601 platform (Roche Diagnostics, Germany) with inter-assay CVs of <5.0 % across their respective analytical ranges. Serum samples were analysed for soluble receptor activator of nuclear factor kappa B ligand (sRANKL), osteoprotegerin (OPG), sclerostin, and bone-specific alkaline phosphatase (bone ALP) by enzyme-linked immunosorbent assays (ELISA). Ampli-sRANKL, OPG, and sclerostin ELISA assays (Biomedica, Austria) had inter-assay CVs < 15.0 % across the assay working range of 0.02–2.00 pmol•L^{-1}, 0.07–20.00 pmol•L^{-1}, and 2.6–240.0 pmol•L^{-1}, respectively. Bone-specific ALP ELISA assay (MicroVue, Quidel Corp., Germany) had inter-assay CVs < 8.0 % across the assay working range of 0.7–149.0 U•L^{-} Serum total 25-hydroxyvitamin D (25(OH)D) was analysed by liquid chromatography tandem mass spectrometry (LC-MS/MS) [27]. Total 25(OH)D was calculated from the sum of the

measurements of 25(OH)D3 and 25(OH)D2 with an inter-assay CV < 10.0 % across the assay working range of 0.1–200.0 nmol•L^{-1}. Total 25(OH)D was only measured at week 1, 6, and 14. Serum calcium, albumin, and phosphate were measured by spectrophotometric methods on the COBAS c501 platform (Roche Diagnostics, Germany) according to the manufacturer's instructions. The inter-assay CVs for total calcium, albumin, and phosphate were ≤ 2.1 % across the assay working ranges of 0.20–5.00 mmol•L^{-1}, 2–60 g•L^{-1}, and 0.81–1.45 mmol•L^{-1}, respectively. All biochemical analysis was undertaken by the Bioanalytical Facility at the University of East Anglia under GCP/GLP conditions, and the 25(OH)D method met the certification requirements specified by the Vitamin D External Quality Assessment Scheme (DEQAS).

2.5. Statistical analyses

These data are secondary analysis from a trial examining sex differences in skeletal adaptations to military training [18]. Sensitivity analysis revealed that our sample size was adequate to detect an interaction effect size of $\eta_p^2 = 0.01$ (Cohen's $f = 0.201$) for pQCT outcomes with an alpha of 0.05 and a power of 80 % (GPower, *v*.3.1.9.2). All data were analysed using the R programming language (*v*.4.2.2). Participant characteristics were compared between hormonal contraceptive groups with a one-way ANOVA with post-hoc Holm-Bonferroni adjusted pairwise comparisons for significant effects of group. Chi-squared tests were used to compare the prevalence of vitamin D deficiency between groups. Linear mixed effect models with restricted maximum likelihood estimation (*lme4 package v*.1.1–31) were used to examine differences between hormonal contraceptive groups for pQCT outcomes and biochemical markers of bone and calcium metabolism. Group (non-users vs COCP vs DMPA), time (pQCT outcomes: pre-training vs post-training; bone and calcium metabolism outcomes: week 1 vs week 2 vs week 4 vs week 6 vs week 10 vs week 14), and their interaction were included as fixed effects to examine the effect of hormonal contraceptive use. Random intercepts were assigned to each participant to account for within participant correlation for repeated measures. Significance of the fixed effects from each model were determined with Sattherwaite degrees of freedom (*lmerTest package v*.3.1–3). Normality of the residuals for each model were checked visually by plotting the residuals against the fitted values and from Q-Q plots. In the event of a significant main effect of time, group, or significant interaction, pairwise comparisons with Holm-Bonferroni corrections and Kenward-Roger degrees of freedom were used on the linear mixed effects model to identify differences between time points or group (*emmeans package v*.1.8.3). Pooled data were used for main effects with no interaction, and each group was analysed independently for interaction effects. Effect sizes are presented as partial eta-squared (η_p^2) for main effects and interactions and paired Hedges' g for within-group paired comparisons (*effectsize package v*.0.8.2). Significance was accepted as $p < 0.05$; these analyses were exploratory and so *p* values were not corrected for multiple outcomes.

3. Results

Twenty-six participants failed to complete training due to voluntary discharge, medical discharge, entry to rehabilitation, or poor course performance and were lost to follow-up. A further five participants were excluded for movement artefact or alignment error in at least one pQCT scan. Complete pQCT data were available for 116 women (Fig. 1). Mean length of COCP use was 39 months (1 to 132 months); 59 % were taking Microgynon, 8 % Yasmin, 7 % Cerezette, and the remaining 26 % other brands including Dianette and Cilest. Mean time of DMPA use was 13 months (range 1 to 48 months). Average age at menarche was 13 ± 2 years (range 9 to 17 years). Of the women who were not currently using any form of hormonal contraception, 83 % reported regular periods at baseline, and 20 % reported menstrual changes during training including irregular or lighter periods. There were no differences between non-users, COCP users, and DMPA users for age, height, body mass, or 2.4 km run time ($p \geq 0.403$) (Table 1). There was a higher prevalence of vitamin D deficiency in DMPA users than COCP users ($p = 0.025$), but COCP users and DMPA users were not different than non-users ($p \geq 0.056$).

Tibial density, geometry, and strength indices are presented in Table 2 with mean absolute changes presented in Table 3.

3.1. Volumetric bone mineral density

Training increased trabecular vBMD at the 4 % site (main effect of time, $p < 0.001$, $\eta_p^2 = 0.496$), with no differences between hormonal contraceptive groups (group × time interaction, $p = 0.843$, $\eta_p^2 = 0.003$;

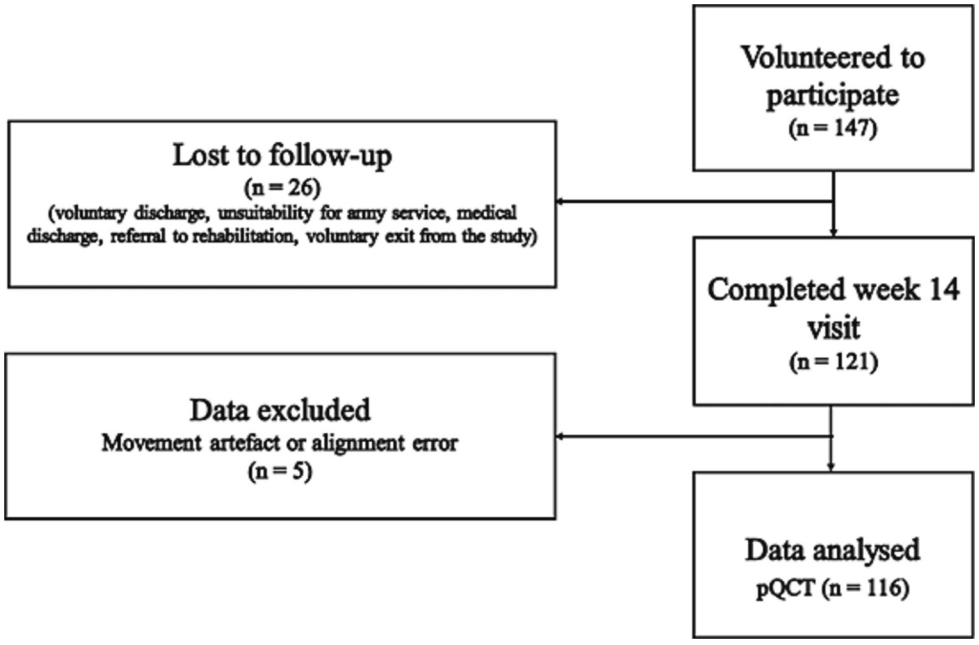

Fig. 1. Participant flow through the study.

Table 1
Participant demographics. Data are mean ± SD.

	COCP (n = 38)	DMPA (n = 21)	Non-users (n = 57)
Age (years)	20 ± 3	20 ± 2	21 ± 3
Height (m)	1.63 ± 0.05	1.64 ± 0.08	1.65 ± 0.07
Body Mass (kg)	61.8 ± 6.9	63.0 ± 9.9	63.5 ± 8.2
2.4 km Run Time (s)	772 ± 92	773 ± 77	775 ± 77
Total 25(OH)D ($mmol•L^{-1}$)	72.4 ± 37.3	50.5 ± 34.2	47.9 ± 24.0
Vitamin D Deficient (%)	11	40	30

COCP, combined oral contraceptive pill; DMPA, depot medroxyprogesterone acetate; Total 25(OH)D, total 25-hydroxyvitamin D.
Missing 2.4 km Run Time (s) data: COCP (n = 4), Depot (n = 3), Non-users (n = 4).

main effect of group, $p = 0.403$, $\eta_p^2 = 0.016$). There was a group × time interaction for trabecular vBMD at the 14 % site ($p = 0.022$, $\eta_p^2 = 0.066$). Trabecular vBMD increased in non-users ($p = 0.005$, $g = 0.34$), but not COCP ($p = 0.205$, $g = 0.24$) or DMPA users ($p = 0.717$, $g = 0.08$). Trabecular vBMD was not different between any two groups at any time-point ($p \geq 0.399$). Training had no effect on cortical vBMD at any site (main effects of time, $p \geq 0.676$, $\eta_p^2 \leq 0.002$), with no differences between hormonal contraceptive groups (group × time interactions, $p \geq 0.098$, $\eta_p^2 \leq 0.040$; main effects of group, $p \geq 0.514$, $\eta_p^2 \geq 0.012$).

3.2. Geometry

Training increased total area at all sites (main effects of time, $p \leq 0.009$, $\eta_p^2 \geq 0.059$), with no differences between hormonal contraceptive groups (group × time interaction, $p \geq 0.127$, $\eta_p^2 \leq 0.036$; main effect of group, $p \geq 0.085$, $\eta_p^2 \leq 0.043$). Training increased cortical area at all sites (main effects of time, $p \leq 0.001$, $\eta_p^2 \geq 0.118$), with no difference between hormonal contraceptive groups (group × time interaction, $p \geq 0.156$, $\eta_p^2 \leq 0.018$). There was a main effect of group for cortical area at the 38 % site ($p = 0.050$, $\eta_p^2 = 0.052$), but not 14 % or 66 % sites ($p \geq 0.108$, $\eta_p^2 \leq 0.039$). Cortical area was larger in non-users than COCP users ($p = 0.045$), but DMPA users were not different to COCP users or non-users ($p \geq 0.654$). Training increased cortical thickness at all sites (main effects of time, $p \leq 0.005$, $\eta_p^2 \geq 0.066$), with no differences between hormonal contraceptive groups (group × time interactions, $p \geq 0.244$, $\eta_p^2 \leq 0.025$; main effects of group, $p \geq 0.065$, $\eta_p^2 \leq 0.047$). Training had no effect on PPm at the 4 % site (main effect of time, $p = 0.168$, $\eta_p^2 = 0.017$), but increased PPm at the 14 % and 66 % sites (main effects of time, $p \leq 0.002$, $\eta_p^2 \geq 0.061$), with no differences between hormonal contraceptive groups (group × time interactions, $p \geq 0.412$, $\eta_p^2 \leq 0.016$; main effects of group, $p \geq 0.106$, $\eta_p^2 \leq 0.039$). There was a group × time interaction for PPm at the 38 % site ($p = 0.003$, $\eta_p^2 = 0.099$). PPm increased at the 38 % site in COCP ($p < 0.001$, $g = 0.84$) and DMPA ($p < 0.001$, $g = 1.00$) users, but not non-users ($p = 0.058$, $g = 0.25$). PPm at the 38 % site was not different between any groups at any time-point ($p \geq 0.181$). Training had no effect on trabecular area at the 14 % site or EPm at any site (main effects of time, $p \geq 0.053$, $\eta_p^2 \leq$

Table 2
Tibial density, geometry, and strength indices in women separated by hormonal contraceptive use in response to British Army basic training. Data are mean ± SD.

	COCP (n = 38)		DMPA (n = 21)		Non-users (n = 57)	
	Pre-training	Post-training	Pre-training	Post-training	Pre-training	Post-training
4 % site						
Trabecular vBMD ($mg•cm^{-3}$)	276 ± 25	280 ± 24[a]	276 ± 22	280 ± 21[a]	282 ± 29	287 ± 29[a]
Periosteal Perimeter (mm^{-1})	114.3 ± 6.3	114.4 ± 6.6	115.7 ± 7.1	115.9 ± 7.4	115.3 ± 7.6	115.6 ± 7.6
14 % site						
Trabecular vBMD ($mg•cm^{-3}$)	253 ± 19	252 ± 19	253 ± 16	254 ± 16	256 ± 22	258 ± 21[b]
Cortical vBMD ($mg•cm^{-3}$)	1147 ± 20	1148 ± 21	1145 ± 21	1143 ± 21	1150 ± 18	1150 ± 18
Total Area (mm^{-2})	392 ± 51	393 ± 51[a]	418 ± 74	419 ± 73[a]	406 ± 58	408 ± 57[a]
Trabecular Area (mm^{-2})	153 ± 49	154 ± 49	160 ± 57	160 ± 58	160 ± 53	162 ± 55
Cortical Area (mm^{-2})	141 ± 18	142 ± 19[a]	145 ± 14	147 ± 13[a]	150 ± 20	150 ± 20[a]
Cortical Thickness (mm^{-1})	1.79 ± 0.36	1.81 ± 0.37[a]	1.81 ± 0.32	1.83 ± 0.31[a]	1.90 ± 0.33	1.91 ± 0.33[a]
Periosteal Perimeter (mm^{-1})	72.3 ± 4.1	72.3 ± 4.1[a]	74.1 ± 6.2	74.2 ± 6.2[a]	73.2 ± 4.9	73.3 ± 4.8[a]
Endosteal Perimeter (mm^{-1})	60.7 ± 5.6	60.6 ± 5.7	62.6 ± 7.7	62.5 ± 7.7	61.0 ± 5.7	61.1 ± 5.7
Bone Strength Index ($g•cm^{-4}$)	1.76 ± 0.30	1.77 ± 0.30[a]	1.92 ± 0.39	1.94 ± 0.40[a]	1.92 ± 0.43	1.93 ± 0.43[a]
Stress-Strain Index (mm^{-3})	1251 ± 166	1260 ± 165[a]	1336 ± 187	1345 ± 187[a]	1337 ± 232	1344 ± 229[a]
38 % site						
Cortical vBMD ($mg•cm^{-3}$)	1185 ± 21	1184 ± 19	1188 ± 14	1187 ± 14	1187 ± 23	1188 ± 20
Total Area (mm^{-2})	326 ± 34	328 ± 33[a]	333 ± 35	335 ± 34[a]	340 ± 41	341 ± 41[a]
Cortical Area (mm^{-2})	247 ± 29	249 ± 29[a]	255 ± 27	257 ± 28[a]	263 ± 34[c]	265 ± 34[ac]
Cortical Thickness (mm^{-1})	4.38 ± 0.53	4.41 ± 0.52[a]	4.55 ± 0.49	4.58 ± 0.49[a]	4.65 ± 0.57	4.68 ± 0.57[a]
Periosteal Perimeter (mm^{-1})	68.9 ± 3.5	69.2 ± 3.5[b]	69.4 ± 4.1	69.7 ± 4.1[b]	70.6 ± 4.6	70.7 ± 4.5[b]
Endosteal Perimeter (mm^{-1})	37.5 ± 3.4	37.5 ± 3.4	37.3 ± 3.9	37.3 ± 4.0	37.3 ± 4.2	37.2 ± 4.1
Bone Strength Index ($g•cm^{-4}$)	2.24 ± 0.42	2.28 ± 0.43[a]	2.33 ± 0.47	2.36 ± 0.47[a]	2.48 ± 0.61	2.50 ± 0.61[a]
Stress-Strain Index (mm^{-3})	1490 ± 205	1509 ± 209[a]	1554 ± 241	1569 ± 246[a]	1616 ± 285	1629 ± 291[a]
66 % site						
Cortical vBMD ($mg•cm^{-3}$)	1155 ± 20	1154 ± 20	1151 ± 19	1152 ± 18	1150 ± 20	1151 ± 18
Total Area (mm^{-2})	451 ± 51	454 ± 51[a]	466 ± 52	467 ± 52[a]	480 ± 68	481 ± 68[a]
Cortical Area (mm^{-2})	260 ± 29	261 ± 28	269 ± 32	271 ± 31	273 ± 35	274 ± 35
Cortical Thickness (mm^{-1})	3.40 ± 0.41	3.42 ± 0.41[a]	3.50 ± 0.48	3.51 ± 0.48[a]	3.50 ± 0.47	3.53 ± 0.47[a]
Periosteal Perimeter (mm^{-1})	84.1 ± 4.7	84.2 ± 4.6	85.0 ± 5.1	85.2 ± 5.1	86.5 ± 5.9	86.6 ± 5.9
Endosteal Perimeter (mm^{-1})	58.5 ± 5.3	58.5 ± 5.4	58.8 ± 6.2	58.9 ± 6.2	60.3 ± 6.5	60.2 ± 6.4
Bone Strength Index ($g•cm^{-4}$)	4.02 ± 0.81	4.05 ± 0.80[a]	4.22 ± 0.84	4.27 ± 0.84[a]	4.46 ± 1.10	4.50 ± 1.09[a]
Stress-Strain Index (mm^{-3})	2082 ± 318	2091 ± 313[a]	2170 ± 338	2191 ± 336[a]	2239 ± 410	2254 ± 407[a]

COCP, combined oral contraceptive pill; DMPA, depot medroxyprogesterone acetate; vBMD, volumetric bone mineral density.
[a] $p < 0.05$ vs pre-training (main effect of time).
[b] $p < 0.05$ vs pre-training (post-hoc within group).
[c] $p < 0.05$ vs COCP users (main effect of group).

Table 3
Mean absolute difference and 95 % confidence intervals for tibial density, geometry, and strength indices in women separated by hormonal contraceptive use in response to British Army basic training.

	COCP (n = 38)		DMPA (n = 21)		Non-users (n = 57)	
	Difference	95 % CI	Difference	95 % CI	Difference	95 % CI
4 % site						
Trabecular vBMD (mg•cm^{-3})	5	3, 6	4	2, 6	4	3, 6
Periosteal Perimeter (mm^{-1})	0.1	−0.3, 0.5	0.2	−0.4, 0.8	0.3	−0.2, 0.7
14 % site						
Trabecular vBMD (mg•cm^{-3})	−1	−3, 4	0	−2, 3	2	0, 4
Cortical vBMD (mg•cm^{-3})	0	−2, 3	−2	−5, 1	0	−2, 3
Total Area (mm^{-2})	1	−1, 2	1	−1, 3	2	1, 3
Trabecular Area (mm^{-2})	1	−1, 3	1	−2, 3	2	0, 4
Cortical Area (mm^{-2})	1	0, 2	1	0, 2	1	0, 1
Cortical Thickness (mm^{-1})	0.02	0.00, 0.03	0.02	0.00, 0.04	0.01	0.00, 0.02
Periosteal Perimeter (mm^{-1})	0.0	−0.1, 0.1	0.1	0.0, 0.3	0.1	0.0, 0.2
Endosteal Perimeter (mm^{-1})	−0.1	−0.3, 0.0	−0.1	−0.4, 0.1	0.1	−0.1, 0.2
Bone Strength Index (g•cm^{-4})	0.01	0.00, 0.03	0.02	0.00, 0.04	0.01	0.00, 0.02
Stress-Strain Index (mm^{-3})	9	3, 15	9	2, 17	7	2, 12
38 % site						
Cortical vBMD (mg•cm^{-3})	0	−2, 1	−1	−3, 1	2	0, 4
Total Area (mm^{-2})	3	1, 4	2	0, 5	1	0, 3
Cortical Area (mm^{-2})	2	1, 3	2	1, 4	1	0, 3
Cortical Thickness (mm^{-1})	0.04	0.01, 0.06	0.03	0.00, 0.06	0.03	0.01, 0.05
Periosteal Perimeter (mm^{-1})	0.3	0.2, 0.4	0.3	0.2, 0.5	0.1	0.0, 0.2
Endosteal Perimeter (mm^{-1})	−0.1	−0.2, 0.1	0.0	−0.2, 0.2	−0.1	−0.2, 0.0
Bone Strength Index (g•cm^{-4})	0.04	0.03, 0.05	0.03	0.01, 0.06	0.02	0.01, 0.03
Stress-Strain Index (mm^{-3})	19	11, 26	15	3, 27	13	5, 21
66 % site						
Cortical vBMD (mg•cm^{-3})	−1	−4, 1	1	−1, 3	0	−1, 2
Total Area (mm^{-2})	3	1, 5	1	−1, 2	1	−1, 2
Cortical Area (mm^{-2})	1	0, 2	2	1, 3	2	1, 3
Cortical Thickness (mm^{-1})	0.02	0.00, 0.04	0.00	−0.02, 0.02	0.03	0.01, 0.04
Periosteal Perimeter (mm^{-1})	0.1	0.0, 0.3	0.2	0.0, 0.4	0.1	0.0, 0.2
Endosteal Perimeter (mm^{-1})	−0.1	−0.2, 0.1	0.1	−0.1, 0.4	−0.1	−0.3, 0.0
Bone Strength Index (g•cm^{-4})	0.03	0.01, 0.04	0.05	0.02, 0.07	0.04	0.02, 0.06
Stress-Strain Index (mm^{-3})	9	2, 15	21	10, 32	15	7, 24

CI, confidence interval; COCP, combined oral contraceptive pill; DMPA, depot medroxyprogesterone acetate; vBMD, volumetric bone mineral density.

0.033), with no differences between hormonal contraceptive groups (group × time interactions, $p \geq 0.062$, $\eta_p^2 \leq 0.048$; main effects of group, $p \geq 0.347$, $\eta_p^2 \leq 0.019$).

3.3. Bone strength

Training increased BSI and SSI at all sites (main effects of time, $p < 0.001$, $\eta_p^2 \geq 0.162$) with no differences between hormonal contraceptive groups (group × time interactions, $p \geq 0.152$, $\eta_p^2 \leq 0.025$; main effects of group, $p \geq 0.075$, $\eta_p^2 \leq 0.045$).

3.4. Biochemical markers of bone and calcium metabolism

Biochemical markers of bone metabolism can be seen in Figs. 2 to 3. Sclerostin, OPG, sRANKL, and OPG:sRANKL were log transformed following visual inspection of the distribution of the residuals. There was a group × time interaction for PINP ($p < 0.001$, $\eta_p^2 = 0.069$). PINP was higher in weeks 2, 4, 6, 10, and 14 than week 1 for non-users ($p < 0.001$, $g \geq 0.57$), COCP users ($p < 0.001$, $g \geq 0.75$), and DMPA users ($p \leq 0.008$, $g \geq 0.51$). PINP was not different between groups at any time-point ($p \geq 0.082$). There was a main effect of time for βCTX, bone ALP, osteocalcin, and sclerostin ($p < 0.001$, $\eta_p^2 \geq 0.060$), but no difference between hormonal contraceptive groups (group × time interaction, $p \geq 0.104$, $\eta_p^2 \leq 0.030$; main effect of group, $p \geq 0.112$, $\eta_p^2 \leq 0.038$). βCTX was lower in week 2, 4, and 6 than week 1 ($p \leq 0.001$, $g \geq 0.39$), with weeks 10 and 14 not different from week 1 ($p \geq 0.157$, $g \leq 0.19$). Bone ALP increased from week 1 to week 14 ($p = 0.005$, $g = 0.23$), but weeks 2, 4, 6, and 10 were not different from week 1 ($p \geq 0.066$, $g \leq 0.29$). Osteocalcin was lower in week 2 and 4 than week 1 ($p \leq 0.010$, $g \geq 0.29$) and higher in weeks 10 and 14 than week 1 ($p \leq 0.010$, $g \geq 0.26$), with week 1 and 6 not different ($p = 0.182$, $g = 0.13$). Sclerostin increased from week 1 to week 14 ($p = 0.008$, $g = 0.16$), but weeks 2, 4, 6, and 10 were not different from week 1 ($p \geq 0.061$, $g \leq 0.17$). There was a main effect of time ($p < 0.001$, $\eta_p^2 = 0.083$) and group ($p = 0.004$, $\eta_p^2 = 0.092$) for OPG, but no group × time interaction ($p = 0.478$, $\eta_p^2 = 0.018$). OPG was higher in weeks 2, 4, 6, and 10 than week 1 ($p \leq 0.014$, $g \geq 0.30$), but weeks 1 and 14 were not different ($p = 0.156$, $g = 0.13$). OPG was higher in COCP users than non-users ($p = 0.003$), but DMPA users were not different to non-users and COCP users ($p \geq 0.173$). Training had no effect on sRANKL or OPG:sRANKL (main effects of time, $p \geq 0.111$, $\eta_p^2 \leq 0.015$, group × time interactions, $p \geq 0.583$, $\eta_p^2 \leq 0.016$). There was a main effect of group for sRANKL ($p = 0.022$, $\eta_p^2 = 0.066$), but not for OPG:sRANKL ($p = 0.055$, $\eta_p^2 = 0.051$). sRANKL was lower in DMPA users than non-users ($p = 0.024$), but COCP users were not different to DMPA users or non-users ($p \geq 0.227$).

Biochemical markers of calcium metabolism can be seen in Fig. 4. There was a group × time interaction for iPTH ($p = 0.027$, $\eta_p^2 = 0.038$). iPTH was higher in weeks 4 and 6 than week 1 in non-users ($p < 0.001$, $g \geq 0.52$), with weeks 2, 10, and 14 not different from week 1 ($p \geq 0.304$, $g \leq 0.26$); there was no change in iPTH for COCP users ($p \geq 0.059$, $g \leq 0.38$) or DMPA users ($p \geq 0.524$, $g \leq 0.40$). iPTH was higher in non-users than COCP users at week 4 ($p = 0.005$). There was a group × time interaction for total 25(OH)D ($p = 0.024$, $\eta_p^2 = 0.053$). Total 25(OH)D was lower in week 6 than week 1 for non-users ($p = 0.048$, $g = 0.47$), but week 1 and week 14 were not different ($p = 0.259$, $g = 0.12$). Total 25(OH)D was lower in week 6 and 14 than week 1 for COCP users ($p \leq 0.003$, $g \geq 0.49$), but did not change in DMPA users ($p \geq 0.538$, $g \leq 0.20$). Total 25(OH)D was higher in COCP users than non-users and DMPA users at week 1 ($p \leq 0.004$), and non-users at week 6 ($p = 0.001$). There was a main effect of time for albumin-adjusted calcium and phosphate ($p < 0.001$, $\eta_p^2 \geq 0.053$), but no difference between hormonal contraceptive groups (group × time interaction, $p \geq 0.698$, $\eta_p^2 \leq 0.014$;

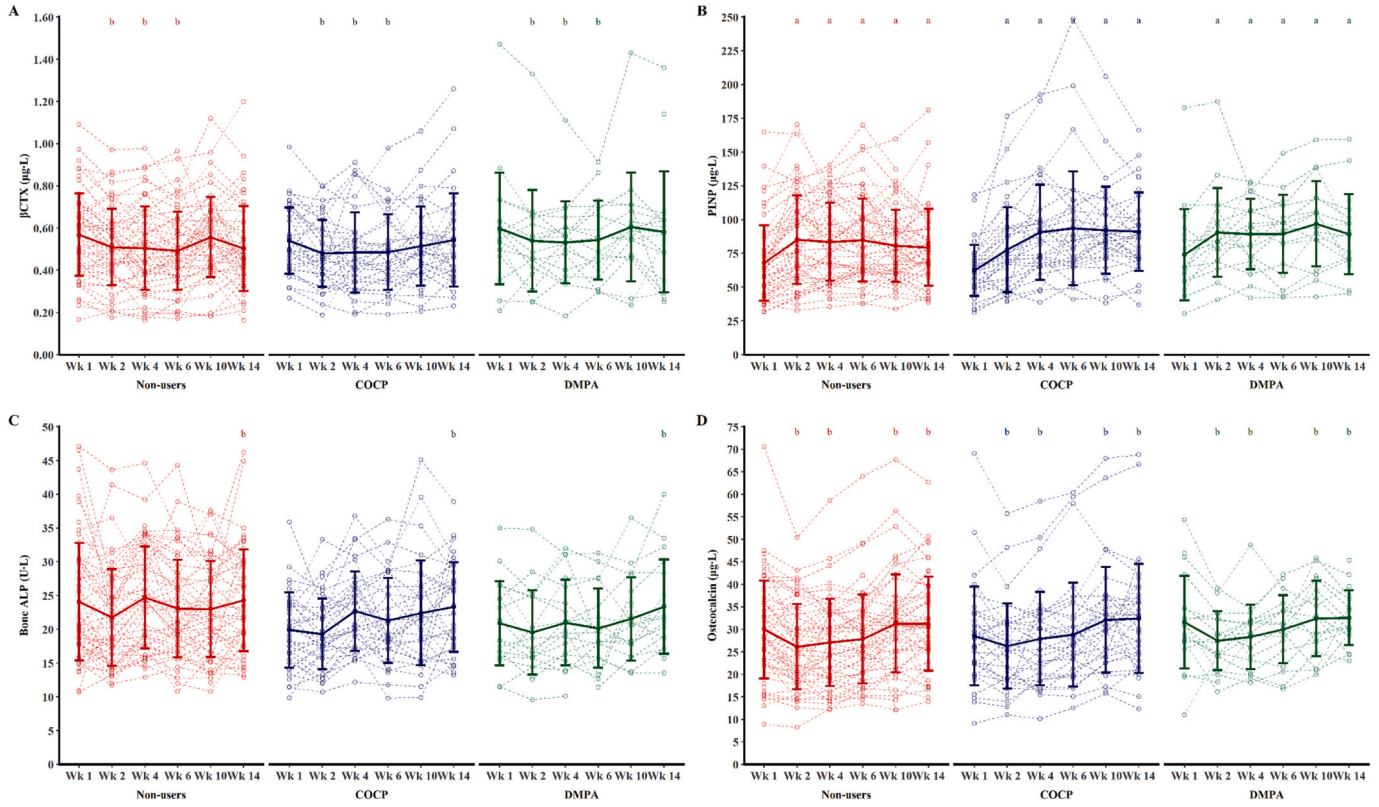

Fig. 2. Biochemical markers of bone resorption and formation during 14 weeks British Army basic training in non-users, combined oral contraceptive pill (COCP) users, and depot medroxyprogesterone acetate (DMPA) users. Data are mean ± standard deviation with individual data. βCTX, c-terminal cross-links telopeptide of type 1 collagen; Bone ALP, bone-specific alkaline phosphatase; PINP, procollagen I N-terminal propeptide.
[a]$p < 0.05$ vs week 1 (post-hoc within group); [b]$p < 0.05$ vs week 1 (main effect of time).

main effect of group, $p \geq 0.251$, $\eta_p^2 \leq 0.025$). Albumin-adjusted calcium decreased from week 1 to week 6 ($p = 0.001$, $g = 0.36$), with weeks 2, 4, 10, and 14 not different from week 1 ($p \geq 0.065$, $g \leq 0.22$). Phosphate was higher in weeks 2, 10, and 14 than week 1 ($p \leq 0.002$, $g \geq 0.26$), but weeks 4 and 6 were not different to week 1 ($p \geq 0.151$, $g \leq 0.20$).

4. Discussion

This study compared tibial adaptations and biochemical markers of bone and calcium metabolism between women using different hormonal contraceptives and women not using any hormonal contraceptives during 14 weeks of basic military training. Basic military training is physically demanding [25,28,29], consists of high volumes of weight-bearing activities like load carriage [25,28] and military drill [30,31], and puts recruits at risk of lower limb stress fractures [32]. Training increased trabecular density at the distal tibia, and bone size and estimated bone strength across the length of the tibia. Hormonal contraceptive use was associated with blunted adaptation of trabecular density (at the 14 % site) but increased periosteal expansion. Hormonal contraceptive use also had effects on the bone and calcium metabolic response to military training. Several studies have observed changes in tibial geometry and density within 14 weeks of military training in men and women [13–17,20], but the effects of hormonal contraceptives on tibial adaptations to military training are poorly understood. The measurement of tibial structure across four skeletal sites and comprehensive assessment of bone and calcium metabolic markers provide new insight into the effect of hormonal contraceptive use on the mechanobiology of bone.

4.1. Volumetric bone mineral density

Training increased trabecular vBMD at the 4 % site (1.6 % increase), with no differences between hormonal contraceptive groups. An increase in trabecular vBMD is an early adaptation to mechanical loading [33] and improves resistance to the compressive forces at the metaphysis [34]. This new bone formation is likely the result of physically demanding weight-bearing activities like running, resistance exercise, load carriage, circuit training, obstacle courses, and military drill [25,28,30,31]. Previous pQCT [13,15] and HR-pQCT [14,16] studies have shown similar increases (0.9 to 2.0 %) in trabecular vBMD at the tibial metaphysis following 8 to 13 weeks of military training in men and women, with larger increases (3.0 %) following 44 weeks military training in women [19]. This increase in trabecular vBMD is likely due to adaptation of the trabecular microarchitecture [16,19,20]. Trabecular vBMD increased at the 14 % site in non-users (0.8 %), but not COCP or DMPA users, demonstrating blunted trabecular adaptation with hormonal contraceptive use. The very high compressive forces at the 4 % site may have masked any effects of hormonal contraceptives. The similar height, body mass, and 2.4 km run time between groups suggests the lack of trabecular adaptation in COCP and DMPA users was not due to differences in baseline aerobic fitness or body size, which might influence loading. Depot medroxyprogesterone acetate is a progestin-only contraceptive, whereas COCPs provide synthetic (ethinyl) oestradiol and progestins. Hormonal contraceptives suppress the hypothalamic-pituitary-ovarian axis and decrease endogenous oestradiol [4,5]. Progestin-only contraceptive use has been associated with blunted trabecular microarchitecture adaptations to military training [19]. Transdermal oestradiol increased trabecular number over 12 months of training to a greater extent than the COCP in young oligomenorrhoeic athletes, although there were no differences between oestradiol and no

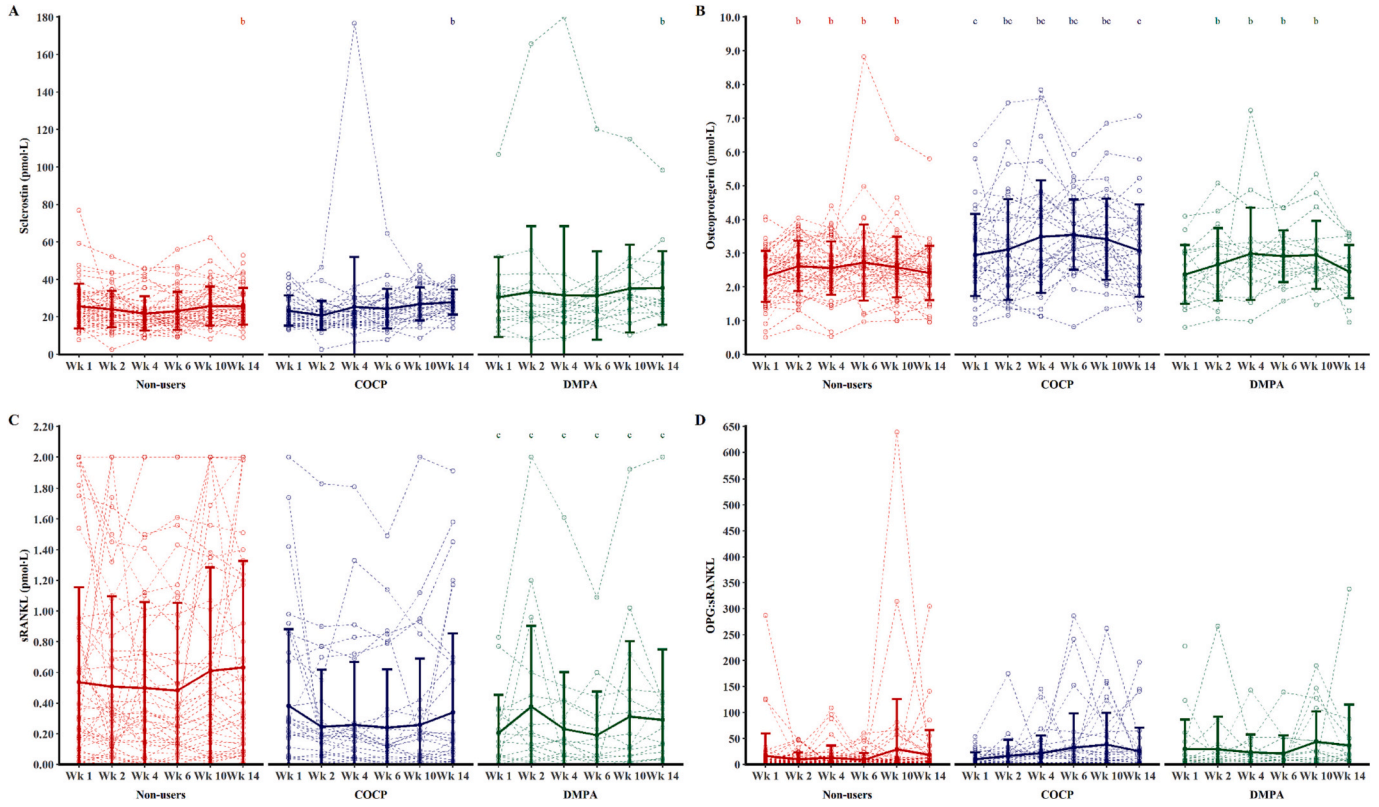

Fig. 3. Biochemical markers of bone metabolism during 14 weeks British Army basic training in non-users, combined oral contraceptive pill (COCP) users, and depot medroxyprogesterone acetate (DMPA) users. Data are mean ± standard deviation with individual data.
OPG, osteoprotegerin; sRANKL, soluble receptor activator of nuclear factor kappa B ligand.
[a]$p < 0.05$ vs week 1 (post-hoc within group); [b]$p < 0.05$ vs week 1 (main effect of time); [c]$p < 0.05$ vs non-users (main effect of group).

treatment suggesting an inhibitory effect of COCPs [35]. Low oestradiol increases the rate of remodelling on the trabecular surfaces [2,36], can result in trabecular thinning [37], and can impair the adaptive response to loading through actions on the ERα in trabecular bone [1,38]. These data suggest hormonal contraceptives inhibit trabecular adaptation to loading, likely by suppressing endogenous oestradiol.

Training had no effect on cortical vBMD, with no differences between hormonal contraceptive groups. Data on cortical vBMD adaptations to military training are inconsistent. Previous pQCT data show increased (0.1 to 0.5 %) [13,15,18] or unchanged [17] cortical vBMD across the 14 %, 38 %, and 66 % sites in men and women. High-resolution pQCT studies provide some evidence of a sex difference; increased cortical vBMD at the 4 % site (0.6 to 0.9 %) in men [14] and decreased cortical vBMD at the 4 % site (−0.3 %) and the 30 % site (−0.7 to −0.3 %) in women [16,19] have been observed. A direct comparison of men and women found greater increases in cortical vBMD at the 4 % site in men than women (0.6 vs 0.4 %) [20]. The tibial diaphysis experiences high bending and torsion stresses during locomotion [39] and an increase in cortical vBMD increases stiffness and susceptibility to microdamage [40,41]. Cortical vBMD is higher for women than men across the length of the tibia [18,20], and so cortical vBMD may be less likely to increase in women to prevent increasing susceptibility to microdamage. Low oestradiol does not affect the response of cortical bone to loading in animals [1], with evidence in humans that trabecular, but not cortical, bone is impacted by low oestradiol [6,7]. Oestrogens act differently at trabecular and cortical sites [1], and trabecular bone may be more susceptible to low oestradiol.

4.2. Geometry

Training increased total area (0.3 to 0.6 %), cortical thickness (0.6 to 0.8 %), and cortical area (0.6 to 0.8 %) at all sites, with no difference in the training response between hormonal contraceptive groups. The increased periosteal perimeter circumference at the 14 % and 66 % sites (0.1 to 0.2 %) and unchanged endosteal perimeter circumference demonstrates that the increase in cortical thickness and area were due to periosteal expansion, consistent with previous military training pQCT studies in men [13]. Periosteal expansion and an increase in cross-sectional area improves strength and resistance to bending during weight bearing activity as the tibial cortex is placed further from the neutral axis [2,3]. Adaptations to geometry following loading provide a greater contribution to increased strength than increases in density [42] and likely contributed to the training-induced increase in estimated strength (0.7 to 1.0 % across the tibia length). The periosteal perimeter increased at the 38 % site in COCP (0.4 %) and DMPA (0.5 %) users, but not in non-users. No change in periosteal perimeter in non-users could be due to the inhibition of periosteal expansion by endogenous oestradiol [2,3], whereas endogenous oestradiol was suppressed in hormonal contraceptive users; the effect of ethinyl oestradiol on the loading response is not clear. Cortical area at the 38 % site was, however, higher in non-users compared with COCP users at baseline, which may result in greater potential for periosteal expansion adaptation in COCP users. These data are also consistent with mechanical loading being the dominant stimulus in tibial adaptations compared with any effect of sex hormones.

4.3. Biochemical markers of bone and calcium metabolism

The bone resorption marker βCTX decreased in the early part of training (week 2, 4, and 6), with no differences between hormonal contraceptive groups. The decrease in βCTX demonstrates that training decreased type I collagen degradation by osteoclasts, consistent with

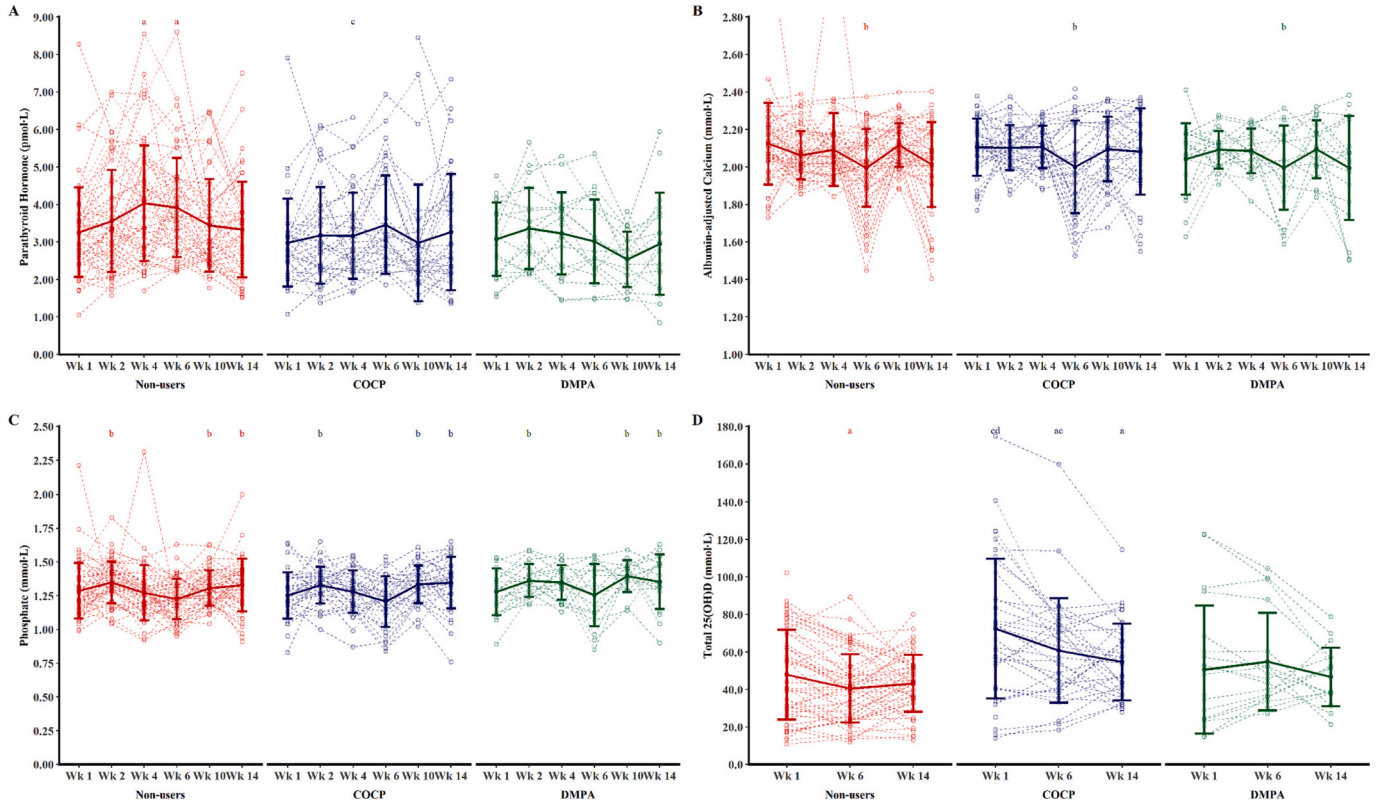

Fig. 4. Biochemical markers of calcium metabolism during 14 weeks British Army basic training in non-users, combined oral contraceptive pill (COCP) users, and depot medroxyprogesterone acetate (DMPA) users. Data are mean ± standard deviation with individual data.
Total 25(OH)D, total 25-hydroxyvitamin D. Albumin-adjusted calcium y-axis is truncated at 2.80 mmol•L^{-1} for clarity.
[a]$p < 0.05$ vs week 1 (post-hoc within group); [b]$p < 0.05$ vs week 1 (main effect of time); [c]$p < 0.05$ vs non-users (post-hoc sex × time interaction); [d]$p < 0.05$ vs DMPA users (post-hoc sex × time interaction).

other military training studies over 8 to 16 weeks [14,17,43]; although increases in βCTX have also been reported [44,45]. Comparisons between military studies must be made with caution due to differences in timing and method of analysis of samples, and differences in training course duration and demands (e.g., exercise intensity, nutrition, sleep deprivation). The decrease in βCTX is consistent with a change in remodelling leading to adaptive bone formation [13,14,16]. Training increased OPG—consistent with a decrease in bone resorption—but sRANKL and OPG:sRANKL did not change with training, however many sRANKL measurements were below the limit of detection. Soluble receptor activator of nuclear factor kappa B ligand is essential for osteoclast functioning by promoting osteoclast differentiation and action, and preventing apoptosis, whereas OPG acts as a decoy receptor competitively binding to and inhibiting sRANKL activity [46]. The COCP is associated with decreased, and DMPA with increased, markers of bone resorption and formation [4,23]. Osteoprotegerin was higher in COCP users than non-users—likely due to the ethinyl oestradiol increasing OPG expression [47]—and could contribute to lower bone resorption. Similarly, sRANKL was lower in DMPA users than non-users; low oestradiol can increase sRANKL [48] and so it is not clear why sRANKL was lower in DMPA users, however, OPG:sRANKL was not different between groups and the sRANKL assay may be too insensitive to detect robust differences between groups. It is unclear why bone resorption and formation markers were not different between groups, however, not all non-users were eumenorrheic, measurements were not standardised around the menstrual cycle or contraceptive use, and high levels of physical activity may mask differences between groups.

Markers of bone formation—PINP and bone ALP—increased during training, consistent with the new bone formation measured by pQCT. Procollagen type I N-terminal propeptide is a marker of type I collagen synthesis [49] and PINP increased early in training and remained increased. Bone ALP is a marker of osteoblast activity and mineralisation [49] and increased late in training. Early type I collagen formation with later mineralisation is consistent with adaptive bone formation [12]. Osteocalcin—a bone matrix protein synthesised by mature osteoblasts [49]—decreased early in training before increasing later in training, but osteocalcin may not be a sensitive indicator of bone formation with exercise [50]. Military training studies report increased [43–45] or unchanged [14–17] PINP, increased [15,16,44,45] or unchanged [17] bone ALP, and decreased [15,17] or unchanged [16] osteocalcin in men and women. Changes in markers of bone formation were largely similar between hormonal contraceptive groups. The COCP decreases insulin-like growth factor I [51], an important regulator of bone formation, but we did not find an association between COCP use and decreased bone formation markers. Sclerostin—a glycoprotein secreted by osteocytes—increased from week 1 to week 14. Military studies report decreased sclerostin [16–18] with downregulation of sclerostin with loading promotes bone formation through disinhibition of the Wnt signalling pathway [52]. We observed an increase in sclerostin despite evidence of bone formation, but an increase in bone mass may have contributed to increased sclerostin secretion by osteocytes and a decrease in oestradiol during training can increase sclerostin [53]. The relationship between oestradiol, sclerostin, and bone formation markers during mechanical loading is not clear [53], and requires further investigation.

Parathyroid hormone increased early in training in non-users, but there was no change in iPTH for COCP or DMPA users. Parathyroid hormone secretion is regulated by serum ionized calcium and continuous increased iPTH stimulates bone resorption to mobilise calcium, whereas intermittent increases in iPTH are osteogenic [54]. Albumin-

adjusted calcium decreased and phosphate increased during training—which both stimulate iPTH production and release and likely explain the increase in iPTH—with no difference between hormonal contraceptive groups. Exercise acutely decreases ionized calcium and increases phosphate and iPTH production [55,56], but a decrease in calcium could also be due to reduced bone resorption and increased calcium uptake into bone during bone formation. Total 25(OH)D decreased for non-users and COCP users, but total 25(OH)D did not change in DMPA users. Total 25(OH)D was higher in COCP users, likely due to exogenous ethinyl oestradiol [23,57], which increases vitamin D binding protein [57,58]. The similar total 25(OH)D at the end of training between groups might be due to the standardisation of vitamin D production due to identical geographical location, clothing, and outdoor activity. It is also possible the decrease in total 25(OH)D in non-users was due to a decline in oestradiol with training, but the unchanged total 25(OH)D in DMPA users was because this group already had lower overall oestradiol [23]; we did not measure oestradiol in this study and cannot confirm this supposition. The active 25(OH)D metabolite—1,25 dihydroxyvitamin D ($1,25(OH)_2D$)—provides negative feedback on iPTH secretion [54]. Oestradiol can increase calcium absorption from the gut and renal calcium resorption—resulting in suppressed iPTH [37]—and can modulate the end organ effects of $1,25(OH)_2D$ and iPTH [36]. An increase in iPTH increases bone resorption, predominantly in cortical bone, by stimulating sRANKL and inhibiting OPG [54], which may explain the lack of periosteal expansion in non-users. In support of this supposition, supplementation with calcium and vitamin D prevents the increase in iPTH during military training and augments increases in cortical thickness [15], but the anabolic and catabolic actions of iPTH are complex [54].

4.4. Limitations

The attrition during military training resulted in some loss to follow-up and our data are subject to survivor bias. We did not measure sex steroid hormones, which would help further explain some of our findings. Our data are observational—not from a randomised controlled trial—and so our data are at risk of bias. These data were exploratory, so we did not control for multiple comparisons and the likelihood of type I error must be considered when interpreting these data. The nature of military training meant we were unable to standardise measurements around the menstrual cycle or contraceptive use. Dietary intake data were not recorded and energy, calcium, and vitamin D intake can affect the skeletal response to training. Ethnicity was not recorded yet can affect bone adaptation to military training [20]. Finally, our data are limited by the low number of women per hormonal contraceptive group, and wide variability within each group in length of contraceptive use and contraceptive preparation.

5. Conclusion

Arduous weight bearing exercise in the form of basic military training increased the density, size, and *estimated* strength of the tibia. These adaptations were similar between women using different hormonal contraceptives, however, hormonal contraceptive use was associated with inhibited adaptation of trabecular bone but increased periosteal expansion, consistent with the effects of lower endogenous oestradiol.

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CRediT authorship contribution statement

Thomas J. O'Leary: Writing – original draft, Visualization, Software, Formal analysis. **Rachel M. Izard:** Writing – review & editing, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jonathan C.Y. Tang:** Writing – review & editing, Methodology, Data curation. **William D. Fraser:** Writing – review & editing, Methodology, Data curation. **Julie P. Greeves:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors have no conflicts of interest relevant to this manuscript.

Data availability

Data will be made available on request.

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