Tibial macrostructure and microarchitecture adaptations in women during 44-weeks of arduous military training

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Running Title: Female soldier bone health

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Competing Interests

The authors have no competing interests to declare.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Abstract

Bone adapts to unaccustomed, high-impact loading but loses mechanosensitivity quickly. Short periods of military training (≤ 12 weeks) increase the density and size of the tibia in women. The effect of longer periods of military training, where the incidence of stress fracture is high, on tibial macrostructure and microarchitecture in women is unknown. This observational study recruited fifty-one women (aged 19 to 30 years) at the start of 44-weeks of British Army Officer training. Tibial volumetric bone mineral density (vBMD), geometry, and microarchitecture were measured by HR-pQCT. Scans of the right tibial metaphysis (4% site) and diaphysis (30% site) were performed at weeks 1, 14, 28, and 44. Measures of whole-body areal bone mineral density (aBMD) were obtained using DXA. Blood samples were taken at weeks 1, 28, and 44, and analysed for markers of bone formation and resorption. Trabecular vBMD increased from week 1 to 44 at the 4% site (3.0%, $P < 0.001$). Cortical vBMD decreased from week 1 to 14 at the 30% site (−0.3%, $P < 0.001$). Trabecular area decreased at the 4% site (−0.4%); trabecular bone volume fraction (3.5%), cortical area (4.8%), and cortical thickness (4.0%) increased at the 4% site; and, cortical perimeter increased at the 30% site (0.5%) from week 1 to 44 ($P \leq 0.005$). Trabecular number (3.5%) and thickness (2.1%) increased, and trabecular separation decreased (−3.1%), at the 4% site from week 1 to 44 ($P < 0.001$). Training increased failure load at the 30% site from week 1 to 44 (2.5%, $P < 0.001$). Training had no effect on aBMD or markers of bone formation or resorption. Tibial macrostructure and microarchitecture continued to adapt across 44-weeks of military training in young women. Temporal decreases in cortical density support a role of intracortical remodelling in the pathogenesis of stress fracture.

Key words: Bone Modeling and Remodeling; DXA; Exercise; HR-pQCT; Nutrition.

Introduction

Changes in bone morphology and density at the weight-bearing tibia are observed within 13 weeks of dynamic and high-impact loading during military training.[1-5] Tibial density, cortical thickness, periosteal perimeter, and estimated mechanical strength are increased after 8 to 13 weeks of basic military training.[1-5] The sudden increase in mechanical loading with military training can overload bone and lead to stress fractures through remodelling of fatigue damage.[6] Stress fractures at the hip, tibia and metatarsals are most commonly presented by military recruits, reflecting sites of highest mechanical stress.[7-10] Women are typically at three-fold greater risk of stress fracture than men in basic military training,[9] but this risk increases to more than 6-fold as training intensity and duration increases.[11] Studies of tibial adaptation in women have focussed on basic military training of ≤ 12 weeks;[2,3,5] measuring the tibial response to prolonged arduous training may provide important insight into the aetiology of stress fracture.

High-resolution peripheral quantitative computed tomography (HR-pQCT) assesses bone microarchitecture. Trabecular microarchitecture and cortical porosity are important contributors to mechanical strength,[12-16] and, therefore, HR-pQCT offers important insight in determining fracture risk.[17,18] There are few prospective longitudinal HR-pQCT studies examining the response of bone macrostructure and microarchitecture to exercise training in humans. Eight weeks of US Army basic training increased cortical thickness, trabecular volumetric bone mineral density (vBMD), trabecular thickness, and trabecular number at the tibial metaphysis in women.[3] Training also decreased cortical vBMD at the tibial metaphysis and diaphysis, consistent with intracortical remodelling.[3] Conversely, 13-weeks British Army basic training increased cortical vBMD, and also trabecular vBMD, cortical thickness, and cortical area at the tibial metaphysis in men.[4] Differences in cortical vBMD responses between

these studies may underpin sex differences in susceptibility to fracture or could be due to differences in the length of training. Data are lacking on the temporal pattern of tibial adaptations to longer periods of military training in women but would aid our understanding of mechanobiology of bone and stress fractures.

This observational study examined the tibial macrostructure and microarchitecture in women undergoing the 44-week British Army Officer Commissioning Course. The Officer Commissioning Course is the most arduous and prolonged basic military training course in the British Army and is characterised by a high incidence of lower limb stress fractures in women (11.4%).[11] Secondary aims were to examine changes in areal bone mineral density (aBMD) and biochemical markers of bone resorption and bone formation. We also compared women by hormonal contraceptive use in exploratory analyses because of the reported effects of some contraceptives on the hypothalamic pituitary ovarian axis and bone metabolism;[19] the effects of hormonal contraceptives on mechanotransduction is not clear.

Materials and Methods

Participants

All women starting British Army Officer training between May 2017 and January 2018 were invited to take part in this study. All participants were recruited during their pre-course instructional briefing held 6 to 20 weeks before starting the 44-week British Army Officer Commissioning Course at the Royal Military Academy, Sandhurst, United Kingdom. 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; or musculoskeletal injury. All participants passed an initial military medical assessment and were confirmed injury free and

medically fit to train. Each participant had the study procedures and risks fully explained verbally and in writing before providing written informed consent. This study was approved by the Ministry of Defence Research Ethics Committee (Ref: 790/MoDREC/16).

Study Design

All participants were undergoing the 44-week British Army Officer Commissioning Course. The Officer Commissioning Course is a 44-week basic military training course comprising three 14-week terms. Each term is separated by 2 or 3 weeks of leave with 2 weeks of adventure training after the second term. The Officer Commissioning Course teaches soldiering skills and military leadership, and is physically[20] and psychologically[21] arduous. Officer Cadets complete aerobic endurance training, strength and conditioning, military specific fitness training (obstacle courses, circuit training), military drill, progressive loaded marching, learn basic military skills (weapon handling), and complete several arduous field exercises. Officer Cadets wear trainers for physical training, drill shoes for military drill, and military boots for all other activities. We have reported energy expenditure and activity levels during training in these same women.[20] Total daily energy expenditures — measured by doubly labelled water over 10 days — were 3332 ± 424, 3849 ± 363, and 3041 ± 286 $kcal \cdot d^{-1}$ during weeks 9 to 10, weeks 19 to 20, and weeks 35 to 36. Energy expenditures from moderate and vigorous physical activity — estimated using wrist-worn accelerometery — were 1865 ± 312, 2253 ± 536, and 1513 ± 336 $kcal \cdot d^{-1}$ during weeks 9 to 10, weeks 19 to 20, and weeks 35 to 36. We have previously reported the menstrual function of our participants not using hormonal contraceptives; 25%, 65%, and 43% of women experienced oligomenorrhoea or amenorrhoea between weeks 1 and 14, weeks 15 and 28, and weeks 29 and 44.[22]

Tibial vBMD, geometry, and microarchitecture were measured by HR-pQCT at the start of training (week 1) and at the end of each term (week 14, 28, and 44). At the same timepoints, whole-body DXA scans were obtained for the assessment of aBMD and body composition. Blood samples were drawn at week 1, 28, and 44 for analysis of bone formation and bone resorption. Contraceptive use during training was determined by questionnaire at the beginning of training and the end of each term. Women were grouped as: i) combined oral contraceptive pill (COCP) users (*e.g.*, Microgynon 30); ii) progestogen-only contraceptive (POC) users, including the progestogen-only pill (*e.g.*, Micronor), implant (*e.g.*, Nexplanon), and injection (*e.g.*, depot medroxyprogesterone acetate); iii) no hormonal contraception (non-users), and; iv) other (intrauterine device, changed contraceptive during training, or unknown).

Tibial Volumetric Bone Mineral Density, Geometry, and Microarchitecture

A three-dimensional HR-pQCT system (XtremeCT II, Scanco Medical AG, Switzerland) was used to assess vBMD, geometry, and microarchitecture of the right tibia. The tibial adaptation to basic military training is not dependent on leg dominance.[4] A three-dimensional representation of 10.2 cm of the right tibia in the axial direction, at both the metaphysis (4% site) and diaphysis (30% site), were obtained from 168 CT slices with an isotropic voxel size of 61 μm. Tibial length was measured before the first scan in week 1, taken as the distance between the medial malleolus and the tibial end plate. The leg of each participant was fitted into a carbon fibre shell and immobilised within the gantry of the scanner for the duration of the scan. A reference line was placed at the tibial endplate, with the first CT slice taken 4% and 30% of the tibia length from the reference line. For follow-up measurements at the 4% site, automatic algorithms matched the volumes of interest between baseline and follow-up scans using the cross-sectional area within the periosteal boundary, so only the bone volume common to the baseline scans were assessed;[23] only scans with a common region of ≥ 80% were

included in the analyses. Of an initial 168 slices, on average 152 ± 8 (134 to 166) slices were analysed on follow-up. The automated matching algorithms were disabled for analysis at the 30% site.[3,24] Daily quality control scans were performed using the manufacturer-issued phantom that contained rods of hydroxyapatite (HA). The quality of each HR-pQCT scan was reviewed by a single operator and any scans judged to be of poor quality, as per the manufacturer visual grading of image quality, were excluded from the analyses; one baseline scan was re-performed. The methods used to process the data have been previously described.[23,25,26] The standard evaluation procedure provided by the manufacturer was used to derive: total vBMD (mg HA·cm^3), trabecular vBMD (mg HA·cm^3), cortical vBMD (mg HA·cm^3), trabecular area (mm^2), trabecular bone volume fraction (%), cortical area (mm^2), cortical thickness (mm), trabecular thickness (mm), trabecular number (1·mm), trabecular separation (mm), cortical porosity (%) and cortical pore diameter (mm). Micro-finite element analysis was performed to calculate stiffness [kN·mm] and failure load [kN] under uniaxial compression.[27] These outcomes are sensitive to changes following shorter periods of military training[3,4] and are recommended as commonly reported HR-pQCT outcomes to describe bone.[28] All evaluations were performed by a single investigator to ensure consistency of periosteal and endosteal contouring. The coefficient of variations (CV) and least significant changes (LSC)[29] for this HR-pQCT at the 4% site are 0.2% CV and 0.5% LSC for total vBMD, 0.4% CV and 1.0% LSC for trabecular vBMD, 0.9% CV and 2.4% LSC for cortical vBMD, ≤ 1.3% CV and ≤ 3.3% LSC for geometry, ≤ 2.1% CV and ≤ 5.8% LSC for trabecular microarchitecture, 7.8% CV and 21.9% LSC for cortical porosity, and ≤ 3.2% CV and ≤ 9.0% LSC for stiffness and failure load (unpublished data from our laboratory). The CV and LSC at the 30% site are 0.3% CV and 0.9% LSC for total vBMD, 0.2% CV and 0.4% LSC for cortical vBMD, ≤ 0.8% CV and ≤ 2.3% LSC for geometry, 4.9% CV and 13.8% LSC for cortical

porosity, and ≤ 0.7% CV and ≤ 2.0% LSC for stiffness and failure load (unpublished data from our laboratory).

Body Composition

Whole-body aBMD, lean mass, and fat mass was assessed using DXA (Lunar iDXA, GE Healthcare, UK) with participants wearing shorts and a t-shirt. Regional analysis of aBMD for the arms, legs, trunk, pelvis, ribs, and spine were derived from the whole-body scan. The CV and LSC for this DXA for whole-body aBMD is 0.5% CV and 1.5% LSC with regional sites for aBMD ≤ 1.5% CV and ≤ 4.2% LSC. The CV for lean mass and fat mass is 0.5% and 1.1%. Semi-nude body mass was measured on calibrated scales (Seca 869, Seca, UK).

Biochemical Markers of Bone Formation and Bone Resorption

A venous blood sample was taken between 05:30 and 06:30 after an overnight fast. Blood was collected in EDTA, serum-separating gel and fluoride oxalate tubes (Monovette®, Sarstedt, Germany) and centrifuged at 5,000 rpm for 10 mins. Plasma and serum were separated and stored at −80°C prior to analysis. Serum bone-specific alkaline phosphatase (bone ALP) and sclerostin were analysed by ELISA using proprietary kits (Quidel, USA and Biomedica Medizinprodukte GmbH, Austria). Plasma procollagen 1 N-terminal propeptide (P1NP) and beta C-telopeptide cross-links of type 1 collagen (βCTX) were measured by Roche® Cobas e411 (Roche Diagnostics, UK). Serum total 25-hydroxyvitamin D (25(OH)D) was measured using liquid chromatography / tandem mass spectrometry using automated solid phase extraction.[30] Serum phosphate, calcium and albumin were measured using commercial kits (Alpha Laboratories, UK) adapted for use on a Cobas Fara centrifugal analyser. Inter-assay CVs were < 10% for ELISAs and < 4% for e411 and Fara. Data were excluded for one participant for P1NP and one participant for bone ALP due to insufficient sample at week 44.

Statistical Analysis

All data were analysed using SPSS (v.24, SPSS Inc., USA) and checked for normality using the Shapiro-Wilk test. Participants who completed the study were compared with those lost to follow-up at week 1 with independent-samples t-tests or Mann-Whitney *U* tests for non-parametric data (trabecular thickness, trabecular separation, and cortical porosity at the 4% site, cortical porosity and cortical pore diameter at the 30% site, and bone ALP, sclerostin, and P1NP). Our primary analysis examined changes in HR-pQCT outcomes, aBMD, and markers of bone formation and resorption during training in all women with a one-way repeated measures (main effect of time) ANOVA (week 1 *vs* week 14 *vs* week 28 *vs* week 44) (markers of bone formation and resorption not measured at week 14). Non-normally distributed data (trabecular thickness, trabecular separation, cortical porosity, and cortical pore diameter at the 4% site, cortical porosity and cortical pore diameter at the 30% site, and P1NP, bone ALP, βCTX, and sclerostin) were analysed with a Friedman's ANOVA. Significant effects of time were followed up with Bonferroni corrected pairwise *post-hoc* comparisons (or Wilcoxon signed-rank tests for Friedman's ANOVA) to compare week 14, 28, and 44 with week 1, and the previous time-point. Secondary analysis compared women by contraceptive use with 3 × 4 repeated-measures ANOVAs (contraception [COCP users *vs* POC users *vs* non-users] × time [week 1 *vs* week 14 *vs* week 28 *vs* week 44]) (markers of bone formation and resorption not measured at week 14). Women who used an intrauterine system or changed contraception during training were excluded from this analysis. Significant contraception × time interactions were followed up with a separate one-way repeated measures ANOVA (main effect of time) or Friedman's ANOVA for non-parametric data for each contraceptive group and one-way between groups ANOVA or Kruskal-Wallis tests for non-parametric data at each time point. Significant effects of time were followed up with Bonferroni corrected *post-hoc* pairwise

comparisons (or Wilcoxon signed-rank tests for Friedman's ANOVA) to compare week 14, 28, and 44 with week 1, and the previous time-point. Significant effects of group were followed up with Bonferroni corrected independent-samples t-tests (or Mann-Whitney *U* tests for Kruskal-Wallis tests) at each time-point. Statistical significance was accepted at $P \leq 0.05$ and Bonferroni corrected P values are presented for *post-hoc* tests. Effect sizes were calculated using eta squared (η^2) or partial eta squared (η_p^2) for ANOVAs, Cohen's d_z for pairwise comparisons and paired-samples t-tests, and Cohen's d for independent samples t-tests. A minimum of 48 participants were necessary to detect changes in trabecular and cortical vBMD, cortical area and thickness, trabecular microarchitecture, and stiffness and failure load in response to military training (f = 0.196 to 0.716)[4] with an alpha of 0.05 and power of 90%.

Results

Seventy-seven women were invited to take part and screened eligible. Sixty-one women volunteered and completed baseline measures with fifty-one women completing the study (Figure 1). Two women were medically discharged from the Army, six women were removed from training for injury (one tibial stress fracture, one medial tibial stress syndrome, with the remaining injuries overuse injuries of the knee and back or shoulders), one woman voluntarily left the Army, and one woman was unavailable at the time of follow-up visits. Data were excluded for eight women for HR-pQCT scans at the 4% site (insufficient matching, $n = 4$; movement artifact, $n = 4$) and four women for HR-pQCT scans at the 30% site (movement artifact, $n = 4$) (Figure 1). Demographic and bone data are presented in Table 1 for those who completed the study and those lost to follow-up. There was no difference in those lost to follow-up and the final sample for demographic ($P \geq 0.160$) or aBMD ($P \geq 0.596$) outcomes. Cortical area, cortical thickness, trabecular thickness, stiffness and failure load at the 4% site were lower in those who completed the study compared with those who were lost to follow-up ($P \leq 0.021$);

there were no differences in other HR-pQCT outcomes ($P \geq 0.071$). Phosphate was higher in those who completed the study compared with those who were lost to follow-up ($P = 0.046$), but there were no differences between groups for other markers of bone formation or bone resorption ($P \geq 0.133$). There were no differences in age, height, body mass, or lean body mass between contraception groups ($P \geq 0.140$).

Body Composition

All body composition data are shown in Table 2 with mean absolute changes and 95% confidence intervals shown in Table 3. Regional aBMD data are presented in Supplemental Figure 1, with aBMD separated by contraceptive use presented in Supplemental Table 1. There was a main effect of time for body mass ($P = 0.025$, $\eta^2 = 0.064$), lean mass ($P = 0.009$, $\eta^2 = 0.082$), and fat mass ($P < 0.001$, $\eta^2 = 0.242$). Body mass increased from week 14 to week 28 ($P = 0.005$, $d_z = 0.484$) and decreased from week 28 to week 44 ($P = 0.025$, $d_z = -0.412$). Lean mass increased from week 1 to week 14 ($P = 0.045$, $d_z = 0.382$) and decreased from week 14 to week 28 ($P = 0.030$, $d_z = 0.399$). Fat mass decreased from week 1 to week 14 ($P < 0.001$, $d_z = -0.587$), increased from week 14 to week 28 ($P < 0.001$, $d_z = 1.327$), and decreased from week 28 to week 44 ($P = 0.015$, $d_z = 0.444$). There was a main effect of time for whole-body aBMD ($P = 0.009$, $\eta^2 = 0.082$) and aBMD of the arms ($P = 0.016$, $\eta^2 = 0.074$) and ribs ($P = 0.026$, $\eta^2 = 0.060$), but not legs, trunk, pelvis, or spine ($P \geq 0.061$, $\eta^2 \leq 0.053$). Whole-body and arms aBMD were not different between any time-points after Bonferroni correction. Ribs aBMD decreased from week 1 to week 14 (mean absolute change [95% confidence interval] −0.01 [−0.02, 0.00] g·cm^2, $P = 0.020$, $d_z = 0.450$). There were no contraception × time interactions or main effects of contraception for aBMD at any site ($P \geq 0.109$, $\eta_p^2 \leq 0.076$).

Volumetric Bone Mineral Density

Tibial vBMD data are presented in Figure 2A for the 4% site and Figure 3A for the 30% site, with mean absolute changes and 95% confidence intervals shown in Table 3. Data separated by contraceptive use are presented in Supplemental Table 2 and 3. There was a main effect of time for total vBMD ($P < 0.001$, $\eta^2 = 0.370$) and trabecular vBMD ($P < 0.001$, $\eta^2 = 0.291$) but not cortical vBMD ($P = 0.295$, $\eta^2 = 0.029$) at the 4% site. Training increased total vBMD from week 1 to week 14 ($P < 0.001$, $d_z = 0.819$), week 28 ($P = 0.005$, $d_z = 0.555$), and week 44 ($P < 0.001$, $d_z = 1.109$), and from week 28 to week 44 ($P < 0.001$, $d_z = 0.642$). Training increased trabecular vBMD from week 1 to week 14 ($P < 0.001$, $d_z = 0.676$), week 28 ($P = 0.025$, $d_z = 0.454$), and week 44 ($P < 0.001$, $d_z = 0.966$), and from week 28 to week 44 ($P = 0.005$, $d_z = 0.544$). There was a main effect of time for cortical vBMD ($P = 0.008$, $\eta^2 = 0.099$) but not total vBMD ($P = 0.100$, $\eta^2 = 0.047$) at the 30% site. Training decreased cortical vBMD from week 1 to week 14 ($P < 0.001$, $d_z = 0.605$). There were no contraception × time interactions or main effects of contraception for any measure of vBMD ($P \geq 0.302$, $\eta_p^2 \leq 0.060$).

Geometry

Tibial geometry data are presented in Figure 2B for the 4% site and Figure 3B for the 30% site, with mean absolute changes and 95% confidence intervals shown in Table 3. Data separated by contraceptive use are presented in Supplemental Table 2 and 3. There was a main effect of time for trabecular area ($P < 0.001$, $\eta^2 = 0.511$), trabecular bone volume fraction ($P < 0.001$, $\eta^2 = 0.252$), cortical area ($P < 0.001$, $\eta^2 = 0.523$), cortical thickness ($P < 0.001$, $\eta^2 = 0.274$), and cortical perimeter ($P = 0.024$, $\eta^2 = 0.102$) at the 4% site. Training decreased trabecular area from week 1 to week 14 ($P < 0.001$, $d_z = 0.673$), week 28 ($P < 0.001$, $d_z = 0.928$), and week 44 ($P < 0.001$, $d_z = 1.277$), from week 14 to week 28 ($P < 0.001$, $d_z = 0.699$), and from week 28 to week 44 ($P < 0.001$, $d_z = 0.708$). Training increased trabecular bone volume fraction from week 1 to week 14 ($P = 0.030$, $d_z = 0.438$) and week 44 ($P < 0.001$, $d_z = 0.999$), and from week

28 to week 44 ($P < 0.001$, $d_z = 0.592$). Training increased cortical area from week 1 to week 14 ($P < 0.001$, $d_z = 0.716$), week 28 ($P < 0.001$, $d_z = 0.970$), and week 44 ($P < 0.001$, $d_z = 1.303$), from week 14 to week 28 ($P < 0.001$, $d_z = 0.713$), and from week 28 to week 44 ($P < 0.001$, $d_z = 0.703$). Training increased cortical thickness from week 1 to week 14 ($P < 0.001$, $d_z = 0.646$), week 28 ($P = 0.020$, $d_z = 0.466$), and week 44 ($P < 0.001$, $d_z = 0.855$), and from week 28 to 44 ($P < 0.001$, $d_z = 0.724$). Cortical perimeter was not different between any-time points. There was a main effect of time for cortical area ($P = 0.038$, $\eta^2 = 0.064$) and cortical perimeter ($P < 0.001$, $\eta^2 = 0.150$) but not cortical thickness ($P = 0.195$, $\eta^2 = 0.035$) at the 30% site. Training increased cortical area from week 1 to week 28 ($P = 0.030$, $d_z = 0.421$). Training increased cortical perimeter from week 1 to week 28 ($P = 0.005$, $d_z = 0.549$) and week 44 ($P = 0.005$, $d_z = 0.535$). There were no contraception × time interactions or main effects of contraception for any measure of geometry ($P \geq 0.194$, $\eta_p^2 \leq 0.083$).

Microarchitecture

Trabecular microarchitecture and cortical porosity data are presented in Figure 2C for the 4% site and Figure 3C for the 30% site, with mean absolute changes and 95% confidence intervals shown in Table 3. Data separated by contraceptive use are presented in Supplemental Table 2 and 3. There was a main effect of time for trabecular number ($P < 0.001$, $\eta^2 = 0.175$), trabecular thickness ($P < 0.001$), trabecular separation ($P < 0.001$), and cortical pore diameter size ($P = 0.012$) but not cortical porosity ($P = 0.155$) at the 4% site. Training increased trabecular number from week 1 to week 28 ($P < 0.001$, $d_z = 0.682$) and week 44 ($P < 0.001$, $d_z = 0.608$). Training increased trabecular thickness from week 1 to week 28 ($P < 0.001$, $d_z = 0.670$) and week 44 ($P < 0.001$, $d_z = 0.828$), and from week 14 to week 28 ($P = 0.015$, $d_z = 0.493$). Training decreased trabecular separation from week 1 to week 14 ($P = 0.010$, $d_z = 0.473$), week 28 ($P < 0.001$, $d_z = 0.742$), and week 44 ($P < 0.001$, $d_z = 0.756$). Training decreased cortical pore diameter size

from week 1 to week 28 (P = 0.015, d_z = 0.510) and from week 14 to week 28 (P = 0.040, d_z = 0.313). Training had no effect on cortical porosity (main effect of time, P = 0.115) or cortical pore diameter (main effect of time, P = 0.169) at the 30% site. There was a contraception × time interaction for trabecular thickness at the 4% site (P = 0.013, η_p^2 = 0.137). Trabecular thickness increased in COCP users from week 1 to week 28 (0.005 [0.002, 0.009] mm, P = 0.040, d_z = 0.854) and week 44 (0.006 [0.004, 0.009] mm, P = 0.005, d_z = 1.408), and from week 14 to week 28 (0.006 [0.002, 0.010] mm, P = 0.040, d_z = 0.846). There was a main effect of time for trabecular thickness in non-users (P = 0.002) but no difference between individual time-points after Bonferroni corrections. Trabecular thickness did not change in POC users. Trabecular thickness was not different between contraceptive groups at any time-point (P ≥ 0.283). There were no contraception × time interactions and no main effects of contraception for trabecular number, trabecular separation, cortical porosity, or cortical pore diameter at the 4% site (P ≥ 0.161, $\eta_p^2 \leq 0.083$). There were contraception × time interactions for cortical porosity (P = 0.033, η_p^2 = 0.109) and cortical pore diameter (P = 0.026, η_p^2 = 0.125) at the 30% site. Cortical porosity decreased from week 14 to week 28 in COCP users only (−0.13 [−0.21, −0.05] %, P = 0.030, d_z = 0.823) but was not different between groups at any time-point (P ≥ 0.703). Training did not change cortical pore diameter size in any contraceptive group but was higher in non-users compared with COCP and POC users at week 1, and higher in non-users than COCP users at week 28 (P ≤ 0.024, d ≥ 1.048).

Estimated Mechanical Strength

All mechanical property data are presented in Figure 4 with mean absolute changes and 95% confidence intervals shown in Table 3. Data separated by contraceptive use are presented in Supplemental Table 4. Training had no effect on stiffness (main effect of time, P = 0.486, η^2 = 0.017) or failure load (main effect of time, P = 0.305, η^2 = 0.028) at the 4% site, or stiffness at

the 30% site (main effect of time, $P = 0.223$, $\eta^2 = 0.032$). There was a main effect of time for failure load at the 30% site ($P < 0.001$, $\eta^2 = 0.302$). Training increased failure load from week 1 to week 14 ($P = 0.005$, $d_z = 0.524$), week 28 ($P < 0.001$, $d_z = 0.717$), and week 44 ($P < 0.001$, $d_z = 0.888$), and from week 14 to week 28 ($P = 0.015$, $d_z = 0.456$). There were no contraception × time interactions or main effects of contraception for stiffness or failure load at the 4% or 30% sites ($P \geq 0.172$, $\eta_p^2 \leq 0.093$).

Biochemical Markers of Bone Formation and Bone Resorption

All markers of bone formation and bone resorption are presented in Figure 5, with data separated by contraceptive use presented in Supplemental Table 5. There was a main effect of time for adjusted calcium ($P = 0.013$, $\eta^2 = 0.165$) but not P1NP, bone ALP, βCTX, sclerostin, phosphate, or total 25(OH)D ($P \geq 0.096$, $\eta^2 \leq 0.044$). Adjusted calcium increased from week 1 to week 28 ($P = 0.045$, $d_z = 0.368$) and week 44 ($P = 0.035$, $d_z = 0.396$). There was a contraception × time interaction for P1NP ($P = 0.050$, $\eta^2 = 0.044$) but P1NP did not change in any contraceptive group. P1NP was higher in POC users than COCP users at week 1 ($P = 0.012$, $d = 1.022$). There were no contraception × time interactions for bone ALP, sclerostin, βCTX, albumin-adjusted calcium, phosphate, or total 25(OH)D ($P \geq 0.053$, $\eta^2 \leq 0.111$). There was a main effect of contraception for sclerostin ($P = 0.039$, $\eta_p^2 = 0.140$) and phosphate ($P = 0.023$, $\eta_p^2 = 0.164$) but not bone ALP, βCTX, albumin-adjusted calcium, or total 25(OH)D ($P \geq 0.054$, $\eta_p^2 \leq 0.130$). Sclerostin was higher in POC users than COCP users at week 1 and week 44 ($P \leq 0.018$, $d \geq 0.840$). Phosphate was not different between groups at any-time point.

Discussion

This study reports the temporal adaptations of the tibial macrostructure and microarchitecture in women during 44 weeks of basic military training. Basic military training is physically and

psychologically arduous, consists of prolonged periods of weight-bearing activity and nutritional restriction,[20,21,31-33] and is characterised by a high incidence of stress fractures in women.[11] The incidence of stress fracture in this cohort was 1.6% and lower than the incidence previously reported in female military recruits (9.2%)[9] and in women during this training course (11.2%).[11] The low incidence in this study could be due to recent training modifications — including physical training in groups based on physical fitness and reducing the amount of marching around the military camp between lessons — or a recruitment bias. We recently demonstrated these women are exposed to periodic low energy availability[20] and have impaired hypothalamic pituitary gonadal (HPG) axis function and menstrual disturbances,[22] but protected hypothalamic pituitary adrenal (HPA) axis function.[21] We hypothesised adaptations to the tibial macrostructure and microarchitecture would be consistent with continuous bone modelling across 44-weeks of training. Training resulted in continual and site-specific adaptation of the tibia: increases in trabecular vBMD, and adaptations to geometry (increases in trabecular bone volume fraction, cortical thickness, and cortical area) and microarchitecture (increases in trabecular number and thickness) at the metaphyseal site, and; initial temporary decreases in cortical vBMD, and increases in cortical perimeter and estimated mechanical strength at the diaphyseal site. Training had no effect on whole-body aBMD or biochemical markers of bone formation or bone resorption at the measured time-points. These findings demonstrate that unaccustomed exercise in women, in the form of basic military training, continues to be osteogenic far beyond durations previously studied (8 to 12 weeks).[2,3,5] Our HR-pQCT measurement protocol, concurrent assessment of aBMD and bone metabolic markers, and previously published measures of energy availability,[20] HPA axis function,[21] and HPG axis function[22] provides novel and comprehensive insight into the skeletal adaptations to prolonged arduous exercise with concomitant nutritional and

psychological stress in women. These data are also applicable to female endurance athletes and women undergoing endurance training.

Volumetric Bone Mineral Density

We observed continual increases in trabecular vBMD at the 4% site with a total increase of 3.0% by week 44. An increase in trabecular vBMD is an early adaptation to mechanical loading,[34,35] and improves resistance to the compressive forces at the metaphysis.[36] Previous pQCT[1,2] and HR-pQCT[3,4] studies have demonstrated increases of 0.9 to 2.0% in trabecular vBMD at the tibial metaphysis following 8 to 13 weeks of basic military training in men and women. Exercise training is osteogenic where the mechanical stress is greatest[37] and basic military training involves an increase in the volume and frequency of irregular and high magnitude tibial impacts during weight-bearing activities like heavy load carriage[31,33] and military drill.[38,39] Bone rapidly becomes desensitised to repetitive and prolonged mechanical loading,[37] but this varied, multi-directional, and high-impact loading stimulus, likely contributed to the osteogenic potential of military training across 44 weeks. The magnitude of change we report here is 1.5 to 3-fold higher than any previous military study,[1-4] probably as a result of the high intensity (total energy expenditures of ~3,500 kcal·d^{-1}[20]) and long duration of training, which results in fracture in approximately one in ten women.[11] The increase in trabecular vBMD (and geometry and microarchitecture) we report here are comparable to or greater than those observed with 12 to 24 months of treatment with osteoporotic drugs.[40-42] Although the magnitude of the changes we report for all outcomes are comparable or larger to those previously published with HR-pQCT, and generally larger than the LSC of our HR-pQCT, some changes are close to or below the LSC; therefore, our data should be interpreted in context of the LSC for each outcome.

Cortical vBMD was unchanged at the 4% site but decreased from week 1 to 14 (−0.3%) at the 30% site before recovering. This decrease was, however, close to the LSC (0.4%) we report for this measure. In contrast to our data, cortical vBMD at the 4% site measured by HR-pQCT increased in men (0.6 to 0.9%) following 13-weeks British Army basic training[4] and decreased in women (−0.3%) following 8-weeks US Army basic training.[3] The small contribution of cortical vBMD to mechanical strength at the tibial metaphysis in young athletic women[43] may explain why mechanical loading does not produce a consistent pattern of adaptation at this site. The tibial metaphysis and diaphysis are mainly trabecular and cortical bone, respectively, which reflects differences in loading profile at these sites.[36] This difference in loading profile between sites may explain why cortical vBMD and failure load only changed at the 30% site. Most tibial stress fractures in military training occur at the diaphysis,[8] where high bending and torsion stresses are experienced during locomotion.[44] The median time to stress fracture in women during this training course was reported as 102 days,[11] which is similar to when cortical vBMD decreased at the 30% site and supports intracortical remodelling as an important process in the pathogenesis of stress fractures.[45] Cortical porosity can increase from the removal of fatigue damage,[45] and may contribute to stress fracture risk[6] by facilitating the propagation of microcracks[14] and decreasing mechanical strength,[15,46] although training had no effect on cortical porosity in this study and estimated mechanical strength increased at the 30% site. In agreement with our data, female US Army recruits had decreased cortical vBMD at the 30% site (−0.7%) following 8-weeks training, which may reflect the unmineralised nature of new bone or a lag between bone formation and bone resorption.[3] The temporary decrease followed by recovery of cortical vBMD in our study supports this supposition, but our circulating markers of bone formation and resorption were unchanged. Increases in markers of bone formation (P1NP or bone ALP), bone resorption (βCTX), and / or decreases in sclerostin have previously been reported in women during 8- to 16-weeks of basic military

training.[2,3,5,47,48] We did not measure markers of bone formation or resorption before week 28 and so acute changes will have been missed, however, calcium increased with training. The mechanism is unclear but an increase in circulating calcium could support the formation of new bone by promoting mineralisation or inhibiting parathyroid hormone.[2,5]

Geometry

Training continually increased cortical area and thickness at the 4% site, resulting in increases of 4.8% and 4.0% by week 44. There was no change in cortical perimeter, and a decrease in trabecular area (0.4%), demonstrating increases in the size of the cortical bone were due to endosteal contraction not periosteal expansion, consistent with previous military HR-pQCT studies.[3,4] The decrease in trabecular area could be due to remodelling of trabecular bone, with the increase in cortical area a result of new cortical bone formation, or corticalisation of trabecular bone, on the endosteal surface. Cortical perimeter increased by 0.5% from week 1 to 44 at the 30% site demonstrating periosteal expansion at the diaphysis. An increase in cross-sectional area improves resistance to bending during weight bearing activity as the tibial cortex is placed further from the neutral axis,[1,35] and is responsible for increased strength where only modest improvements in vBMD are observed.[1] The increase in estimated mechanical strength at the 30% site supports this supposition. The tibial metaphysis is predominantly trabecular bone and the forces are mainly compressive.[36] Accordingly, increases in strength at the tibial metaphysis following mechanical loading are likely mediated by changes in trabecular microarchitecture rather than geometry,[3,4] whereas increases in geometry are more important for increasing strength at the tibial diaphysis where bending forces are high.[1]

Microarchitecture

Training resulted in adaptation of the trabecular microarchitecture: trabecular number and thickness increased by 3.5% and 2.1% at the 4% site by week 44. Adaptations to the trabecular microarchitecture were not evident until week 28 whereas adaptations to density and geometry were evident at week 14. These data provide first evidence of a difference in time course between the macrostructure and microarchitecture responses to exercise in humans. The mechanism is unclear but may reflect site-specific differences in the loading profile[36] or regulation of bone by osteoblast progenitors and osteoclasts in response to mechanical and hormonal stimuli.[49] There are few data examining human trabecular microarchitecture in response to loading. Cross-sectional HR-pQCT studies have reported no difference in trabecular microarchitecture between female athletes in weight-bearing sports with athletes in non-weight bearing sports or controls,[50,51] a higher trabecular number in female alpine skiers compared with controls,[52] and a positive association between physical activity history and trabecular microarchitecture in young men and women.[53] Longitudinal data show no adaptation in the trabecular microarchitecture following a 61-day Antarctic traverse in women[24] and 13 weeks British Army basic training in men,[4] and an increase in trabecular thickness and number following 8 weeks US Army basic training in women.[3] Our data confirm that the trabecular microarchitecture adapts to military training in women and provide the first evidence that microarchitecture continues to adapt across longer training durations. The trabecular network aligns parallel to the mechanical stress axis, and absorbs and distributes mechanical stress to the cortex.[54] Trabecular microarchitecture is an important contributor to bone strength,[12-14,55] yet, despite these adaptations to trabecular microarchitecture, estimated mechanical strength at the 4% site was unchanged. This lack of an increase in failure load at the 4% site despite adaptations to density, geometry, and microarchitecture could be due to a lack of sensitivity in detecting changes in estimated mechanical strength at this site, supported by the higher CV and LSC compared with the 30% site. Improvements in density, geometry,

and / or trabecular microarchitecture without increases in failure load have also been reported in response to exercise[4] and osteoporosis treatment.[42]

In our exploratory analyses, trabecular thickness did not increase in POC users, providing the first evidence that POCs may inhibit adaptation of the trabecular microarchitecture in response to mechanical loading. The mechanism is unconfirmed, but low total oestradiol exposure (low exogenous and endogenous oestradiol) with POC use likely contribute. Trabecular thickness increased in COCP users and we observed a similar increase in non-users (COCP users, $d_z \geq 0.883$; non-users, $d_z \geq 1.090$). The increase in trabecular thickness in non-users was not significant after correcting for multiple comparisons, but data from this group must treated with caution due to the smaller number of non-users (n = 10) compared with COCP (n = 16) and POC (n = 14) users, and, therefore, higher risk of type II error. Procollagen 1 N-terminal propeptide (week 1) and sclerostin (week 1 and 44) were also higher in POC users compared with COCP users. Sclerostin — a glycoprotein secreted by osteocytes — inhibits the formation of new bone in response to mechanical loading through inhibition of the WNT signalling pathway.[56] Progestogen only contraceptives — depending on type — can suppress oestradiol by inhibition of the HPO axis,[57] whereas COCPs provide synthetic (ethinyl) oestradiol. Synthetic oestradiol in the COCP might explain the decrease in cortical porosity experienced by this group, but the mechanism is unclear. Low oestradiol increases sclerostin,[58] bone formation and bone resorption, and results in trabecular thinning.[59] Young oligomenorrhoeic athletes who received transdermal oestradiol increased trabecular number over 12 months of training to a greater extent than those who received a COCP providing some support for the role of oestradiol in trabecular microarchitecture adaptations to mechanical loading in young women, however, there were no differences between the oestradiol patch and no treatment.[60] Whilst low oestradiol is a plausible mechanism to explain differences in trabecular adaptation,

P1NP, and sclerostin between groups, there was no difference in oestradiol between contraceptive groups.[22] We did not, however, standardise measurements around the menstrual cycle or contraceptive use, or measure ethinyl oestradiol. The non-users also had a high prevalence of oligo/amenorrhoea and anovulatory cycles by week 28,[22] and so are not a eumenorrhoeic comparison. The relationship between oestradiol, sclerostin, and bone formation during mechanical loading is not clear,[58] and the effect of contraceptive use on adaptation to mechanical loading requires further investigation with larger sample sizes.

Areal Bone Mineral Density

Whole-body aBMD did not change with training, but aBMD decreased for the ribs from week 1 to 14 (−1.5%). A decrease in axial aBMD may result from insufficient calcium intake[61] or prolonged low energy availability,[24] however we did not perform hip or spine scans, which are more clinically relevant than regional analysis from whole-body scans. Low energy availability stimulates bone resorption by decreasing oestradiol, and decreases bone formation by increasing cortisol and decreasing 3,5,3-triiodothyronine (T3), leptin, and insulin like growth factor I (IGF-I).[62-65] Military training in energy deficit increases bone resorption,[66] decreases bone formation,[66,67] and decreases whole-body bone mineral content[68] in men. Amenorrhoeic or oligomenorrhoeic athletes and military recruits have lower aBMD (whole-body, axial, and appendicular sites) than their eumenorrhoeic counterparts.[50,69-72] There is also some evidence that amenorrhoeic or oligomenorrhoeic athletes have lower tibial trabecular number,[50] but differences in radial, and not tibial, structure or strength are most often reported between groups, suggesting a protective effect of mechanical loading with low oestradiol.[43,69,71,73] We previously reported that the women in this study are exposed to periods of low energy availability,[20] and have increased cortisol,[21] suppressed responsiveness of the HPG axis, menstrual disturbances, increased sex hormone binding globulin, and unchanged

T3, IGF-I, leptin, and oestradiol.[22] Preservation of whole-body aBMD and favourable adaptation to tibial microstructure and microarchitecture suggests that low energy availability had no maladaptive effects on the bone response to military training and mechanical loading was protective.

Limitations

Due to attrition during military training, we were only able to follow-up those individuals who completed training and our data are subject to survivor bias; therefore, women who developed a stress fracture were excluded. We were unable to include a non-exercising control group, however, this is typical of observational mechanical loading studies,[1,3,4] and we do not think this affects the interpretation of the data. We also used 2D image registration to match repeat scans at the 4% site, which limits out ability to detect changes in bone size and does not control for angular differences between scans unlike 3D image registration;[28] however, 2D image registration is typically used in exercise studies[3,4] and 3D image registration is not recommended for micro-finite element analysis.[28] Finally, our contraceptive data are limited by the low number of women per group, wide variability within each group in length of contraceptive use and contraceptive preparation, and should be considered as exploratory analyses.

Conclusion

Prolonged periods of basic military training (44-weeks) result in continual and site-specific adaptation of tibial density, geometry, microarchitecture, and estimated mechanical strength in women. Temporal decreases in cortical density support a role of intracortical remodelling in the pathogenesis of stress fracture. Military training remained osteogenic across a prolonged

training duration despite impaired HPG function, likely due to the frequent dynamic, high-impact, and episodic loading.

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Table 1. Participant demographics at week 1 in those who completed the study and those lost to follow-up. Data are mean ± standard deviation or median [interquartile range].

	Completed (n = 51)[a]	Lost to Follow-up (n = 10)[b]
Demographics		
Age (years)	24 ± 2	24 ± 3
Height (m)	1.66 ± 0.06	1.69 ± 0.05
Body Mass (kg)	64.3 ± 7.7	66.6 ± 3.8
Lean Body Mass (kg)	46.0 ± 5.3	48.4 ± 3.3
Combined Oral Contraceptive Pill Users (n, [%])	18 (35%)	2 (20%)
Progestogen-only Contraceptive Users (n, [%])	17 (33%)[c]	0 (0%)
Non-Contraceptive Users (n, [%])	11 (22%)	5 (50%)
Intrauterine System Users (n, [%])	4 (8%)	0 (0%)
Other Contraceptive User (n, [%])[d]	1 (2%)	3 (30%)
aBMD ($g \cdot cm^2$)		
Arms	0.87 ± 0.10	0.86 ± 0.07
Legs	1.26 ± 0.09	1.28 ± 0.16
Trunk	1.04 ± 0.09	1.04 ± 0.08
Ribs	0.89 ± 0.06	0.89 ± 0.07
Pelvis	1.12 ± 0.09	1.12 ± 0.09
Spine	1.12 ± 0.11	1.14 ± 0.11
Whole-body	1.23 ± 0.08	1.24 ± 0.10
Tibial Metaphysis (4% site)		
Total vBMD (mg $HA \cdot cm^3$)	243 ± 23	270 ± 52
Trabecular vBMD (mg $HA \cdot cm^3$)	200 ± 20	220 ± 45
Cortical vBMD (mg $HA \cdot cm^3$)	747 ± 45	763 ± 49
Trabecular Area (mm^2)	965 ± 123	960 ± 120
Trabecular Bone Volume Fraction (%)	28.1 ± 3.3	31.5 ± 6.9
Cortical Area (mm^2)	82 ± 10*	96 ± 13
Cortical Thickness (mm)	0.72 ± 0.12*	0.88 ± 0.16
Cortical Perimeter (mm)	129.6 ± 8.4	131.1 ± 6.5
Trabecular Number (1·mm)	1.74 ± 0.17	1.75 ± 0.19
Trabecular Thickness (mm)	0.234 [0.228, 0.244]*	0.252 [0.234, 0.281]
Trabecular Separation (mm)	0.530 [0.490, 0.554]	0.510 [0.485, 0.590]
Cortical Porosity (%)	1.0 [0.9, 1.3]	1.6 [1.0, 2.1]
Cortical Pore Diameter (mm)	0.178 ± 0.015	0.198 ± 0.028
Stiffness (kN·mm)	174 ± 37*	214 ± 64
Failure Load (kN)	9.5 ± 1.9*	11.4 ± 3.2
Tibial Diaphysis (30% site)		

Total vBMD (mg HA·cm^3)	777 ± 44	771 ± 83
Cortical vBMD (mg HA·cm^3)	1012 ± 21	1009 ± 21
Cortical Area (mm^2)	254 ± 29	267 ± 17
Cortical Thickness (mm)	5.67 ± 0.48	5.85 ± 0.82
Cortical Perimeter (mm)	73.8 ± 4.5	77.0 ± 2.8
Cortical Porosity (%)	0.7 [0.4, 0.9]	0.8 [0.6, 1.6]
Cortical Pore Diameter (mm)	0.227 [0.204, 0.248]	0.229 [0.226, 0.252]
Stiffness (kN·mm)	269 ± 31	281 ± 16
Failure Load (kN)	15.1 ± 1.7	15.6 ± 0.8
Markers of Bone Formation and Bone Resorption		
Bone ALP (µg·l^{-1})	18.4 [16.2, 21.2]	19.8 [17.7, 25.6]
Sclerostin (pmol·l^{-1})	37.4 [31.9, 43.9]	32.7 [29.1, 39.3]
P1NP (µg·l^{-1})	70.7 [56.5, 86.6]	77.7 [57.7, 106.3]
βCTX (µg·l^{-1})	0.54 ± 0.19	0.64 ± 0.23
Total 25(OH)D (nmol·l^{-1})	71.5 ± 26.8	84.6 ± 36.4
Phosphate (mmol·l^{-1})	1.61 ± 0.14*	1.51 ± 0.13
Adjusted Calcium (mmol·l^{-1})	2.49 ± 0.11	2.50 ± 0.10

[a]n = 43 for tibial metaphysis (4% site), n = 47 for tibial diaphysis (30% site), n = 50 for P1NP and bone ALP; [b]n = 8 for tibial metaphysis (4% site) and tibial diaphysis (30% site); [c]n = 1 for depot medroxyprogesterone acetate, n = 6 for progestogen only pill, n = 10 for implant; [d]changed methods during training or unknown.

aBMD, areal bone mineral density; vBMD, volumetric bone mineral density; bone ALP, bone-specific alkaline phosphatase; P1NP, procollagen 1 N-terminal propeptide; βCTX, beta C-telopeptide cross-links of type 1 collagen; total 25(OH)D, total 25-hydroxyvitamin D; adjusted calcium, albumin-adjusted calcium.

*P ≤ 0.05 *vs* lost to follow-up.

Table 2. Body composition in women during 44-weeks of British Army Officer training (n = 51).

	Week 1	**Week 14**	**Week 28**	**Week 44**
Body mass (kg)	64.3 ± 7.7	63.8 ± 7.8	64.9 ± 7.7[b]	64.1 ± 7.7[c]
Lean mass (kg)	46.0 ± 5.3	46.7 ± 5.0[a]	46.1 ± 4.9[b]	46.3 ± 4.7
Fat mass (kg)	15.7 ± 3.9	14.6 ± 3.7[a]	16.3 ± .3.8[b]	15.7 ± 3.9[c]
aBMD ($g \cdot cm^2$)	1.23 ± 0.08	1.23 ± 0.09	1.22 ± 0.08	1.21 ± 0.09

[a]P ≤ 0.05 *vs* Week 1; [b]P ≤ 0.05 *vs* Week 14; [c]P ≤ 0.05 *vs* Week 28.

Table 3. Mean absolute change and 95% confidence intervals for body composition, volumetric bone mineral density, geometry, microarchitecture, and estimated mechanical properties of the tibial metaphysis (4% site) and diaphysis (30% site), and biochemical markers of bone formation and bone resorption.

	Main Effect	Week 1 vs Week 14		Week 1 vs Week 28		Week 1 vs Week 44		Week 14 vs Week 28		Week 28 vs Week 44	
	P	Mean change (95% CI)	P*	Mean change (95% CI)	P*	Mean change (95% CI)	P*	Mean change (95% CI)	P*	Mean change (95% CI)	P*
Body Mass (kg)	0.025	−0.5 (−1.2 0.2)	0.840	0.6 (0.1, 1.3)	0.515	−0.3 (−1.1, 0.6)	1.000	1.1 (0.4, 1.7)	0.005	−0.8 (−1.4, −0.3)	0.025
Lean Mass (kg)	0.009	0.7 (0.2, 1.2)	0.045	0.1 (−0.3, 0.4)	1.000	0.3 (−0.2, 0.7)	1.000	−0.6 (−0.2, −1.0)	0.030	0.2 (−0.1, 0.5)	1.000
Fat Mass (kg)	<0.001	−1.1 (−0.6, −1.6)	<0.001	−0.6 (−1.2, 0.0)	0.205	0.0 (−0.6, 0.7)	1.000	1.7 (1.3, 2.1)	<0.001	−0.6 (−0.9, −0.2)	0.015
aBMD ($g \cdot cm^2$)	0.009	0.01 (0.00, 0.01)	1.000	−0.01 (−0.02, 0.01)	1.000	−0.01 (−0.03, 0.00)	0.170	−0.01 (−0.02, 0.00)	0.185	−0.01 (−0.02, 0.00)	0.465
Tibial Metaphysis (4% site)											
Total vBMD (mg HA·cm^3)	<0.001	3 (2, 4)	<0.001	4 (2, 6)	0.005	8 (5, 10)	<0.001	1 (−1, 3)	1.000	4 (2, 5)	<0.001
Trabecular vBMD (mg HA·cm^3)	<0.001	2 (1, 3)	<0.001	3 (1, 5)	0.025	6 (4, 7)	<0.001	1 (−2, 1)	1.000	3 (1, 4)	0.005
Cortical vBMD (mg HA·cm^3)	0.295	—	—	—	—	—	—	—	—	—	—
Trabecular Area (mm^2)	<0.001	−1 (−2, −1)	<0.001	−3 (−4, −2)	<0.001	−4 (−5, −3)	<0.001	−1 (−2, −1)	<0.001	−1 (−2, −1)	<0.001
Trabecular Bone Volume Fraction (%)	<0.001	0.3 (0.1, 0.5)	0.030	0.3 (0.0, 0.7)	0.290	0.9 (0.6, 1.2)	<0.001	0.0 (−0.3, 0.3)	1.000	0.6 (0.3, 0.9)	<0.001
Cortical Area (mm^2)	<0.001	1 (1, 2)	<0.001	3 (2, 4)	<0.001	4 (3, 5)	<0.001	1 (1, 2)	<0.001	1 (1, 2)	<0.001
Cortical Thickness (mm)	<0.001	0.01 (0.01, 0.02)	<0.001	0.02 (0.01, 0.03)	0.020	0.03 (0.02, 0.04)	<0.001	0.00 (−0.01, 0.01)	0.760	0.01 (0.01, 0.02)	<0.001
Cortical Perimeter (mm)	0.024	0	1.000	1	0.220	0	1.000	1	0.080	−1	0.220

		(−1, 0)		(0, 3)		(0, 1)		(0, 3)		(0, −2)	
Trabecular Number (1·mm)	<0.001	0.03	0.100	0.07	<0.001	0.06	<0.001	0.05	0.085	−0.01	1.000
		(0.00, .05)		(0.04, 0.10)		(0.03, 0.09)		(0.01, 0.09)		(−0.5, 0.2)	
Trabecular Thickness (mm)	<0.001	0.001	1.000	0.004	<0.001	0.005	<0.001	0.003	0.015	0.001	1.000
		(−0.001, 0.002)		(0.002, 0.006)		(0.003, 0.007)		(0.001, 0.005)		(−0.001, 0.003)	
Trabecular Separation (mm)	<0.001	−0.007	0.010	−0.018	<0.001	−0.017	<0.001	−0.011	0.135	0.001	1.000
		(−0.011, −0.002)		(−0.026, −0.011)		(−0.024, −0.010)		(−0.020, −0.003)		(−0.007, 0.009)	
Cortical Porosity (%)	0.155	—	—	—	—	—	—	—	—	—	—
Cortical Pore Diameter (mm)	0.012	−0.001	1.000	−0.006	0.015	−0.003	1.000	−0.005	0.040	0.003	0.325
		(−0.005, 0.003)		(−0.009, −0.002)		(−0.007, 0.001)		(−0.010, 0.000)		(−0.001, 0.007)	
Stiffness (kN·mm)	0.486	—	—	—	—	—	—	—	—	—	—
Failure Load (kN)	0.305	—	—	—	—	—	—	—	—	—	—
Tibial Diaphysis (30% site)											
Total vBMD (mg HA·cm^3)	0.100	—	—	—	—	—	—	—	—	—	—
Cortical vBMD (mg HA·cm^3)	0.008	−3	<0.001	−2	1.000	4	0.475	2	1.000	6	0.105
		(−5, −2)		(−5, 2)		(−1, 9)		(−2, 6)		(1, 10)	
Cortical Area (mm^2)	0.038	1	1.000	2	0.030	2	0.410	1	0.255	0	1.000
		(−1, 2)		(1, 3)		(0, 3)		(0, 2)		(−2, 1)	
Cortical Thickness (mm)	0.195	—	—	—	—	—	—	—	—	—	—
Cortical Perimeter (mm)	<0.001	0.2	0.090	0.4	0.005	0.4	0.005	0.2	0.055	0.0	1.000
		(0.0, 0.3)		(0.2, 0.6)		(0.2, 0.6)		(0.0, 0.4)		(−0.2, 0.2)	
Cortical Porosity (%)	0.115	—	—	—	—	—	—	—	—	—	—
Cortical Pore Diameter (mm)	0.169	—	—	—	—	—	—	—	—	—	—

Stiffness (kN·mm)	0.223	—	—	—	—	—	—	—	—	—	—
Failure Load (kN)	<0.001	0.2 (0.1, 0.3)	0.005	0.3 (0.2, 0.5)	<0.001	0.4 (0.3, 0.5)	<0.001	0.2 (0.1, 0.3)	0.015	0.0 (−0.1, 0.0)	1.000
Markers of Bone Formation and Bone Resorption											
Bone ALP ($\mu g \cdot l^{-1}$)	0.108	—	—	—	—	—	—	—	—	—	—
Sclerostin ($pmol \cdot l^{-1}$)	0.484	—	—	—	—	—	—	—	—	—	—
P1NP ($\mu g \cdot l^{-1}$)	0.096	—	—	—	—	—	—	—	—	—	—
βCTX ($\mu g \cdot l^{-1}$)	0.133	—	—	—	—	—	—	—	—	—	—
Total 25(OH)D ($nmol \cdot l^{-1}$)	0.124	—	—	—	—	—	—	—	—	—	—
Phosphate ($mmol \cdot l^{-1}$)	0.571	—	—	—	—	—	—	—	—	—	—
Adjusted Calcium ($mmol \cdot l^{-1}$)	0.013	—	—	0.05 (0.01, 0.10)	0.045	0.05 (0.01, 0.10)	0.035	—	—	0.00 (−0.04, 0.04)	1.000

aBMD, areal bone mineral density; vBMD, volumetric bone mineral density; bone ALP, bone-specific alkaline phosphatase; P1NP, procollagen 1 N-terminal propeptide; βCTX, beta C-telopeptide cross-links of type 1 collagen; total 25(OH)D, total 25-hydroxyvitamin D; adjusted calcium, albumin-adjusted calcium.

*P values are after Bonferonni correction.

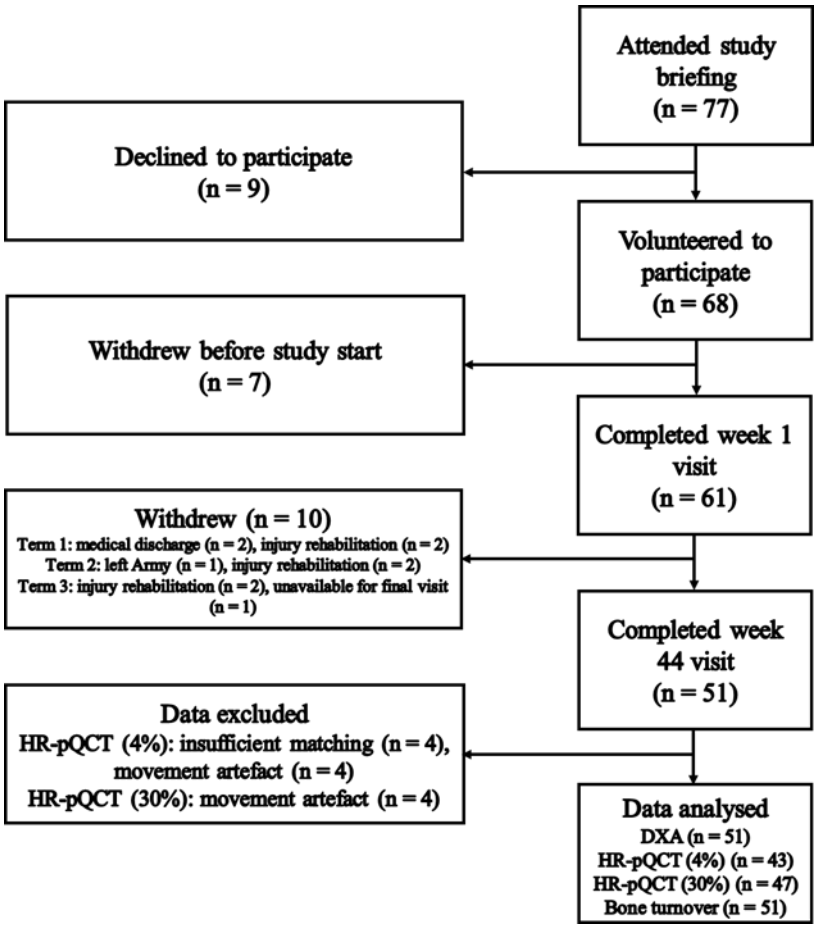


Figure 1. Participant flow through the study.

DXA, dual energy X-ray absorptiometry

HR-pQCT, high-resolution peripheral quantitative computed tomography

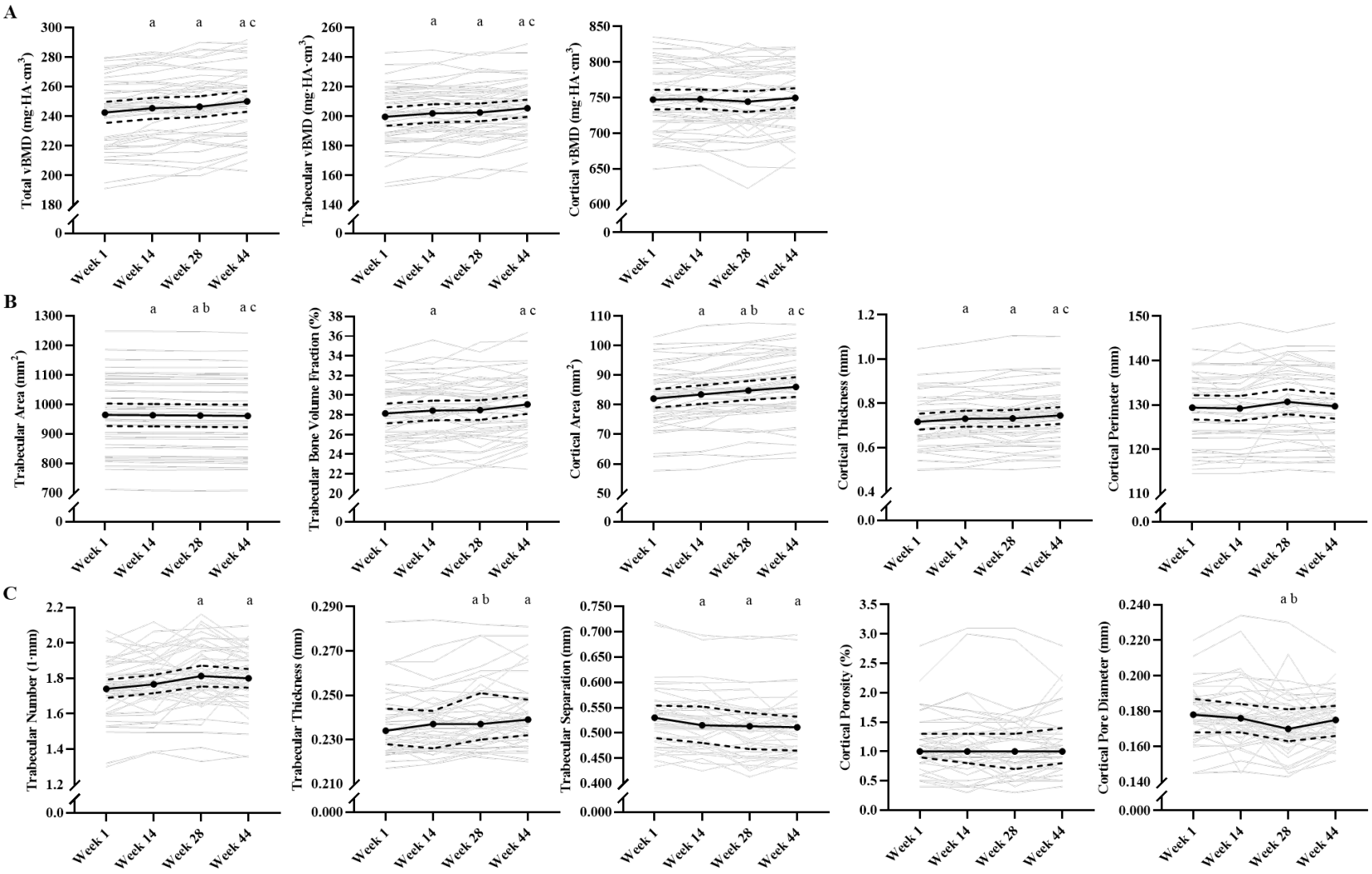

A
Total vBMD (mg·HA·cm3)
Trabecular vBMD (mg·HA·cm3)
Cortical vBMD (mg·HA·cm3)
B
Trabecular Area (mm2)
Trabecular Bone Volume Fraction (%)
Cortical Area (mm2)
Cortical Thickness (mm)
Cortical Perimeter (mm)
C
Trabecular Number (1·mm)
Trabecular Thickness (mm)
Trabecular Separation (mm)
Cortical Porosity (%)
Cortical Pore Diameter (mm)
Week 1
Week 14
Week 28
Week 44

Figure 2. Volumetric bone mineral density (A), geometry (B) and microarchitecture (C) of the tibial metaphysis (4% site) in women during 44-weeks of British Army Officer training (n = 43).

Data are presented as mean (solid lines with circles) with upper and lower 95% confidence intervals (dashed lines), and individual data (faded lines). Non-parametric data (trabecular thickness, trabecular separation, cortical porosity, cortical pore diameter) are presented as median (solid lines with circles) with upper and lower interquartile range (dashed lines), and individual data (faded lines).

vBMD, volumetric bone mineral density.

[a]$P \leq 0.05$ *vs* Week 1; [b]$P \leq 0.05$ *vs* Week 14; [c]$P \leq 0.05$ *vs* Week 28.

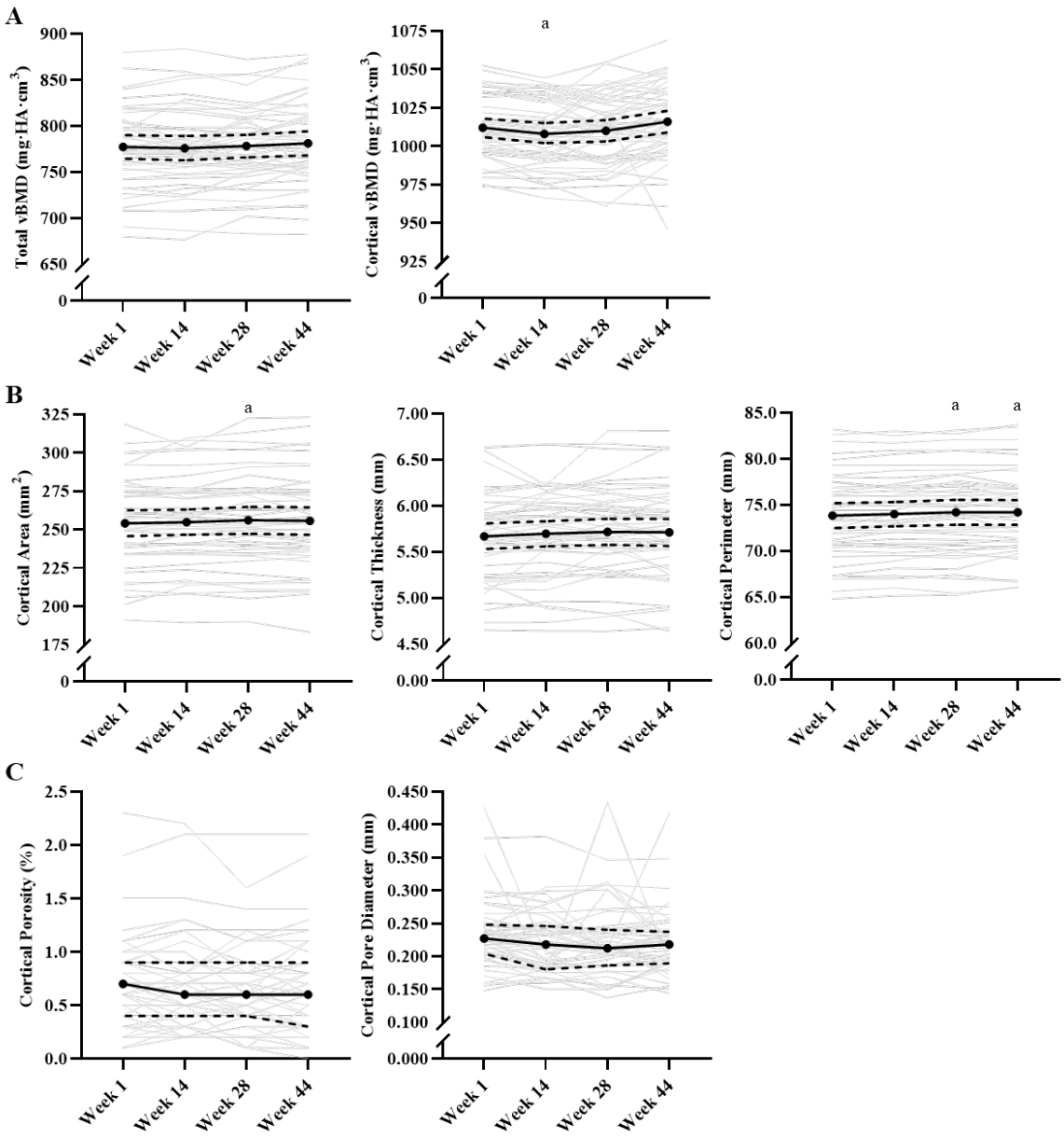


Figure 3. Volumetric bone mineral density (A), geometry (B) and microarchitecture (C) of the tibial diaphysis (30% site) in women during 44-weeks of British Army Officer training (n = 47).

Data are presented as mean (solid lines with circles) with upper and lower 95% confidence intervals (dashed lines), and individual data (faded lines). Non-parametric data (cortical porosity, cortical pore diameter) are presented as median (solid lines with circles) with upper and lower interquartile range (dashed lines), and individual data (faded lines).

vBMD, volumetric bone mineral density.

[a]P ≤ 0.05 *vs* Week 1; [b]P ≤ 0.05 *vs* Week 14; [c]P ≤ 0.05 *vs* Week 28.

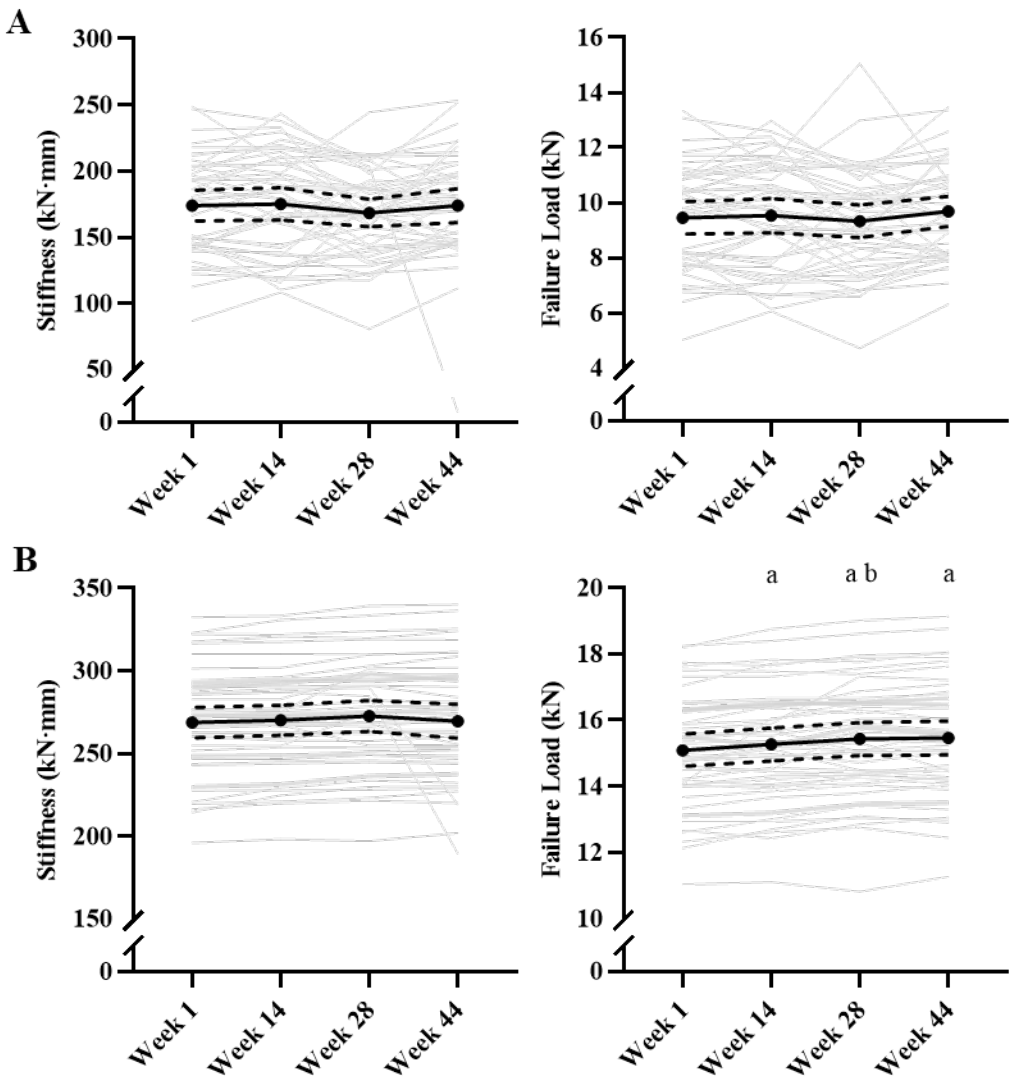


Figure 4. Stiffness and failure load at the tibial metaphysis (A, 4% site, n = 43) and diaphysis (B, 30% site, n = 47) in women during 44-weeks of British Army Officer training. Data are presented as mean (solid lines with circles) with upper and lower 95% confidence intervals (dashed lines) and individual data (faded lines).

[a]P ≤ 0.05 *vs* Week 1; [b]P ≤ 0.05 *vs* Week 14.

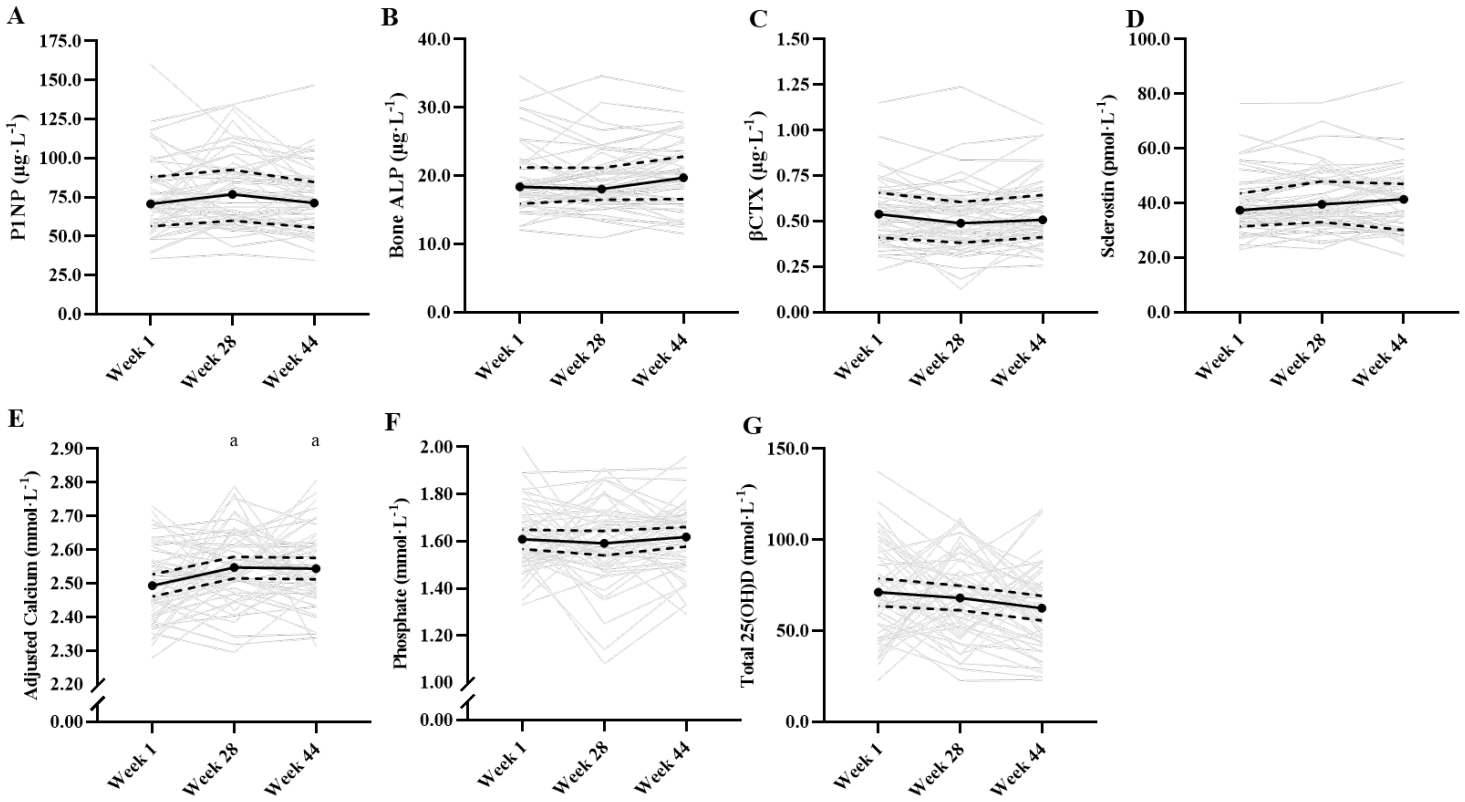


Figure 5. Biochemical markers of bone formation and bone resorption in women during 44-weeks of British Army Officer training (n = 51).

Data are presented as mean (solid lines with circles) with upper and lower 95% confidence intervals (dashed lines), and individual data (faded lines). Non-parametric data (P1NP, bone ALP, βCTX and sclerostin) are presented as median (solid lines with circles) with upper and lower interquartile range (dashed lines), and individual data (faded lines).

A, procollagen 1 N-terminal propeptide (P1NP); B, bone-specific alkaline phosphatase (bone ALP); C, beta C-telopeptide cross-links of type 1 collagen (βCTX); D, sclerostin; E, albumin-adjusted calcium (adjusted calcium); F, phosphate; G, total 25-hydroxyvitamin D (total 25(OH)D).

[a]$P \leq 0.05$ *vs* Week 1.

Supplemental Table 1. Regional and whole-body areal bone mineral density in women separated by contraceptive use during 44-weeks of British Army Officer training. Data are mean ± standard deviation.

	Week 1	Week 14	Week 28	Week 44
Arms aBMD ($g \cdot cm^2$)				
All	0.87 ± 0.10	0.89 ± 0.10	0.88 ± 0.12	0.83 ± 0.13
COCP	0.88 ± 0.09	0.88 ± 0.10	0.84 ± 0.12	0.78 ± 0.13[c]
POC	0.85 ± 0.11	0.91 ± 0.11	0.88 ± 0.15	0.87 ± 0.13[c]
None	0.87 ± 0.11	0.89 ± 0.11	0.92 ± 0.08	0.85 ± 0.12[c]
Legs aBMD ($g \cdot cm^2$)				
All	1.26 ± 0.09	1.26 ± 0.10	1.25 ± 0.09	1.25 ± 0.08
COCP	1.25 ± 0.08	1.25 ± 0.10	1.24 ± 0.08	1.24 ± 0.09
POC	1.29 ± 0.10	1.29 ± 0.12	1.27 ± 0.10	1.28 ± 0.10
None	1.24 ± 0.10	1.24 ± 0.08	1.23 ± 0.07	1.24 ± 0.07
Trunk aBMD ($g \cdot cm^2$)				
All	1.04 ± 0.09	1.04 ± 0.09	1.04 ± 0.09	1.04 ± 0.09
COCP	1.02 ± 0.10	1.02 ± 0.10	1.02 ± 0.10	1.02 ± 0.10
POC	1.06 ± 0.10	1.06 ± 0.10	1.06 ± 0.09	1.06 ± 0.09
None	1.02 ± 0.06	1.02 ± 0.06	1.02 ± 0.06	1.02 ± 0.06
Ribs aBMD ($g \cdot cm^2$)				
All	0.89 ± 0.06	0.87 ± 0.07[a]	0.88 ± 0.07	0.88 ± 0.07
COCP	0.87 ± 0.07	0.86 ± 0.08	0.86 ± 0.08	0.87 ± 0.08
POC	0.90 ± 0.06	0.89 ± 0.07	0.89 ± 0.06	0.89 ± 0.06
None	0.87 ± 0.06	0.86 ± 0.06	0.88 ± 0.05	0.87 ± 0.05
Pelvis aBMD ($g \cdot cm^2$)				
All	1.12 ± 0.12	1.13 ± 0.11	1.13 ± 0.11	1.13 ± 0.11
COCP	1.11 ± 0.14	1.11 ± 0.13	1.12 ± 0.13	1.11 ± 0.12
POC	1.15 ± 0.14	1.15 ± 0.13	1.15 ± 0.13	1.14 ± 0.14
None	1.08 ± 0.07	1.10 ± 0.07	1.09 ± 0.07	1.10 ± 0.07
Spine aBMD ($g \cdot cm^2$)				
All	1.12 ± 0.11	1.12 ± 0.10	1.12 ± 0.10	1.11 ± 0.11
COCP	1.09 ± 0.11	1.10 ± 0.10	1.10 ± 0.09	1.08 ± 0.11
POC	1.15 ± 0.12	1.13 ± 0.11	1.14 ± 0.11	1.13 ± 0.10
None	1.11 ± 0.09	1.13 ± 0.09	1.12 ± 0.08	1.12 ± 0.10
Whole-body aBMD ($g \cdot cm^2$)				
All	1.23 ± 0.08	1.23 ± 0.09	1.22 ± 0.08	1.21 ± 0.09
COCP	1.22 ± 0.09	1.22 ± 0.10	1.21 ± 0.10	1.19 ± 0.10
POC	1.25 ± 0.07	1.26 ± 0.08	1.24 ± 0.09	1.24 ± 0.09
None	1.21 ± 0.08	1.21 ± 0.07	1.21 ± 0.06	1.20 ± 0.07

COCP, combined oral contraceptive pill; POC, progestogen-only contraceptives; None, no hormonal contraceptives; aBMD, areal bone mineral density.

All, n = 51; COCP, n = 18; POC, n = 17; none, n = 11.

[a]$P \leq 0.05$ *vs* Week 1; [b]$P \leq 0.05$ *vs* Week 14; [c]$P \leq 0.05$ *vs* Week 28.

Supplemental Table 2. Volumetric bone mineral density, geometry, and microarchitecture of the tibial metaphysis (4% site) in women during 44-weeks of British Army Officer training. Data are mean ± standard deviation or median [interquartile range].

	Week 1	Week 14	Week 28	Week 44
Total vBMD (mg HA·cm^3)				
All	243 ± 23	245 ± 23[a]	246 ± 23[a]	250 ± 23[a,c]
COCP	240 ± 21	243 ± 21[a]	246 ± 23[a]	250 ± 21[a,c]
POC	242 ± 27	245 ± 27[a]	244 ± 25[a]	248 ± 24[a,c]
None	245 ± 24	248 ± 23[a]	250 ± 25[a]	253 ± 23[a,c]
Trabecular vBMD (mg HA·cm^3)				
All	200 ± 20	202 ± 20[a]	203 ± 20[a]	205 ± 19[a,c]
COCP	197 ± 18	199 ± 16[a]	202 ± 17[a]	204 ± 15[a,c]
POC	199 ± 21	201 ± 22[a]	200 ± 20[a]	203 ± 19[a,c]
None	203 ± 24	205 ± 23[a]	207 ± 24[a]	210 ± 22[a,c]
Cortical vBMD (mg HA·cm^3)				
All	747 ± 45	748 ± 44	744 ± 47	750 ± 45
COCP	748 ± 48	750 ± 48	745 ± 57	754 ± 52
POC	752 ± 45	750 ± 44	749 ± 37	750 ± 42
None	745 ± 34	744 ± 33	739 ± 42	741 ± 34
Trabecular Area (mm^2)				
All	965 ± 123	963 ± 123[a]	962 ± 123[a,b]	961 ± 123[a,c]
COCP	947 ± 127	946 ± 127[a]	945 ± 127[a,b]	944 ± 128[a,c]
POC	969 ± 132	967 ± 133[a]	966 ± 132[a,b]	965 ± 131[a,c]
None	962 ± 110	961 ± 110[a]	959 ± 109[a,b]	957 ± 110[a,c]
Trabecular Volume (%)				
All	28.1 ± 3.3	28.5 ± 3.3[a]	28.5 ± 3.2	29.1 ± 3.1[a,c]
COCP	27.5 ± 2.9	27.8 ± 2.6	28.1 ± 2.8	28.5 ± 2.5[a,c]
POC	27.9 ± 3.6	28.1 ± 3.9	28.0 ± 3.5	28.7 ± 3.1[a,c]
None	29.1 ± 3.5	29.5 ± 3.3	29.6 ± 3.5	30.1 ± 3.4[a,c]
Cortical Area (mm^2)				
All	82 ± 10	83 ± 10[a]	85 ± 11[a,b]	86 ± 11[a,c]
COCP	81 ± 12	83 ± 13[a]	84 ± 13[a,b]	85 ± 13[a,c]

POC	82 ± 9	84 ± 9[a]	85 ± 10[a,b]	86 ± 10[a,c]
None	82 ± 11	83 ± 10[a]	85 ± 10[a,b]	86 ± 11[a,c]
Cortical Thickness (mm)				
All	0.72 ± 0.12	0.73 ± 0.12[a]	0.73 ± 0.12[a]	0.75 ± 0.12[a,c]
COCP	0.72 ± 0.14	0.73 ± 0.15[a]	0.74 ± 0.16[a]	0.75 ± 0.16[a,c]
POC	0.71 ± 0.11	0.73 ± 0.11[a]	0.74 ± 0.11[a]	0.74 ± 0.11[a,c]
None	0.71 ± 0.11	0.72 ± 0.10[a]	0.72 ± 0.09[a]	0.74 ± 0.10[a,c]
Cortical Perimeter (mm)				
All	129.4 ± 8.4	129.2 ± 8.4	130.7 ± 8.5	129.7 ± 8.5
COCP	127.7 ± 8.4	127.6 ± 8.3	128.6 ± 9.2	127.7 ± 8.6
POC	131.1 ± 9.6	131.0 ± 10.2	132.6 ± 7.6	131.2 ± 9.0
None	129.7 ± 7.7	129.5 ± 7.5	131.6 ± 8.8	130.9 ± 8.2
Trabecular Number (1·mm)				
All	1.74 ± 0.17	1.77 ± 0.17	1.81 ± 0.19[a]	1.80 ± 0.17[a]
COCP	1.77 ± 0.16	1.79 ± 0.16[a]	1.85 ± 0.20[a]	1.85 ± 0.17[a]
POC	1.73 ± 0.12	1.77 ± 0.13[a]	1.76 ± 0.13[a]	1.77 ± 0.17[a]
None	1.68 ± 0.23	1.72 ± 0.24[a]	1.80 ± 0.24[a]	1.76 ± 0.20[a]
Trabecular Thickness (mm)*				
All	0.234 [0.228, 0.244]	0.237 [0.226, 0.243]	0.237 [0.230, 0.251][a,b]	0.239 [0.232, 0.248][a]
COCP	0.230 [0.226, 0.244]	0.231 [0.225, 0.240]	0.237 [0.230, 0.257][a,b]	0.238 [0.232, 0.251][a]
POC	0234 [0.225, 0.241]	0.237 [0.230, 0.245]	0.236 [0.227, 0.242]	0.237 [0.229, 0.244]
None	0.239 [0.230, 0.245]	0.242 [0.231, 0.249]	0.251 [0.237, 0.254]	0.248 [0.234, 0.258]
Trabecular Spacing (mm)				
All	0.530 [0.490, 0.554]	0.515 [0.480, 0.552][a]	0.513 [0.468, 0.539][a]	0.511 [0.465, 0.532][a]
COCP	0.534 [0.474, 0.546]	0.524 [0.483, 0.550][a]	0.502 [0.452, 0.543][a]	0.511 [0.463, 0.522][a]
POC	0.520 [0.499, 0.553]	0.509 [0.483, 0.538][a]	0.517 [0.483, 0.541][a]	0.509[0.471, 0.548][a]
None	0.554 [0.473, 0.593]	0.544 [0.466, 0.591][a]	0.520 [0.447, 0.567][a]	0.509 [0.463, 0.560][a]
Cortical Porosity (%)				
All	1.0 [0.9, 1.3]	1.0 [0.8, 1.3]	1.0 [0.7, 1.3]	1.0 [0.8,1.4]
COCP	1.0 [0.7, 1.2]	1.1 [0.7, 1.3]	0.9 [0.5, 1.3]	1.1 [0.6, 1.4]
POC	1.0 [0.9, 1.3]	1.0 [0.8, 1.4]	1.0 [0.8, 1.4]	1.0 [0.9, 1.4]
None	1.1 [0.9, 1.5]	1.0 [1.0, 1.6]	1.0 [0.8, 1.4]	1.0 [1.0, 1.6]
Cortical Pore Diameter (mm)				

All	0.178 [0.168, 0.187]	0.176 [0.168, 0.184]	0.170 [0.163, 0.181][a,b]	0175 [0.166, 0.183]
COCP	0.179 [0.168, 0.189]	0.177 [0.169, 0.190]	0.168 [0.158, 0.185]	0.176 [0.164, 0.187]
POC	0.179 [0.174, 0.184]	0.174 [0.164, 0.179]	0.175 [0.166, 0.181]	0.171 [0.168, 0.181]
None	0.173 [0.163, 0.182]	0.177 [0.165, 0.185]	0.168 [0.161, 0.176]	0.167 [0.166, 0.185]

COCP, combined oral contraceptive pill; POC, progestogen-only contraceptives; None, no hormonal contraceptives; vBMD, volumetric bone mineral density.

All, n = 43; COCP, n = 16; POC, n = 14; None, n = 9.

*$P \leq 0.05$ contraception × time interaction; [a]$P \leq 0.05$ *vs* Week 1; [b]$P \leq 0.05$ *vs* Week 14; [c]$P \leq 0.05$ *vs* Week 28.

Supplemental Table 3. Volumetric bone mineral density, geometry, and microarchitecture of the tibial diaphysis (30% site) in women during 44-weeks of British Army Officer training. Data are mean ± standard deviation or median [interquartile range].

	Week 1	Week 14	Week 28	Week 44
Total vBMD (mg HA·cm^3)				
All	777 ± 44	776 ± 45	778 ± 42	781 ± 45
COCP	779 ± 46	778 ± 49	784 ± 45	783 ± 47
POC	766 ± 43	770 ± 44	767 ± 43	773 ± 47
None	786 ± 42	780 ± 43	787 ± 35	789 ± 41
Cortical vBMD (mg HA·cm^3)				
All	1012 ± 21	1008 ± 21[a]	1010 ± 23	1016 ± 25
COCP	1012 ± 16	1009 ± 17[a]	1016 ± 20	1019 ± 27
POC	1008 ± 25	1005 ± 24[a]	1001 ± 27	1010 ± 26
None	1016 ± 21	1014 ± 20[a]	1019 ± 19	1025 ± 19
Cortical Area (mm^2)				
All	254 ± 29	255 ± 28	256 ± 30[a]	256 ± 30
COCP	246 ± 31	247 ± 20	248 ± 31	247 ± 31
POC	258 ± 23	261 ± 25	261 ± 26	261 ± 26
None	252 ± 35	250 ± 32	253 ± 37	253 ± 38
Cortical Thickness (mm)				
All	5.67 ± 0.48	5.70 ± 0.47	5.72 ± 0.48	5.71 ± 0.50
COCP	5.58 ± 0.45	5.62 ± 0.46	5.60 ± 0.45	5.60 ± 0.45
POC	5.67 ± 0.46	5.74 ± 0.48	5.77 ± 0.45	5.77 ± 0.49
None	5.67 ± 0.58	5.64 ± 0.54	5.69 ± 0.63	5.69 ± 0.63
Cortical Perimeter (mm)				
All	73.8 ± 4.5	74.0 ± 4.4	74.2 ± 4.5[a]	74.2 ± 4.4[a]
COCP	72.1 ± 4.2	72.3 ± 4.2	72.6 ± 4.3[a,b]	72.5 ± 3.9[a]
POC	75.1 ± 4.1	75.3 ± 4.0	75.3 ± 4.1[a,b]	75.4 ± 4.2[a]
None	73.3 ± 5.1	72.5 ± 4.9	73.9 ± 5.3[a,b]	73.8 ± 5.2[a]
Cortical Porosity (%)*				
All	0.7 [0.4, 0.9]	0.6 [0.4, 0.9]	0.6 [0.4, 0.9]	0.6 [0.3, 0.9]
COCP	0.7 [0.4, 0.9]	0.7 [0.5, 0.9]	0.6 [0.4, 0.8][b]	0.7 [0.3, 0.9]

POC	0.6 [0.3, 0.9]	0.6 [0.2, 0.8]	0.5 [0.4, 1.1]	0.6 [0.3, 1.2]
None	0.7 [0.6, 1.1]	0.6 [0.4, 0.9]	0.6 [0.5, 1.1]	0.6 [0.4, 1.0]
Cortical Pore Diameter (mm)*				
All	0.227 [0.204, 0.248]	0.218 [0.180, 0.246]	0.212 [0.186, 0.240]	0.218 [0.189, 0.237]
COCP	0.223 [0.210, 0.235]	0.218 [0.179, 0.244]	0.208 [0.190, 0.216][b]	0.208 [0.180, 0.229]
POC	0.211 [0.185, 0.247]	0.219 [0.167, 0.238]	0.212 [0.178, 0.238]	0.223 [0.189, 0.255]
None	0.270 [0.245, 0.361][c,d]	0.223 [0.201, 0.280]	0.243 [0.220, 0.321][c]	0.228 [0.205, 0.256]

COCP, combined oral contraceptive pill; POC, progestogen-only contraceptives; None, no hormonal contraceptives; vBMD, volumetric bone mineral density.

All, n = 47; COCP, n = 17; POC, n = 15; None, n = 10.

*$P \leq 0.05$ contraception × time interaction; [a]$P \leq 0.05$ *vs* Week 1; [b]$P \leq 0.05$ *vs* Week 14; [c]$P \leq 0.05$ *vs* COCP; [d]$P \leq 0.05$ *vs* POC.

Supplemental Table 4. Estimated mechanical strength at the tibial metaphysis and diaphysis (30% site) in women separated by contraceptive use during 44-weeks of British Army Officer training. Data are mean ± standard deviation.

	Week 1	Week 14	Week 28	Week 44
Tibial Metaphysis (4% site)				
Stiffness (kN·mm)				
All	174 ± 38	175 ± 40	168 ± 34	174 ± 41
COCP	163 ± 42	166 ± 37	159 ± 32	169 ± 32
POC	169 ± 34	167 ± 44	168 ± 35	162 ± 50
None	191 ± 36	191 ± 36	182 ± 35	190 ± 39
Failure Load (kN)				
All	9.5 ± 1.9	9.5 ± 2.0	9.3 ± 1.9	9.7 ± 1.8
COCP	8.9 ± 2.2	9.1 ± 1.9	9.1 ± 2.2	9.2 ± 1.6
POC	9.2 ± 1.7	9.1 ± 2.2	9.1 ± 1.8	9.5 ± 1.6
None	10.3 ± 2.0	10.4 ± 1.8	9.9 ± 1.8	10.3 ± 1.9
Tibial Diaphysis (30% site)				
Stiffness (kN·mm)				
All	269 ± 31	270 ± 31	273 ± 32	269 ± 35
COCP	259 ± 33	261 ± 32	263 ± 32	258 ± 36
POC	275 ± 26	276 ± 27	278 ± 27	272 ± 32
None	266 ± 39	267 ± 38	273 ± 41	274 ± 40
Failure Load (kN)				
All	15.1 ± 1.7	15.3 ± 1.7[a]	15.4 ± 1.7[a,b]	15.5 ± 1.7[a]
COCP	14.6 ± 1.7	14.7 ± 1.7[a]	14.9 ± 1.6[a,b]	14.9 ± 1.7[a]
POC	15.6 ± 1.4	15.6 ± 1.5[a]	15.8 ± 1.5[a,b]	15.8 ± 1.6[a]
None	14.9 ± 2.1	15.1 ± 1.7[a]	15.4 ± 2.2[a,b]	15.4 ± 2.2[a]

COCP, combined oral contraceptive pill; POC, progestogen-only contraceptives; None, no hormonal contraceptives.

Tibial metaphysis: all, n = 43; COCP, n = 16; POC, n = 14; none, n = 9.

Tibial diaphysis: all, n = 47; COCP, n = 17; POC, n = 15; none, n = 10.

[a]$P \leq 0.05$ *vs* Week 1; [b]$P \leq 0.05$ *vs* Week 14.

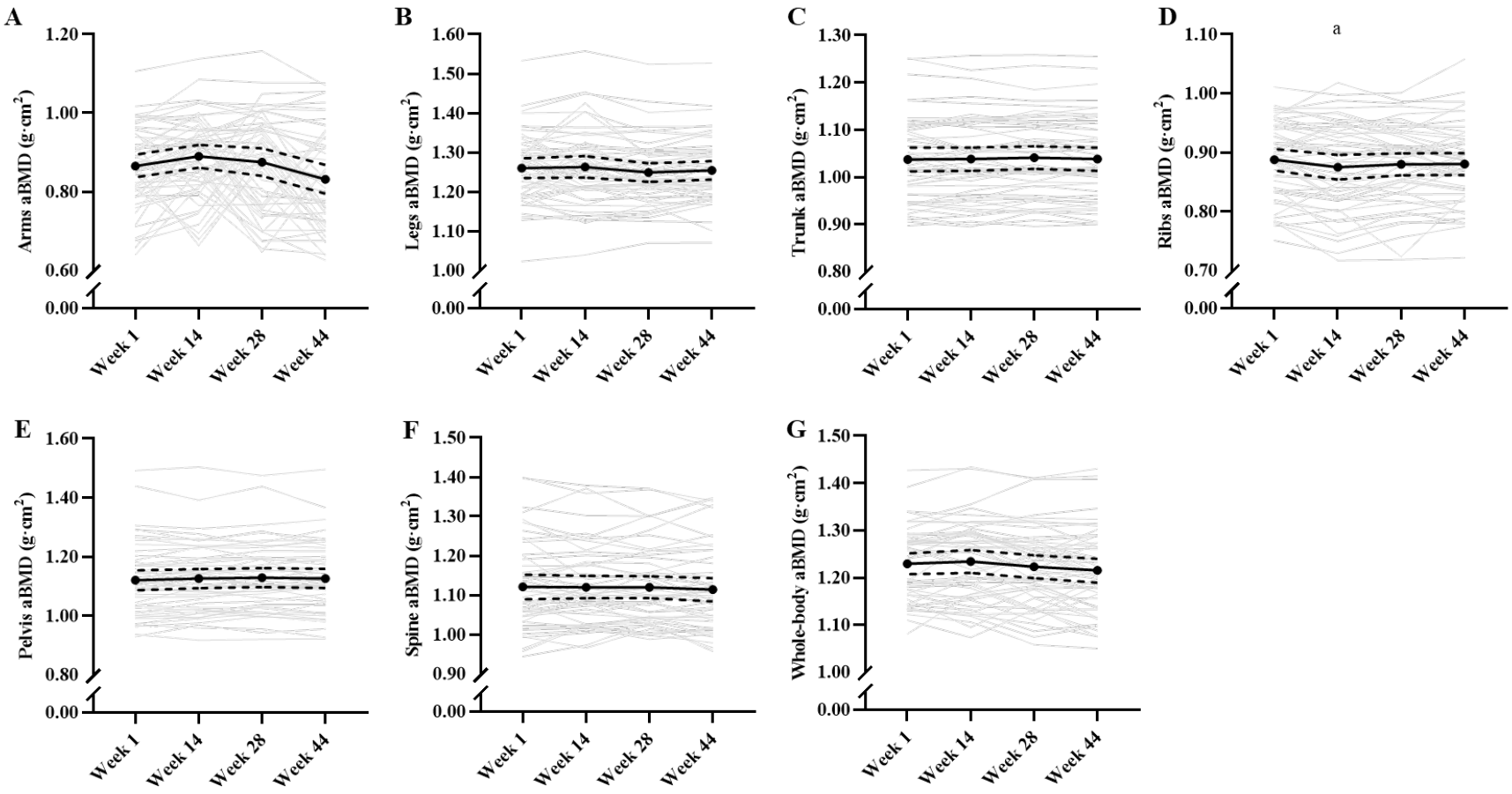
Supplemental Table 5. Markers of bone formation and bone resorption in women separated by contraceptive use during 44-weeks of British Army Officer training. Data are mean ± standard deviation or median [interquartile range].

	Week 1	Week 28	Week 44
P1NP ($\mu g \cdot L^{-1}$)*			
All	70.7 [56.5, 86.6]	76.8 [60.4, 92.7]	71.2 [56.2, 83.5]
COCP	61.3 [50.5, 77.5]	65.9 [54.6, 93.5]	67.7 [57.1, 79.4]
POC	81.8 [68.9, 115.1][b]	78.0 [63.4, 90.7]	75.5 [54.3, 92.2]
None	68.2 [58.1, 84.9]	84.4 [63.7, 105.1]	73.7 [64.6, 80.3]
Bone ALP ($\mu g \cdot L^{-1}$)			
All	18.4 [16.2, 21.2]	18.0 [16.5, 21.1]	19.6 [16.6, 22.8]
COCP	18.1 [15.6, 18.7]	18.4 [17.6, 22.2]	20.1 [16.7, 24.4]
POC	18.4 [16.5, 23.8]	17.5 [16.3, 20.5]	19.5 [17.4, 21.2]
None	19.1 [17.7, 21.7]	20.4 [16.4, 24.2]	21.0 [15.9, 26.4]
βCTX ($\mu g \cdot L^{-1}$)			
All	0.53 [0.41, 0.65]	0.49 [0.38, 0.59]	0.51 [0.42, 0.63]
COCP	0.49 [0.38, 0.59]	0.43 [0.33, 0.60]	0.49 [0.40, 0.59]
POC	0.63 [0.42, 0.74]	0.53 [0.40, 0.63]	0.55 [0.42, 0.76]
None	0.55 [0.42, 0.59]	0.53 [0.36, 0.60]	0.55 [0.44, 0.66]
Sclerostin ($pmol \cdot L^{-1}$)			
All	37.4 [31.9, 43.9]	39.6 [33.8, 48.0]	41.4 [30.3, 47.2]
COCP	33.0 [28.9, 40.6]	36.9 [31.3, 47.9]	30.8 [27.8, 41.7]
POC	40.9 [37.0, 50.6][b]	40.3 [35.8, 50.8]	43.2 [41.3, 47.4][b]
None	36.7 [31.5, 39.6]	35.0 [32.1, 43.4]	36.9 [29.0, 45.4]
Adjusted Calcium ($mmol \cdot L^{-1}$)			
All	2.49 ± 0.11	2.55 ± 0.11[a]	2.54 ± 0.11[a]
COCP	2.48 ± 0.10	2.55 ± 0.09[a]	2.53 ± 0.11[a]
POC	2.51 ± 0.12	2.61 ± 0.11[a]	2.55 ± 0.12[a]
None	2.48 ± 0.12	2.50 ± 0.07[a]	2.57 ± 0.12[a]
Phosphate ($mmol \cdot L^{-1}$)			
All	1.61 ± 0.14	1.59 ± 0.18	1.62 ± 0.15
COCP	1.56 ± 0.10	1.53 ± 0.23	1.55 ± 0.14
POC	1.67 ± 0.15	1.64 ± 0.13	1.64 ± 0.12
None	1.59 ± 0.18	1.62 ± 0.17	1.63 ± 0.16
Total 25(OH)D ($nmol \cdot L^{-1}$)			
All	71.2 ± 27.0	68.1 ± 23.6	62.4 ± 23.7
COCP	77.9 ± 31.0	79.4 ± 24.9	70.5 ± 19.8
POC	74.6 ± 26.7	61.0 ± 21.5	64.7 ± 30.2
None	57.0 ± 16.7	69.7 ± 20.8	53.9 ± 14.8

COCP, combined oral contraceptive pill; POC, progestogen-only contraceptives; None, no hormonal contraceptive; P1NP, procollagen 1 N-terminal propeptide; Bone ALP, bone-specific alkaline phosphatase; βCTX, beta C-telopeptide cross-links of type 1 collagen; Adjusted Calcium, albumin-adjusted calcium; total 25(OH)D.

All, n = 51; COCP, n = 18; POC, n = 17; None, n = 11.

*$P < 0.05$ contraception × time interaction; [a]$P < 0.05$ *vs* Week 1; [b]$P < 0.05$ *vs* COCP users.



Supplemental Figure 1. Regional (A to F) and whole-body (G) areal bone mineral density in women during 44-weeks of British Army Officer training (n = 51). Data are presented as mean (solid lines with circles) with upper and lower 95% confidence intervals (dashed lines), and individual data (faded lines).

aBMD, areal bone mineral density.

[a]P ≤ 0.05 *vs* Week 1; [b]P ≤ 0.05 *vs* Week 14; [c]P ≤ 0.05 *vs* Week 28