Sex differences in tibial adaptations to arduous training: an observational cohort study

Thomas J O'Leary PhD[1,2], Rachel M Izard PhD[3], Jonathan CY Tang PhD[4], William D Fraser MB ChB MD[4,5], Julie P Greeves PhD[1,2,4]

[1]Army Health and Performance Research, Army HQ, Andover, UK; [2]Division of Surgery and Interventional Science, UCL, London, UK; [3]Defence Science and Technology, Porton, UK; [4]Norwich Medical School, University of East Anglia, Norwich, UK; [5]Norfolk and Norwich University Hospital, Norwich, UK

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Corresponding author: Julie P Greeves OBE PhD, Army Health and Performance Research, Andover, Hampshire, SP11 8HT, United Kingdom. Email: julie.greeves143@mod.gov.uk.

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Abstract

Military training increases tibial density and size, but it is unknown if men and women adapt similarly to the same arduous training. Seventy-seven men and 57 women not using hormonal contraceptives 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. Training increased trabecular vBMD (4% site in men; 4% and 14% sites in women), cortical vBMD (38% site), total area (14% and 38% sites), trabecular area (14% site), cortical area and thickness (14%, 38%, and 66% sites), periosteal perimeter (14%, 38%, and 66% sites), and all indices of estimated strength (14%, 38%, and 66% sites); and, decreased endosteal perimeter (66% site) in men and women (all $p \leq 0.045$). The increase in trabecular vBMD (4% and 14% sites) was greater in women and the increases in cortical area and strength (38% site) were greater in men (sex × time interactions, all $p \leq 0.047$). P1NP increased and βCTX and sclerostin decreased during training in men and women, consistent with adaptive bone formation. PTH decreased in men but increased in women. Arduous weight-bearing activity increased the density and size of the tibia after 14 weeks. Women experienced similar tibial adaptations as men, however, a greater increase in trabecular vBMD in women compared with men could be due to higher loading at this skeletal site in women, whereas the small increase in cortical area could be due to inhibitory effects of oestradiol. **Key words:** Bone Modeling and Remodeling; Biochemical Markers of Bone Turnover; Exercise; pQCT; Sex Steroids.

1. Introduction

Sexual dimorphism in long bone growth occurs from puberty. An increase in oestrogens in women inhibits periosteal bone formation and promotes endosteal bone formation, whereas an increase in androgens in men supports continued periosteal expansion and endosteal remodelling [1, 2]. Androgens have a direct effect on bone through the androgen receptor, but oestradiol is the main regulator of bone metabolism in men through peripheral aromatisation of testosterone [3, 4]. Men also develop more lean mass than women that elicits higher mechanical loading of bone [2]. Therefore, adult men have a thicker cortex and larger cortical and total area of the tibia than women [5, 6]. These sex differences in oestradiol and androgen concentrations, and skeletal size, may influence the bone adaptive response to mechanical loading.

Basic military training is characterised by a sudden increase in unaccustomed and repetitive mechanical loading, which promotes remodelling of fatigue damage that can eventually lead to a stress fracture [7]. Sex differences in bone geometry cause women to have a weaker long bone relative to body size [8] and a greater displacement of the cortex from the neutral axis resulting in less resistance to bending stresses [2, 9, 10] than men. Consequently, women typically have a three-fold higher risk of stress fracture than men during arduous training [11]. These high mechanical loads also contribute to adaptive bone formation [9, 12], which because of biomechanical and biochemical sex differences might result in different patterns of skeletal adaptation between men and women. Several studies have reported marked changes in tibial geometry and density within 14-weeks of military training in men and women [13-19], providing evidence of adaptive bone formation and high mechanical stresses at the tibia [9]. Increased [19-22] or unchanged [14-17] biochemical markers of bone formation, and decreased [16, 17, 21] and increased [19, 20, 22] biochemical markers of bone resorption have also been

reported in men and women during military training. One randomised controlled trial investigating calcium and vitamin D supplementation reported similar tibial adaptions to 9 weeks basic military training in men and women [14]. Whether men and women experience similar tibial adaptations and changes in biochemical markers of bone metabolism to military training is poorly understood, but may provide important insight into sex differences in bone mechanobiology during loading.

The primary aim of this study was to examine the sex difference in the adaptive bone response to basic military training. This study compared changes in tibial density and geometry, and biochemical markers of bone metabolism, between men and women during 14-weeks of British Army basic training. We hypothesised that women would experience increased mineralisation to compensate for lower cross-sectional area, but men would experience periosteal expansion. Secondary aims were to examine the effects of basic military training on biochemical markers of bone metabolism. We hypothesised that women would experience greater increases in markers of bone formation and bone resorption during training.

2. Materials and Methods

2.1. Participants

A cohort of 178 British Army recruits (101 men, 77 women) volunteered to participate in this study during week one of their British Army basic military training at the Army Training Centre, Pirbright. Men commenced training between March and July, whereas women commenced training across all months; fewer women enter the British Army than men and so it was necessary to recruit women all year round. 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; amenorrhea or oligomenorrhoea; or 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 [23]. Recruits undertook 43 periods of military drill, 79 physical training periods including 21 loaded marches (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 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, and height (Seca 225, Seca Ltd, UK) and body mass (Seca 770, Seca Ltd, UK) were measured in standard issue military clothing without boots. Women additionally completed a British Army lifestyle questionnaire asking about contraceptive use and age of menarche. Tibial density, geometry, and estimated bone strength indices were measured by pQCT at the start (week 1) and end (week 14) of British Army standard entry basic military training. Blood samples were obtained at weeks 1, 2, 4, 6, 10, and 14 for the assessment of biochemical markers of bone metabolism. All week 1 measurements were made immediately following the initial medical assessment and before any military training had commenced.

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 (≤ 15 min). Initial scout scans were conducted at a speed of 40 $mm \cdot s^{-1}$ 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 $mm \cdot s^{-1}$ 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 $mg \cdot cm^3$. The following outcomes were determined using the Bone Alignment and Measurement Package (BAMPack) (L-3 ATI, USA) as described previously[13]: total area, bone mineral content (BMC), trabecular vBMD, trabecular area, cortical vBMD, cortical area, cortical thickness, periosteal perimeter (PPm), and endosteal perimeter (EPm), polar moment of inertia (i), mass moment of inertia (MM_i), and bone strength index (BSI). Density thresholds above 800 $mg \cdot mm^3$ and below 600 $mg \cdot mm^3$ were used to define cortical and trabecular bone with voxels with density values between 600 and 800 $mg \cdot mm^3$ removed from analysis to delineate between cortical and trabecular regions. Calf muscle area (mm^{-2}) and calf muscle density ($mg \cdot cm^3$) were also calculated [13]. 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 coefficient of variation (CV) of our measures were ≤ 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). 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 1 N-terminal propeptide (P1NP), c-terminal cross-links telopeptide of type 1 collagen (βCTX), intact parathyroid hormone (iPTH), and osteocalcin by electro-chemiluminescence 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); 25-hydroxyvitamin D (25(OH)D) by liquid chromatography tandem mass spectrometry (LC-MS/MS)[24], and; total calcium, albumin, and phosphate (PO_4) on the c501 COBAS platform (Roche). 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 $pmol \cdot L^{-1}$, 0.07 - 20.00 $pmol \cdot L^{-1}$, and 2.6 - 240.0 $pmol \cdot L^{-1}$, respectively. Bone ALP ELISA assays (MicroVue, Quidel Corp., Germany) had inter-assay CVs < 8.0% across the assay working range of 0.7 -149.0 $U \cdot L^{-1}$. 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 $nmol \cdot L^{-1}$. Calcium, albumin, and PO_4 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 PO4 was ≤ 2.1% across the assay working ranges of 0.20 - 5.00 $mmol \cdot L^{-1}$, 2 - 60 $g \cdot L^{-1}$, and 0.81 - 1.45 $mmol \cdot L^{-1}$, respectively. Albumin-

adjusted calcium (ACa) value was calculated as ACa = -0.8 × [Albumin] - 4) + [Total calcium]. All biochemical analysis was undertaken by the Clinical Pathology Accredited Bioanalytical Facility at the University of East Anglia. All analytical processes meet the requirements specified by external national quality assurance schemes.

2.5. Statistical Analyses

Data were analysed using the R Programming Language (v.4.1.0). Distribution of the data were checked using Shapiro-Wilk tests and frequency distribution histograms. Participant characteristics were compared between women and men with independent samples *t*-tests (or Welch's *t*-test for groups with unequal variances) and Mann-Whitney U tests for data that were not normally distributed. Linear mixed effect models with restricted maximum likelihood estimation were used to examine changes in pQCT outcomes and biochemical markers of bone metabolism (*lme4 package v*.1.1-27.1). Sex (Women *vs* Men), time (pQCT: pre-training *vs* post-training, bone metabolism: 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 sex differences. 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. The BSI models did not convergence and were analysed using sex × time ANOVAs. In the event of a significant main effect of time or significant interaction, pairwise comparisons with Bonferroni corrections and Kerward-Roger degrees of freedom were used on the linear mixed effects model to identify differences between time points or group (*emmeans package v*.1.7.0). Pooled data were used for main effects when there was no significant interaction, and each sex was analysed independently when there was a significant interaction. Effect sizes are

presented as partial eta-squared (η_p^2) for main and interaction effects, Cohen's d for between-group comparisons, and paired Cohen's d for within-group paired comparisons (*effectsize package v*.0.5). Figures were drawn in the *ggplot2 package* (*v*.3.3.5). Significance was accepted as $p \leq 0.05$.

3. Results

A total of 44 participants were lost to follow-up (*n* = 24 men, *n* = 20 women) due to failure to complete training. Complete pQCT data were available for 77 men and 57 women (Figure 1). Men were taller, heavier, had a longer tibia, completed the 2.4 km run in a faster time, and had a bigger calf muscle area ($p < 0.001$), but age and calf muscle density were not different ($p \geq 0.258$) (Table 1). Training increased calf muscle area increased in men (8,271 ± 1,019 mm^{-2} *vs* 8,412 ± 1,458 mm^{-2}) and women (6,825 ± 825 mm^{-2} *vs* 7,2302 ± 1,458 mm^{-2}) (main effect of time, $p < 0.001$, $\eta_p^2 = 0.08$) with no difference between sexes (sex × time interaction, $p = 0.093$, $\eta_p^2 = 0.02$). Training had no effect on calf muscle density in either sex. 83% of women reported regular periods at baseline and 20% reported changes during training including irregular or lighter periods.

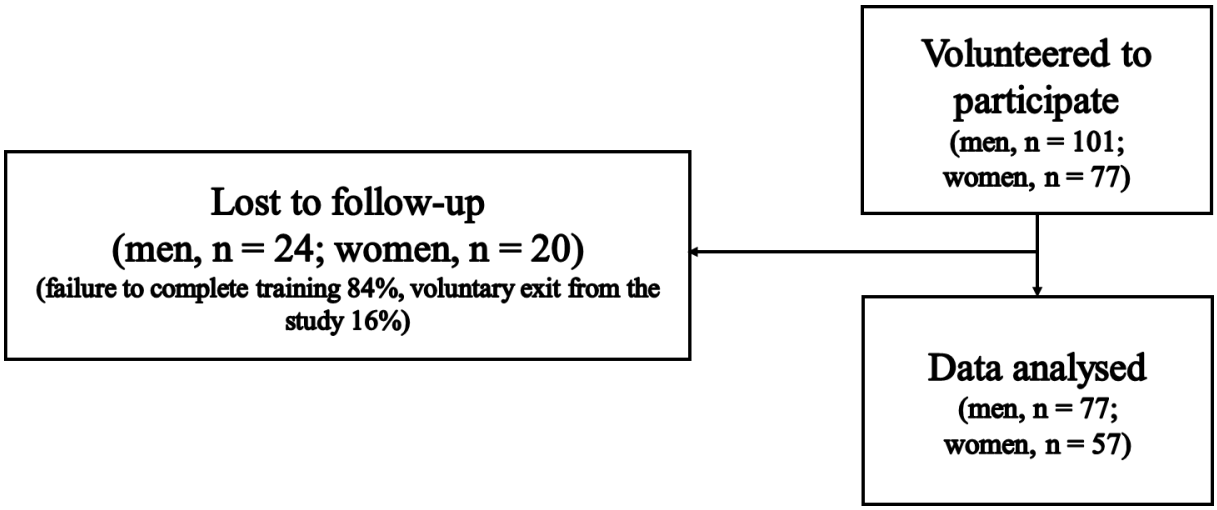

Figure 1. Participant flow through the study.

Table 1. Demographic data. Data are mean ± SD or median [interquartile range].

	Men *n* = 77	Women *n* = 57
Age (years)	20 [19, 23]	20 [18, 23]
Height (m)	1.77 ± 0.07[a]	1.65 ± 0.07
Body Mass (kg)	74.4 ± 9.9[a]	63.5 ± 8.2
Tibia Length (mm)	396 ± 20[a]	372 ± 21
2.4 km Run Time (s)*	618 ± 46[a]	775 ± 77
Muscle Area (mm^{-2})	8,271 ± 1,019[a]	6,825 ± 825
Muscle Density ($mg \cdot cm^{-3}$)	75.9 ± 1.4	75.4 ± 1.4

[a]$p < 0.05$ *vs* women
*n = 30 men and n = 53 women

Tibial density, geometry, and strength indices are presented in Table 2. Mean absolute changes in density, geometry, and strength indices are shown in Supplemental Table 1.

3.1. Volumetric Bone Mineral Density

3.1.2 Trabecular Bone

There was a sex × time interaction for trabecular vBMD at the 4% and 14% sites ($p \leq 0.017$, $\eta_p^2 \geq 0.043$). Trabecular vBMD at the 4% site increased in men and women ($p < 0.001$, $d \geq 0.864$), with greater increases in women. Trabecular vBMD at the 14% site increased for women ($p = 0.004$, $d_z = 0.343$), but not for men ($p = 0.666$, $d_z = 0.055$). Trabecular vBMD was higher for men than women at the 4% and 14% site ($p < 0.001$, $d \geq 0.701$).

3.1.3 Cortical Bone

Training increased cortical vBMD at the 38% site (main effect of time, $p = 0.032$, $\eta_p^2 = 0.034$), but not at the 14% or 66% site (main effects of time, $p \geq 0.144$, $\eta_p^2 \leq 0.016$), with no differences between men and women (sex × time interactions, $p \geq 0.243$, $\eta_p^2 \leq 0.010$). Cortical vBMD was higher for women than men at all sites (main effects of sex, $p \leq 0.027$, $\eta_p^2 \geq 0.037$).

3.2. Geometry

Training increased total area at the 14% and 38% sites (main effects of time, $p < 0.001$, $\eta_p^2 \geq 0.093$), but not the 66% site (main effect of time, $p = 0.255$, $\eta_p^2 = 0.010$), with no differences between men and women (sex × time interactions, $p \geq 0.280$, $\eta_p^2 \leq 0.009$). Total area was higher for men than women at all sites (main effects of sex, $p < 0.001$, $\eta_p^2 \geq 0.195$). Training increased PPm at the 14%, 38%, and 66% sites, and decreased EPm at the 66% site (main effects of time, $p \leq 0.010$, $\eta_p^2 \geq 0.050$), but had no effect on PPm or EPm at other sites (main effects of time, $p \geq 0.070$, $\eta_p^2 \leq 0.025$), with no differences between men and women (sex × time interactions, $p \geq 0.120$, $\eta_p^2 \leq 0.018$). PPm and EPm were greater for men than women at all sites (main effects of sex, $p \leq 0.001$, $\eta_p^2 \geq 0.081$).

3.2.2 Trabecular Bone

Training increased trabecular area at the 14% site (main effect of time, $p = 0.010$, $\eta_p^2 = 0.049$), with no difference between men and women (sex × time interaction, $p = 0.386$, $\eta_p^2 = 0.006$). Trabecular area at the 14% site was higher for men than women (main effect of sex, $p = 0.027$, $\eta_p^2 = 0.036$).

3.2.3 Cortical Bone

Training increased cortical area at the 14% and 66% site (main effects of time, $p \leq 0.014$, $\eta_p^2 \geq 0.045$), with no difference between men and women (sex × time interaction, $p \geq 0.451$, $\eta_p^2 \leq 0.004$). There was a significant sex × time interaction for cortical area at the 38% site ($p = 0.047$, $\eta_p^2 = 0.030$). Cortical area increased at the 38% site in men and women ($p \leq 0.017$, $d \geq 0.332$), with the increase greater in men. Cortical area was higher for men than women at all sites (14% and 66% sites, main effects of sex, $p < 0.001$, $\eta_p^2 \geq 0.365$; 38% site, *post-hoc*, $p <$

0.001, d ≥ 1.517). Training increased cortical thickness at all sites (main effects of time, p ≤ 0.028, $\eta_p^2 \geq 0.036$), with no differences between men and women (sex × time interactions, p ≥ 0.179, $\eta_p^2 \leq 0.014$). Cortical thickness was higher for men than women at all sites (main effects of sex, p < 0.001, $\eta_p^2 \geq 0.154$).

3.3. Bone Strength

There was a significant sex × time interactions for BSI at the 38% site (p = 0.024); the increase in BSI was greater for men (p < 0.001, d = 0.587) than women (p = 0.033, d = 0.398). Training increased BSI at the 14% and 66% sites (main effects of time, p ≤ 0.001), with no differences between men and women (sex × time interactions, p ≥ 0.343). BSI was higher for men than women at all sites (14% and 66% site, main effects of sex, p < 0.001; 38% site, *post-hoc*, p < 0.001, d ≥ 1.552).

Table 2. Tibial density, geometry, and strength indices in men and women in response to British Army basic training. Data are mean ± SD or median [interquartile range].

	Men (n = 77)			Women (n = 57)		
	Pre-Training	Post-Training	Change (%)	Pre-Training	Post-Training	Change (%)
4% site						
Tb vBMD ($mg \cdot cm^{-3}$)*	302 ± 24[c]	305 ± 23[b,c]	**1.0 ± 1.2**	282 ± 29	287 ± 29[b]	**1.6 ± 1.5**
PPm (mm^{-1})	129.2 ± 8.0[c]	128.9 ± 8.3[c]	−0.2 ± 1.5	115.3 ± 7.6	115.6 ± 7.6	0.3 ± 1.5
14% site						
Tb vBMD ($mg \cdot cm^{-3}$)*	272 ± 18[c]	272 ± 18[c]	−0.1 ± 1.7	256 ± 22	258 ± 21[b]	**0.8 ± 2.3**
Ct vBMD ($mg \cdot cm^{-3}$)	1141 ± 23	1142 ± 24	0.1 ± 0.6	1150 ± 18	1150 ± 18	0.0 ± 0.7
Total Area (mm^{-2})	474 ± 76[c]	476 ± 76[a,c]	**0.3 ± 1.2**	406 ± 58	408 ± 57[a]	**0.5 ± 1.1**
Tb Area (mm^{-2})	181 ± 52[c]	182 ± 52[a,c]	**0.7 ± 4.2**	160 ± 53	162 ± 55[a]	**1.3 ± 4.0**
Ct Area (mm^{-2})	186 ± 25[c]	186 ± 25[a,c]	**0.4 ± 1.6**	150 ± 20	150 ± 20[a]	**0.4 ± 1.6**
Ct Thickness (mm^{-1})	2.27 ± 0.37[c]	2.28 ± 0.37[a,c]	**0.5 ± 2.2**	1.90 ± 0.33	1.91 ± 0.33[a]	**0.5 ± 2.5**
PPm (mm^{-1})	79.7 ± 6.4[c]	79.8 ± 6.3[a,c]	**0.1 ± 0.4**	73.2 ± 4.9	73.3 ± 4.8[a]	**0.2 ± 0.5**
EPm (mm^{-1})	65.0 ± 7.0[c]	64.9 ± 7.0[c]	−0.1 ± 0.8	61.0 ± 5.7	61.1 ± 5.7	0.1 ± 0.8
BSI ($g \cdot cm^{-4}$)	2.77 ± 0.75[c]	2.79 ± 0.74[a,c]	**0.6 ± 1.4**	1.92 ± 0.43	1.93 ± 0.43[a]	**0.7 ± 1.3**
38% site						
Ct vBMD ($mg \cdot cm^{-3}$)	1167 ± 21[c]	1167 ± 19[a,c]	**0.0 ± 0.5**	1187 ± 23	1188 ± 20[a]	**0.2 ± 0.5**
Total Area (mm^{-2})	418 ± 52[c]	421 ± 52[a,c]	**0.6 ± 1.4**	340 ± 41	341 ± 41[a]	**0.4 ± 1.4**
Ct Area (mm^{-2})*	323 ± 43[c]	326 ± 43[b,c]	**0.9 ± 1.5**	263 ± 34	265 ± 34[b]	**0.6 ± 1.5**
Ct Thickness (mm^{-1})	5.21 ± 0.67[c]	5.25 ± 0.67[a,c]	**0.9 ± 1.4**	4.65 ± 0.57	4.68 ± 0.57[a]	**0.6 ± 1.7**
PPm (mm^{-1})	78.8 ± 5.1[c]	79.0 ± 5.0[a,c]	**0.2 ± 0.6**	70.6 ± 4.6	70.7 ± 4.6[a]	**0.1 ± 0.5**
EPm (mm^{-1})	40.9 ± 5.0[c]	40.9 ± 5.0[c]	−0.1 ± 1.1	37.3 ± 4.2	37.2 ± 4.1	−0.2 ± 1.2
BSI ($g \cdot cm^{-4}$)*	3.55 [3.09, 4.23][c]	3.58 [3.13, 4.33][b,c]	**1.4 ± 2.3**	2.37 [2.08, 2.81]	2.44 [2.11, 2.77][b]	**0.9 ± 2.0**
66% site						

Ct vBMD ($mg \cdot cm^{-3}$)	1134 ± 20[c]	1134 ± 19[c]	0.1 ± 0.5	1150 ± 20	1151 ± 18	0.0 ± 0.5
Total Area (mm^{-2})	584 ± 80[c]	585 ± 80[c]	0.1 ± 1.1	480 ± 68	481 ± 68	0.2 ± 1.3
Ct Area (mm^{-2})	337 ± 47[c]	339 ± 47[a,c]	**0.4 ± 1.0**	273 ± 35	274 ± 35[a]	**0.7 ± 1.3**
Ct Thickness (mm^{-1})	3.97 ± 0.60[c]	4.00 ± 0.61[a,c]	**0.6 ± 1.5**	3.50 ± 0.47	3.53 ± 0.47[a]	**0.8 ± 1.6**
PPm (mm^{-1})	96.7 ± 6.4[c]	96.7 ± 6.3[a,c]	**0.1 ± 0.4**	86.5 ± 5.9	86.6 ± 5.9[a]	**0.1 ± 0.5**
EPm (mm^{-1})	66.8 ± 7.6[c]	66.6 ± 7.5[a,c]	**−0.2 ± 1.0**	60.3 ± 6.5	60.2 ± 6.4[a]	**−0.2 ± 0.8**
BSI ($g \cdot cm^{-4}$)	6.49 [5.46, 7.53][c]	6.49 [5.44, 7.60][a,c]	**0.4 ± 1.3**	4.25 [3.71, 5.03]	4.35 [3.75, 5.13][a]	**0.9 ± 1.8**

BSI, bone strength index; Ct, cortical; EPm, endosteal perimeter; PPm, periosteal perimeter; Tb, trabecular; vBMD, volumetric bone mineral density.
*$p < 0.05$ sex × time interaction; [a]$p < 0.05$ *vs* pre-training (main effect of time); [b]$p < 0.05$ *vs* pre-training (*post-hoc*, within sex); [c]$p < 0.05$ *vs* women.
Bold values represent a significant change ($p < 0.05$)

3.4. Biochemical Markers of Bone Metabolism

Biochemical markers of bone metabolism are shown in Figures 2 and 3 with mean absolute changes presented in Supplemental Table 2. There were sex × time interactions for βCTX, OPG, osteocalcin, and sclerostin ($p \leq 0.043$, $\eta_p^2 \geq 0.018$). βCTX decreased from week 1 to week 2, 4, 6, 10, and 14 in men ($p < 0.001$, $d \geq 0.648$), and decreased from week 1 to week 2, 4, 6, and 14 in women ($p \leq 0.005$, $d \geq 0.359$). βCTX was higher in men than women at all time-points ($p < 0.001$, $d \geq 0.638$). OPG increased from week 1 to week 2, 4, 6, 10 and 14 in men ($p \leq 0.005$, $d \geq 0.488$), and increased from week 1 to week 2, 6, and 10 in women ($p \leq 0.045$, $d \geq 0.359$). OPG was higher in men than women at week 2 and 4 ($p < 0.001$, $d \geq 0.676$). Osteocalcin decreased from week 1 to week 2, 4, and 14 in men ($p \leq 0.045$, $d \geq 0.270$), and decreased from week 1 to week 2 and 4 in women ($p \leq 0.010$, $d \geq 0.403$). Osteocalcin was higher in men than women at week 1, 2, 4, 6, and 10 ($p \leq 0.005$, $d \geq 0.514$). Sclerostin decreased from week 1 to week 4, 6, and 10 in men ($p \leq 0.005$, $d \geq 0.441$) and decreased from week 1 to 4 in women ($p = 0.035$, $d = 0.371$). Sclerostin was higher in men than women at all time-points ($p \leq 0.045$, $d \geq 0.516$). There was a main effect of time ($p < 0.001$, $\eta_p^2 = 0.072$), but no sex × time interaction ($p = 0.230$, $\eta_p^2 = 0.011$) for P1NP. P1NP increased from week 1 to week 2, 4, 6, 10, and 14 in both sexes ($p \leq 0.001$, $d \geq 0.293$). P1NP was higher in men than women (main effect of sex, $p = 0.003$, $\eta_p^2 = 0.063$). There was a main effect of time ($p = 0.019$, $\eta_p^2 = 0.021$), but no sex × time interaction ($p = 0.478$, $\eta_p^2 = 0.007$) for bone ALP. Post-hoc analysis showed no two time-points were different for bone ALP. Bone ALP was higher in men than women (main effect of sex, $p = 0.001$, $\eta_p^2 = 0.074$). There was no effect of training or sex on sRANKL (main effect of time, $p = 0.097$, $\eta_p^2 = 0.015$; main effect of sex, $p = 0.252$, $\eta_p^2 = 0.010$; sex × time interaction, $p = 0.053$, $\eta_p^2 = 0.017$) or OPG:sRANKL (main effect of time, $p = 0.201$, $\eta_p^2 = 0.011$; main effect of sex, $p = 0.096$, $\eta_p^2 = 0.021$; sex × time interaction, $p = 0.424$, $\eta_p^2 = 0.008$).

3.5. Biochemical Markers of Calcium Metabolism

Biochemical markers of calcium metabolism are shown in Figure 4 with mean absolute changes presented in Supplemental Table 2. There were sex × time interactions for iPTH, ACa, PO_4, and total 25(OH)D ($p \leq 0.003$, $\eta_p^2 \geq 0.029$). iPTH decreased from week 1 to week 10 and 14 in men ($p < 0.001$, $d \geq 0.491$), and increased from week 1 to week 4 and 6 in women ($p < 0.001$, $d \geq 0.530$). iPTH was higher in women than men at week 10 and 14 ($p \leq 0.030$, $d \geq 0.605$). ACa decreased from week 1 to week 2, 4, and 14 in men ($p \leq 0.045$, $d \geq 0.379$), and decreased from week 1 to week 2, 4, 6, 10, and 14 in women ($p \leq 0.020$, $d \geq 0.290$). ACa was higher in men than women at week 6 and 10 ($p < 0.001$, $d \geq 0.999$). PO_4 increased from week 1 to week 10 and 14 in men ($p \leq 0.010$, $d \geq 0.437$), but did not change in women for any time-point ($p \geq 0.080$, $d \leq 0.279$). PO_4 was higher in men than women at week 4, 6, 10, and 14 ($p \leq 0.005$, $d \geq 0.574$). Total 25(OH)D increased from week 1 to week 6 and 14 in men ($p < 0.003$, $d \geq 0.448$), but decreased from week 1 to week 6 in women ($p = 0.024$, $d = 0.475$). Total 25(OH)D was higher in men than women at week 6 and 14 ($p \leq 0.020$, $d \geq 0.625$).

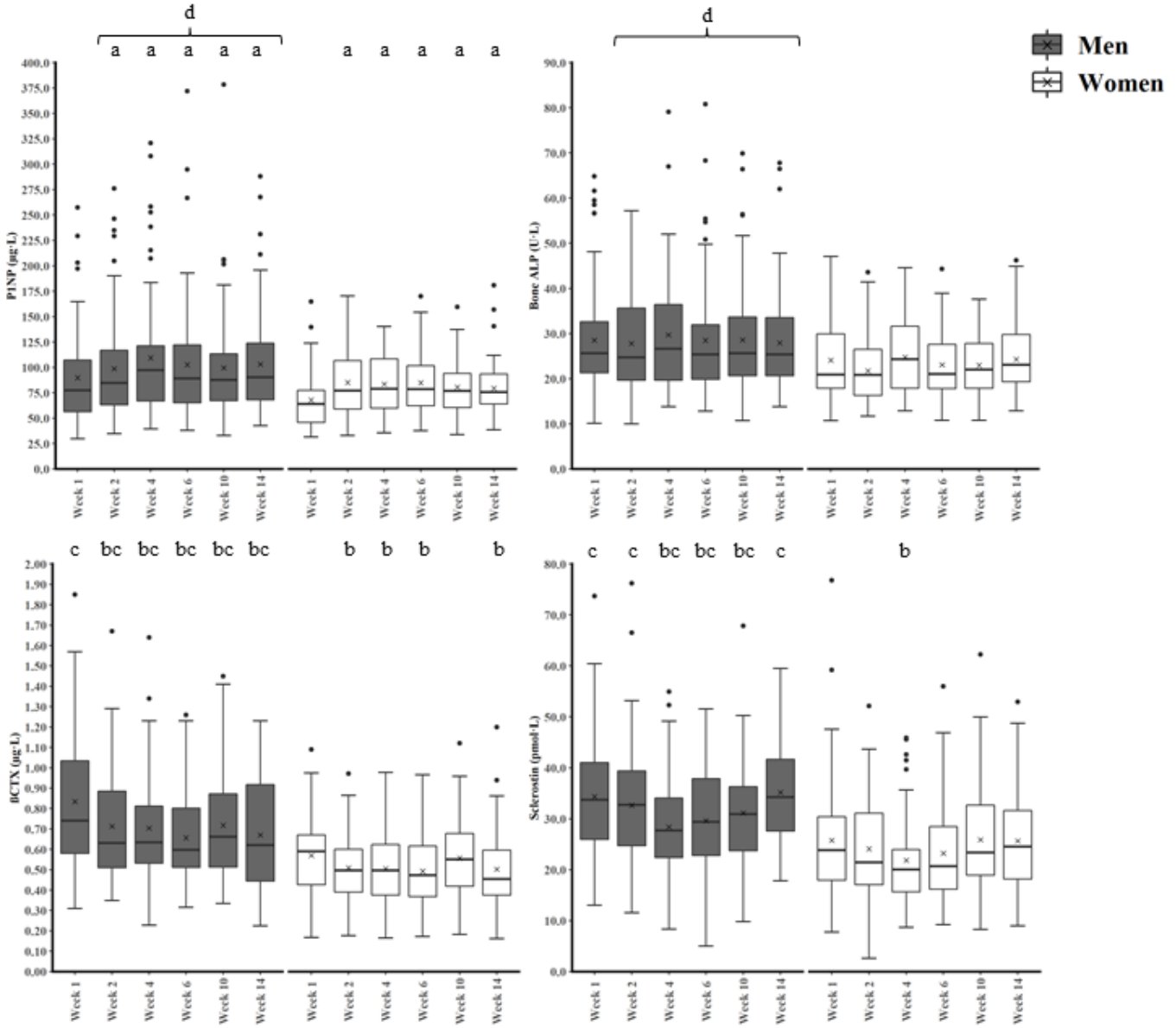

Figure 2. Biochemical markers of bone metabolism during 14-weeks of British Army basic training in men and women. Box and whisker plots represent median, interquartile range, range, and outliers. Mean data are represented by crosses.

βCTX, c-terminal cross-links telopeptide of type 1 collagen; Bone ALP, bone-specific alkaline phosphatase; P1NP, procollagen 1 N-terminal propeptide.

[a]$p < 0.05$ vs week 1 (men and women combined); [b]$p < 0.05$ vs week 1 (within-sex); [c]$p < 0.05$ vs women at the same time-point; [d]$p < 0.05$ vs women (main effect of sex).

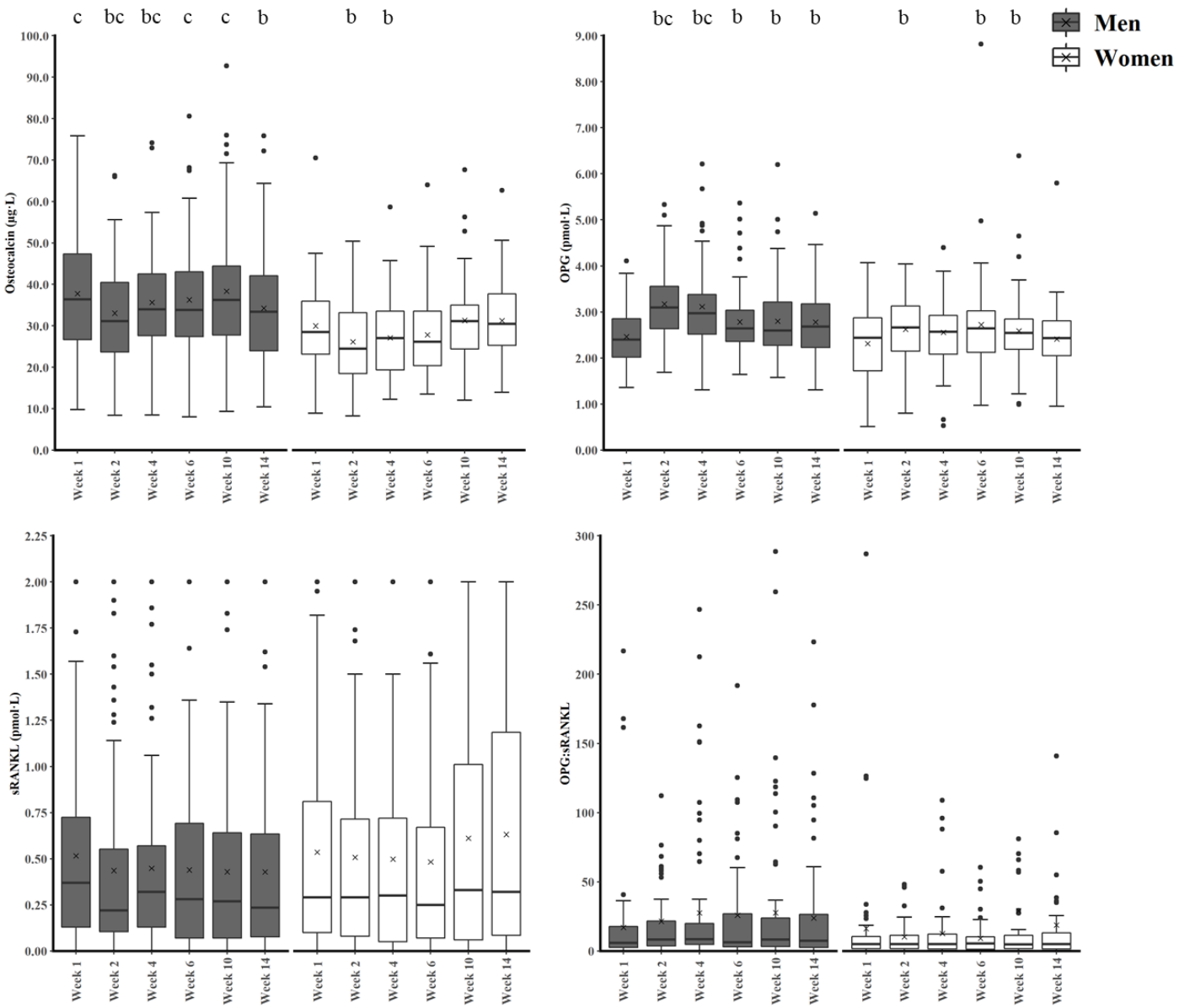

Figure 3. Biochemical markers of bone metabolism during 14-weeks of British Army basic training in men and women. Box and whisker plots represent median, interquartile range, range, and outliers. Mean data are represented by crosses.

OPG, osteoprotegerin; sRANKL, soluble receptor activator of nuclear factor kappa B ligand.

[a]$p < 0.05$ vs week 1 (men and women combined); [b]$p < 0.05$ vs week 1 (within-sex); [c]$p < 0.05$ vs women at the same time-point; [d]$p < 0.05$ vs women (main effect of sex).

The following data points have been removed from the figures for clarity: Men, week 2, OPG:sRANKL = 404; Men, week 6, OPG:sRANKL = 338; Women, week 5, OPG:sRANKL = 305; Women, week 10, OPG:sRANKL = 639; Women, week 10, OPG:sRANKL = 314.

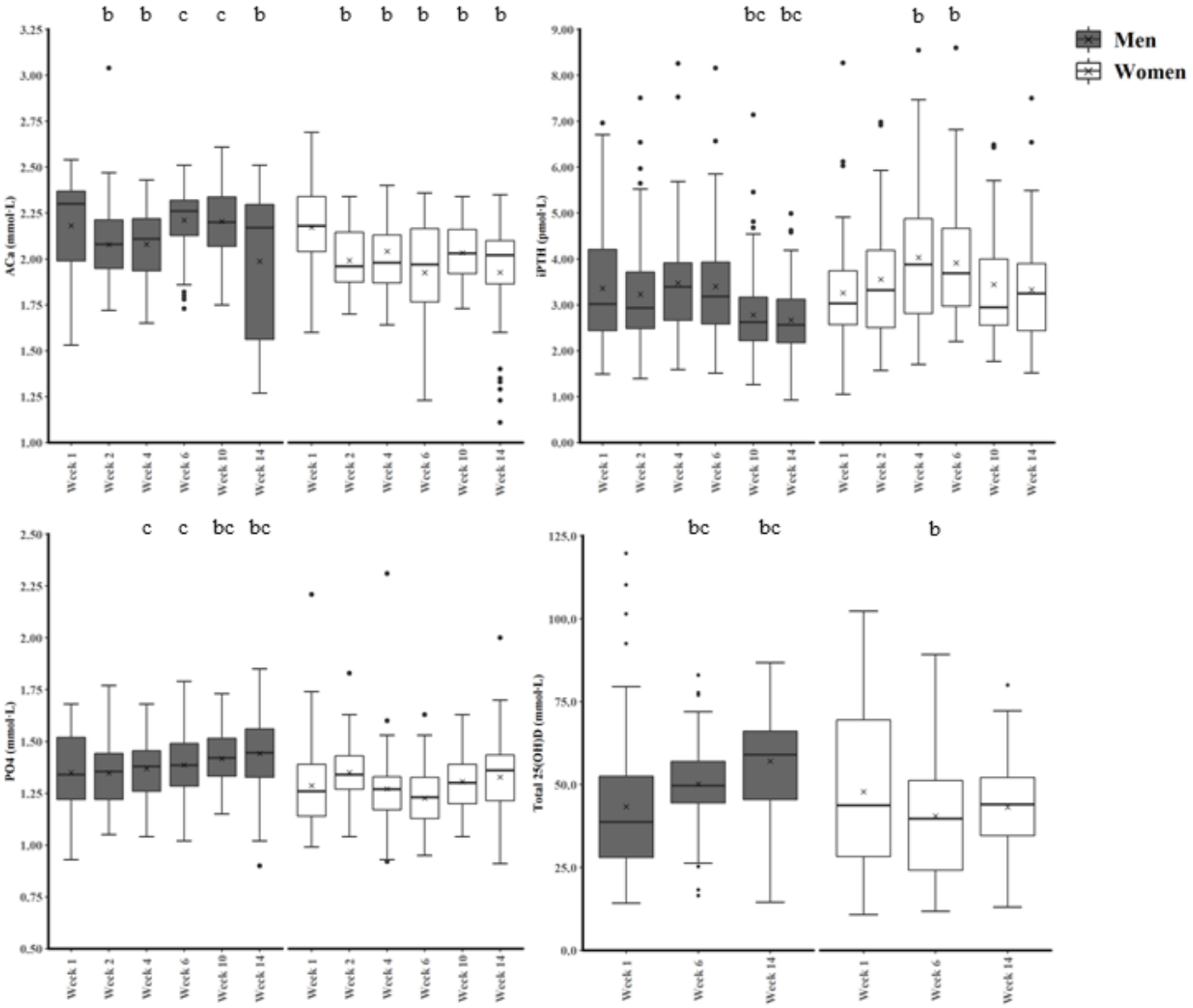

Figure 4. Biochemical markers of calcium metabolism during 14-weeks of British Army basic training in men and women. Box and whisker plots represent median, interquartile range, range, and outliers. Mean data are represented by crosses.

ACa, albumin-adjusted calcium; iPTH, intact parathyroid hormone; PO4, phosphate; Total 25(OH)D, total 25-hydroxyvitamin D.

[a]$p < 0.05$ vs week 1 (men and women combined); [b]$p < 0.05$ vs week 1 (within-sex); [c]$p < 0.05$ vs women at the same time-point; [d]$p < 0.05$ vs women (main effect of sex).

The following outlier data points have been removed from the figures for clarity: Women, week 1, albumin-adjusted calcium = 4.14 $mmol \cdot L^{-1}$; Women, week 4, albumin-adjusted calcium = 3.91 $mmol \cdot L^{-1}$.

4. Discussion

This study compared tibial adaptations and biochemical markers of bone metabolism between men and women during 14-weeks of basic military training. Tibial volumetric density, geometry, and estimated bone strength increased across the length of the tibia. The increase in trabecular vBMD (14% site) was greater in women than men, and the increase in cortical area and strength (38% site) was greater in men than women. The measurement of tibial structure and comprehensive assessment of bone metabolic markers provides novel and comprehensive insight into sex differences in the mechanobiology of bone. The measurement of structural changes across four tibial sites provides new insight into site specific bone adaptions to loading. Basic military training is physically demanding [23, 25, 26], and consists of high volumes of irregular and high magnitude tibial impacts during weight-bearing activities like load carriage [23, 25] and military drill [27, 28]. Several studies have observed changes in tibial geometry and density within 14-weeks of military training in men and women [13-19], but sex differences in tibial adaptations to military training are poorly understood.

4.1. Volumetric Bone Mineral Density

Training increased trabecular vBMD at the distal tibia in both men (1.0% at the 4% site) and women (1.6% and 0.8% at the 4% and 14% site). The increase in trabecular vBMD was higher in women at both sites, with no adaption in men at the 14% site. Similar increases in trabecular vBMD at the tibial metaphysis (0.9 to 2.0%) have been observed following 8 to 13 weeks of basic military training in men and women in previous studies using pQCT [13, 14] and HR-pQCT [15, 16, 19], although we have also previously reported no change in trabecular vBMD in men at the 14% site [13]. The increase in trabecular vBMD (and other tibial adaptations) we report here are comparable to those observed with 12 to 24 months of treatment with osteoporotic drugs [29-31], in female endurance athletes treated with oestradiol patches

performing weight-bearing training over 12 months [32], in adolescent soccer players during 12 weeks training [33], in young women during a 12-week high-impact aerobic training programme [34], and in post-menopausal women completing a range of exercise programmes over 12 months, include high-impact exercise [35]. The clinical significance of the changes reported in this study are unclear but likely contribute to increased stress fracture resistance [12], as small changes in bone contribute to large increases in fatigue fracture resistance [36]. An increase in trabecular vBMD is an early adaptation to mechanical loading [13, 34] and improves resistance to compressive forces at the metaphysis [37]. A greater trabecular adaptation in women is probably due to the lower baseline trabecular vBMD in women [15], and greater relative loading during activities like load carriage as women have lower aerobic capacity and carry relatively heavier weights than men [25]. The increase in trabecular vBMD is likely due to adaptation of the trabecular microarchitecture [15, 18]. An increase in trabecular thickness and number have been observed in women [15, 18], but not men [16], following basic military training, supporting the greater increase in trabecular vBMD in women we observed here. Low oestradiol increases the rate of remodelling on the trabecular surfaces [2, 38], and oestradiol is important in the adaptive response to mechanical loading through actions on the oestrogen receptor alpha (ERα) in trabecular bone [3, 39]. There are likely sex-, site-, and cell-specific effects of oestrogens in the bone response to mechanical loading [3].

Training increased cortical vBMD at the 38% site (pooled mean change of 0.1%), but not 14% or 66% site in men and women. The adaptation to training was similar between men and women. The increase in cortical vBMD was small and not beyond the measurement CV. The evidence for cortical vBMD adaptations to exercise training is inconsistent. Other pQCT data show an increased (0.1 to 0.5%) [13, 14] or unchanged [17] cortical vBMD across the 14%, 38%, and 66% sites in men and women following military training. Increased cortical vBMD

at the 4% site (0.6 to 0.9%) in men [16] and decreased cortical vBMD in women at the 4% site (−0.3%) and the 30% site (−0.7%) in women [15, 18] measured by HR-pQCT have also been reported. The tibial diaphysis experiences high bending and torsion stresses during locomotion [40]. The tibia is narrowest in the distal third [37] and likely to be the site subject to the highest bending an torsional forces, which may explain why most tibial stress fractures in military training occur at the distal third [41] and why cortical vBMD only increased at the 38% site. An increase in cortical vBMD increases stiffness, but may make bones more susceptible to microdamage [42, 43]. Cortical vBMD was higher for women than men at all sites to compensate for a narrower bone, and increased stiffness may contribute to sex differences in susceptibility to microdamage and stress fracture. Nevertheless, adaptations to geometry following loading provide a greater contribution to increased strength than increases in density [44].

4.2. Geometry

Training increased total (14% and 38% site), trabecular (14% site), and cortical (14%, 38%, and 66% sites) area, and cortical thickness (14%, 38%, and 66% sites) (0.1 to 1.3%). The increased periosteal perimeter at the 14%, 38%, and 66% sites (0.1 to 0.2%) demonstrates that periosteal expansion occurred due to an increase in cortical thickness and area, consistent with previous military training pQCT studies in men [13]. The increase in cortical area was greater at the 38% site in men than women, but there were no other sex differences. 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 [1, 2]. Accordingly, bone strength indices increased at all sites and the increases at the 38% site were greater for men than women. Men have higher tibial accelerations than women during military activities like military drill [28], complete a greater distance during basic training [23], and

have a bigger muscle mass, which could contribute to higher loading and cortical area adaptation, but we have no evidence if these sex differences translate to increased bone strain for men. A smaller increase in cortical area at the 38% site in women could be due to inhibition of periosteal expansion by oestradiol [1, 2], but no sex differences were observed.

4.3. Biochemical Markers of Bone Metabolism

Procollagen type 1 N-terminal propeptide and bone ALP were measured as markers of bone formation. Training increased P1NP—a measure of type I collagen synthesis [45]—consistent with adaptive bone formation measured by pQCT. Bone-specific alkaline phosphatase—a measure of osteoblast activity and mineralisation[45]—did not change during 14 weeks of training. Osteocalcin—a bone matrix protein synthesis by mature osteoblasts[45]—decreased early in training demonstrating that osteocalcin may not be a sensitive indicator of bone formation with exercise [46]. Procollagen type 1 N-terminal propeptide, bone ALP, and osteocalcin reflect different processes, which may explain the different time course of their response to training. Sclerostin—a glycoprotein secreted by osteocytes—decreased in training. Downregulation of sclerostin with loading promotes bone formation through disinhibition of the Wnt signalling pathway [47], and here we provide evidence of a time-dependent effect with a novel loading stimulus. Military training studies report increased [19-22] or unchanged [14-17] P1NP, increased [14, 15, 19, 20, 22] or unchanged [17] bone ALP, deceased osteocalcin [14, 15, 17], and decreased sclerostin [15, 17] in men and women. The P1NP, osteocalcin, and sclerostin response to training was similar between men and women. The data in women must be interpreted with caution because we were unable to standardise measurement around the menstrual cycle and not all women were eumenorrheic.

Bone resorption—measured by βCTX—decreased from week 1 across all training weeks for men, but only decreased in the early part of training for women (week 2, 4, and 6). The decrease in βCTX demonstrates that training decreased type I collagen degradation, consistent with other military training studies over 8 to 16 weeks [16, 17, 21]; although increases in βCTX have also been reported [19, 20, 22]. The decrease in βCTX (and increase in P1NP) likely represents the modelling and/or remodelling response to mechanical loading leading to adaptive bone formation [13, 15, 16]. The reason for the sex difference is not clear, but women complete more high-intensity exercise bouts than men when completing identical basic military training courses, particularly in the latter weeks of training when load carriage increases [23, 25]. Moderate-intensity exercise decreases βCTX, whereas βCTX does not change during high-intensity exercise [48]; therefore, the decrease in βCTX in men but not women could be due to the relatively higher intensity exercise for women in the latter weeks of training. However, the ratio between OPG and sRANKL (OPG:sRANKL)—an indication of bone resorption—did not change with training. sRANKL is essential for osteoclast functioning by promoting osteoclast differentiation and action, and preventing apoptosis, whereas OPG competitively binds with and inhibits sRANKL.[49]

Sex-specific changes in biochemical markers of calcium metabolism were observed. Parathyroid hormone decreased by the end of training in men, but increased during the first six weeks of training in women. Parathyroid hormone secretion is regulated by serum ionized calcium and iPTH mobilises skeletal calcium by stimulating bone resorption, although intermittent increases in iPTH are osteogenic [50]. Albumin-adjusted calcium was measured to provide an indication of ionized calcium and ACa decreased more in women than men. A decrease in ACa could be due to reduced bone resorption and increased calcium uptake into bone during adaptive bone formation. Exercise acutely decreases ionized calcium and increases

PO_4 and iPTH production [51, 52], although an acute increase in iPTH only occurs when the exercise intensity is high [48]. The increase in iPTH for women could be due to lower ACa and relatively higher exercise intensity compared with men. Phosphate increased during training for men but did not change in women; the reason for this sex difference is not clear, but could be due to an increase in total 25(OH)D in men [53]. The regulation of PO_4 is achieved through the complex interactions of fibroblast growth factor-23, iPTH, and 1,25 dihydroxyvitamin D [54]. Serum calcium may only partially explain the regulation of iPTH in response to exercise training [50]. Total 25(OH)D increased in men (week 6 and 14) but decreased in women (week 6). This divergent total 25(OH)D response is unsurprising considering men started training in spring and summer whereas women started training across all seasons. The active 25(OH)D metabolite—1,25 dihydroxyvitamin D—provides negative feedback of iPTH secretion [50] and so the increase in iPTH in women could be due to a decline in total 25(OH)D. An increase in iPTH increases bone resorption, predominantly in cortical bone, by stimulating sRANKL and inhibiting OPG [50]. Whilst we did not see a change in sRANKL, OPG was higher in men than women. The increase in iPTH for women may explain the greater adaption of cortical area at the diaphysis in men. 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 [14]. High iPTH has also been associated with increased stress fracture risk in military training [55], but the anabolic and catabolic actions of iPTH are complex [50].

4.4. Limitations

The attrition during military training resulted in some loss to follow-up and our data are subject to survivor bias. Of those who complete the training course, we do not have a measure of how many training sessions were completed, however recruits who miss too many sessions must repeat the course and so compliance was high (> 95%). We did not measure sex steroid

hormones, which would help further explain some of our findings. However, the sex differences in oestradiol and testosterone are well establish and measuring oestradiol in naturally menstruating women at different stages of the menstrual cycle would offer limited insight. We also did not standardise recruitment of men and women according to season, which contributed to differences in total 25(OH)D throughout training. Seasonal variation [17] and vitamin D supplementation [17, 19] has minimal impact on tibial bone adaptations to basic military training and so we do not believe these seasonal differences affected our pQCT data. Finally, all our blood samples were obtained after an overnight fast at the same time of day, but there were have been other factors we could not standardise that influence the bone metabolic response.

4.5. Conclusion

Arduous weight bearing exercise in the form of basic military training increased the volumetric density, size, and strength of the tibia. These adaptations were similar between men and women, however, we observed greater increases in trabecular vBMD in women than men at the metaphysis, but greater increases in cortical area and strength in men at the diaphysis. These findings provide a better understanding of sex differences in the mechanobiology of bone.

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Competing Interests

The authors have no competing interests to declare.

Data Availability Statement

The data and code used to produce this manuscript are available from the corresponding author.

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Supplemental Table 1. Mean absolute change [95% confidence intervals] in tibial density and geometry from week 1 to week 14 of British Army basic training in men and women.

	Men	Women
4% site		
Tb vBMD ($mg \cdot cm^{-3}$)	3 [4, 2]	4 [3, 5]
PPm (mm^{-1})	−0.2 [−0.7, 0.2]	0.3 [−0.2, 0.7]
14% site		
Tb vBMD ($mg \cdot cm^{-3}$)	0 [1, 1]	2 [0, 4]
Ct vBMD ($mg \cdot cm^{-3}$)	1 [−1, 3]	0 [−2, 3]
Total Area (mm^{-2})	2 [0, 3]	2 [1, 3]
Tb Area (mm^{-2})	1 [1, 3]	2 [0, 4]
Ct Area (mm^{-2})	1 [0, 1]	1 [0, 1]
Ct Thickness (mm^{-1})	0.01 [0.00, 0.02]	0.01 [0.00, 0.02]
PPm (mm^{-1})	0.0 [0.0, 0.1]	0.1 [0.0, 0.2]
EPm (mm^{-1})	0.0 [−0.2, 0.1]	0.1 [−0.1, 0.2]
BSI ($g \cdot cm^{-4}$)	0.02 [0.01, 0.02]	0.01 [0.01, 0.02]
38% site		
Ct vBMD ($mg \cdot cm^{-3}$)	1 [1, 2]	2 [0, 3]
Total Area (mm^{-2})	2 [1, 4]	1 [0, 3]
Ct Area (mm^{-2})[c]	3 [2, 4]	1 [0, 3]
Ct Thickness (mm^{-1})	0.05 [0.03, 0.06]	0.03 [0.01, 0.05]
PPm (mm^{-1})	0.2 [0.1, 0.3]	0.1 [0.0, 0.2]
EPm (mm^{-1})	−0.1 [−0.2, 0.0]	−0.1 [−0.2, 0.0]
BSI ($g \cdot cm^{-4}$)	0.05 [0.03, 0.06]	0.02 [0.01, 0.03]
66% site		
Ct vBMD ($mg \cdot cm^{-3}$)	1 [0, 2]	0 [−1, 2]
Total Area (mm^{-2})	1 [−1, 2]	1 [−1, 2]
Ct Area (mm^{-2})	1 [1, 2]	2 [1, 3]
Ct Thickness (mm^{-1})	0.02 [0.01, 0.04]	0.03 [0.01, 0.04]
PPm (mm^{-1})	0.1 [0.0, 0.2]	0.1 [0.0, 0.2]
EPm (mm^{-1})	−0.2 [−0.3, 0.0]	−0.1 [−0.3, 0.0]
BSI ($g \cdot cm^{-4}$)	0.02 [0.00, 0.05]	0.04 [0.02, 0.06]

BSI, bone strength index; Ct, cortical; EPm, endosteal perimeter; PPm, periosteal perimeter; Tb, trabecular; vBMD, volumetric bone mineral density.

Supplemental Table 2. Mean absolute change [95% confidence intervals] in biochemical markers of bone and calcium metabolism from week 1 of training during 14 weeks British Army basic training in men and women.

	Week 2	Week 4	Week 6	Week 10	Week 14
Men					
P1NP ($\mu g \cdot L^{-1}$)	7.1 [−0.1, 14.3]	17.6 [10.3, 24.9]	12.6 [5.3, 19.9]	9.2 [−1.1, 19.5]	11.9 [4.3, 19.5]
Bone ALP ($U \cdot L^{-1}$)	−0.8 [−3.5, 1.9]	1.2 [−0.9, 3.2]	0.0 [−2.1, 2.1]	0.2 [−1.8, 2.3]	−0.7 [−2.5, 1.2]
βCTX ($\mu g \cdot L^{-1}$)	−0.12 [−0.16, −0.09]	−0.14 [−0.17, −0.10]	−0.17 [−0.21, −0.13]	−0.11 [−0.15, −0.07]	−0.17 [−0.21, −0.12]
Sclerostin ($pmol \cdot L^{-1}$)	−2.7 [−5.1, 0.2]	−6.2 [−8.7, −3.8]	−5.4 [−7.9, −2.9]	−3.7 [−5.8, −1.7]	−0.4 [−2.2, 3.0]
Osteocalcin ($\mu g \cdot L^{-1}$)	−4.7 [−6.1, −3.3]	−2.1 [−3.9, −0.3]	−0.7 [−2.5, 1.0]	1.0 [−1.3, 3.3]	−3.4 [−5.6, −1.2]
iPTH ($pmol \cdot L^{-1}$)	−0.14 [−0.37, 0.08]	0.12 [−0.15, 0.38]	−0.02 [−0.24, 0.29]	−0.62 [−0.91, −0.32]	−0.70 [−1.00, −0.40]
ACa ($mmol \cdot L^{-1}$)	−0.10 [−0.16, −0.04]	−0.10 [−0.16, −0.04]	0.04 [−0.03, 0.11]	0.03 [−0.04, 0.10]	−0.19 [−0.30, −0.08]
PO_4 ($mmol \cdot L^{-1}$)	0.00 [−0.05, 0.04]	0.02 [−0.03, 0.07]	0.04 [0.00, 0.09]	0.07 [0.03, 0.11]	0.09 [0.05, 0.13]
Total 25(OH)D ($nmol \cdot L^{-1}$)	—	—	7.9 [3.8, 12.0]	—	13.9 [8.2, 19.6]
OPG ($pmol \cdot L^{-1}$)	0.70 [0.56, 0.84]	0.78 [0.52, 0.84]	0.33 [0.20, 0.46]	0.35 [0.18, 0.51]	0.29 [0.16, 0.42]
sRANKL ($pmol \cdot L^{-1}$)	−0.08 [−0.17, 0.01]	−0.07 [−0.16, 0.02]	−0.09 [−0.18, 0.01]	−0.08 [−0.19, 0.03]	−0.08 [−0.17, 0.00]
OPG:sRANKL	4.32 [−10.09, 18.73]	11.01 [−1.47, 23.51]	8.13 [−4.24, 20.51]	9.52 [−4.69, 23.74]	6.63 [−3.82, 17.09]
Women					
P1NP ($\mu g \cdot L^{-1}$)	17.4 [12.8, 22.1]	16.1 [10.8, 21.4]	17.0 [11.0, 22.9]	12.2 [6.4, 18.0]	12.3 [6.8, 17.7]
Bone ALP ($U \cdot L^{-1}$)	−2.5 [−4.3, −0.7]	0.9 [−0.6, 2.4]	1.2 [−3.1, 0.8]	−1.5 [−3.3, 0.3]	0.1 [−2.2, 2.4]
βCTX ($\mu g \cdot L^{-1}$)	−0.06 [−0.09, −0.03]	−0.06 [−0.09, −0.03]	−0.07 [−0.11, −0.04]	−0.02 [−0.05, 0.02]	−0.07 [−0.12, −0.01]
Sclerostin ($pmol \cdot L^{-1}$)	−1.6 [−4.6, 1.3]	−3.7 [−6.5, −0.9]	−2.8 [−5.8, −0.2]	−0.3 [−2.9, 3.6]	−0.9 [−4.1, 2.3]
Osteocalcin ($\mu g \cdot L^{-1}$)	−4.1 [−5.8, −2.5]	−2.8 [−4.7, −0.9]	−2.2 [−4.4, −0.1]	0.9 [−1.4, 3.2]	1.0 [−0.8, 2.9]
iPTH ($pmol \cdot L^{-1}$)	0.26 [−0.01, 0.53]	0.73 [0.37, 1.08]	0.62 [0.30, 0.95]	0.14 [−0.18, 0.46]	−0.01 [−0.38, 0.36]
ACa ($mmol \cdot L^{-1}$)	−0.18 [−0.29, −0.08]	−0.14 [−0.27, −0.01]	−0.25 [−0.38, −0.13]	−0.14 [−0.25, −0.03]	−0.25 [−0.39, −0.11]
PO_4 ($mmol \cdot L^{-1}$)	0.06 [0.00, 0.13]	−0.01 [−0.09, 0.06]	−0.06 [−0.12, 0.00]	0.02 [−0.05, 0.08]	0.05 [−0.03, 0.12]
Total 25(OH)D ($nmol \cdot L^{-1}$)	—	—	−6.1 [−9.6, −2.6]	—	−2.7 [−8.8, 3.5]
OPG ($pmol \cdot L^{-1}$)	0.34 [0.15, 0.53]	0.26 [0.03, 0.49]	0.39 [0.09, 0.70]	0.28 [0.06, 0.49]	0.11 [−0.14, 0.37]
sRANKL ($pmol \cdot L^{-1}$)	−0.04 [−0.18, 0.09]	−0.06 [−0.17, 0.04]	−0.08 [−0.19, 0.03]	0.06 [−0.07, 0.18]	0.09 [−0.05, 0.24]
OPG:sRANKL	−6.52 [−19.0, 5.95]	−4.45 [−18.16, 9.26]	−7.76 [−20.17, 4.65]	12.26 [−5.82, 30.34]	1.98 [−16.65, 20.60]

ACa, albumin-adjusted calcium; βCTX, c-terminal cross-links telopeptide of type 1 collagen; Bone ALP, bone-specific alkaline phosphatase; iPTH, intact parathyroid hormone; OPG, osteoprotegerin; P1NP, procollagen 1 N-terminal propeptide; PO4, phosphate; sRANKL, soluble receptor activator of nuclear factor kappa B ligand; total 25(OH)D, total 25-hydroxyvitamin D.