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Skeletal macro- and microstructure adaptations in men undergoing arduous military training

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Author contributions: JG designed the study. RMI and TJO collected, and TJO analysed, the imaging data. Biochemical samples were collected by NPW and analysed by JCYT and WDF. TJO produced the manuscript. All authors edited the manuscript and approved the final version.

Running title: Skeletal adaptations to military training.

Abstract

Purpose: Short periods of basic military training increase the density and size of the tibia, but the adaptive response of bone microarchitecture, a key component of bone strength, is not fully understood. *Methods:* Tibial volumetric bone mineral density (vBMD), geometry, microarchitecture and mechanical properties were measured using high-resolution peripheral quantitative computed tomography in 43 male British Army infantry recruits (mean ± SD, age 21 ± 3 years, height 1.76 ± 0.06 m, body mass 76.5 ± 9.4 kg). Bilateral scans were performed at the distal tibia at the start (week 1) and end (week 13) of basic military training. Concurrent measures were obtained for whole-body areal bone mineral density (aBMD) using DXA, and markers of bone metabolism (βCTX, P1NP, PTH, total 25(OH)D and ACa) from venous blood. *Results***:** Training increased areal BMD for total body (1.4%) and arms (5.2%) ($P \leq 0.031$), but not legs and trunk ($P \geq 0.094$). Training increased trabecular (1.3 to 1.9%) and cortical vBMD (0.6 to 0.9%), trabecular volume (1.3 to 1.9%), cortical thickness (3.2 to 5.2%) and cortical area (2.6 to 2.8%), and reduced trabecular area (−0.4 to −0.5%) in both legs ($P < 0.001$). No changes in trabecular number, thickness and separation, cortical porosity, stiffness or failure load were observed ($P \geq 0.188$). βCTX decreased (−0.11 $\mu g \cdot l^{-1}$, $P < 0.001$) and total 25(OH)D increased (9.4 $nmol \cdot l^{-1}$, $P = 0.029$), but no differences in P1NP, PTH or ACa were observed between timepoints ($P \geq 0.233$). *Conclusion:* A short period of basic military training increased density and altered geometry of the distal tibia in male military recruits. The osteogenic effects of basic military training are likely due to an increase in unaccustomed, dynamic and high-impact loading.

Key words: bone remodelling, bone turnover, exercise training, mechanical loading, stress fracture

Abbreviations

25(OH)D, 25-hydroxyvitamin D; aBMD, areal bone mineral density; ACa, albumin-adjusted calcium; βCTX, c-telopeptide cross-links of type 1 collagen; Ct.Area, cortical area; Ct.Pm, cortical perimeter; Ct.Po, cortical porosity; Ct.Th, cortical thickness; Ct.vBMD, cortical volumetric bone mineral density; DXA, dual energy X-ray absorptiometry; HA, hydroxyapatite; HR-pQCT, high-resolution peripheral quantitative computed tomography; P1NP, procollagen type 1 N-terminal propeptide; pQCT, peripheral quantitative computed tomography; PTH, parathyroid hormone; Tb.Area, trabecular area; Tb.BV/TV, trabecular bone volume fraction; Tb.N, number of trabeculae; Tb.Sp, trabecular separation; Tb.Th, trabecular thickness; Tb.vBMD, trabecular volumetric bone mineral density; vBMD, volumetric bone mineral density.

1. Introduction

Bone adapts to mechanical loading by altering its density, geometry, and material properties [1-3]. These skeletal adaptations, mediated by the cellular activity of modelling/remodelling [2, 4-6], increase bone strength to improve resistance to fracture [1, 3]. *In vivo* studies in humans report a rapid response of bone to acute periods of dynamic, high-impact and episodic loading: increased density, cortical thickness, periosteal perimeter and *estimated* mechanical strength in the tibia are reported during basic military training of 8 to 10 weeks [7-9]. Excessive, or sudden increases in repetitive loading, can weaken bone strength due to remodelling of fatigue damage, leading to stress fractures at sites of highest mechanical stress. Stress fractures at principal weight-bearing sites, the hip, tibia and metatarsals, are commonly seen in athletes and military recruits [10-12]. Understanding the response of bone to loading may help identify the aetiology of skeletal injury in exercising populations.

Dual energy x-ray absorptiometry (DXA) measures areal bone mineral density (aBMD), but cannot distinguish between cortical and trabecular bone, or quantify volumetric bone mineral density (vBMD), geometry, microarchitecture and mechanical properties [13, 14]. Dual energy x-ray absorptiometry is, therefore, insensitive for detecting changes in bone strength with exercise training, which can occur with little or no change in aBMD [15]. Peripheral quantitative computed tomography (pQCT) provides measurement of cortical and trabecular vBMD and geometry, but the low resolution prohibits measurement of bone microstructure. The recent advancement of high-resolution pQCT (HR-pQCT) allows detailed assessment of bone microstructure, both trabecular microarchitecture and cortical porosity, which are important contributors to bone strength [4, 6, 16-18]. The dense trabecular network in the broad end of the long bones has an ansiotropic distribution aligned parallel to the mechanical stress axis [2, 19, 20], which absorb and distribute mechanical stresses to the cortex [21]. Greater

porosity of cortical bone can occur from the removal of fatigue damage [2], facilitating the propagation of microcracks [6] and reducing mechanical strength [17, 22]. Concurrent assessment of bone macro- and microstructure improves the ability to assess mechanical properties [23, 24] and skeletal responses to an intervention [4, 14, 19], and therefore, HR-pQCT appears to have greater sensitivity in determining fracture risk than other *in vivo* methods [13, 14].

High-resolution pQCT has been used in cross-sectional studies of different athlete groups to examine the relationship between exercise and bone macro- and microstructure [25-27], but there are few longitudinal data in humans examining bone microstructure in direct response to exercise training. Animal studies show that the increase in bone strength induced with running training is mediated by changes in trabecular microarchitecture in addition to changes in density and geometry [28]. Increased cortical thickness, trabecular vBMD, trabecular thickness and trabecular number, reduced trabecular separation and cortical vBMD, and unchanged cortical porosity at the distal *non-dominant* tibia, have been reported following 8 weeks US Army basic military training in female recruits [9]. These adaptations contributed to an increase in *estimated* strength under axial loading. We recently reported that a 61 day Antarctic traverse had no effect on tibial macro- or microstructure in six women [29]. The response of the bone microstructure to arduous military training has yet to be investigated in men. The aim of this study was to examine *bilateral* tibial macro- and microstructure, whole-body aBMD, and markers of bone metabolism in *men* undergoing 13 weeks of the British Army's infantry basic military training course. Infantry training is considered the British Army's most arduous basic military training course and is characterised by a high incidence of tibial stress injuries [10].

2. Materials and Methods

2.2. Participants

One hundred male British Army infantry recruits volunteered to take part in this study. All participants were recruited during their first week of British Army basic training at the Infantry Training Centre, Catterick. All participants passed an initial medical assessment and were declared 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 (16/Gen/10).

2.3. Experimental Protocol

Whole-body aBMD measured by DXA, and vBMD, geometry, microarchitecture and *estimated* mechanical strength at the distal tibial measured by HR-pQCT, were assessed at the start (week 1) and end (week 13) of infantry basic military training. Blood samples were obtained at the same timepoints for the assessment of biochemical markers of bone metabolism. Baseline measurements were made immediately following the initial medical assessment and before any military training had commenced. All participants were undergoing the 26-week British Army infantry basic training course, broken into two phases (Phase One: 14 weeks basic training; Phase Two: 12 weeks specialist infantry training). The first 14 weeks of British Army infantry basic military training involves periods of aerobic endurance training, strength and conditioning, military specific fitness training (obstacle course, circuit training), military drill, progressive loaded marching and basic military skills (field exercise, weapon handling). Week 14 involves a decrease in typical military activities and an increase in the administrative burden as recruits prepare to complete the basic training component of their course; post-training measurements were, therefore, taken in week 13.

2.4. Areal Bone Mineral Density

Whole-body aBMD 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 and trunk were derived from the whole-body scan.

2.5. Tibial Volumetric Bone Mineral Density, Geometry and Microarchitecture

A three-dimensional HR-pQCT system (XtremeCT, Scanco Medical AG, Switzerland) was used to assess vBMD, geometry and microarchitecture of the distal tibia. Limb dominance was self-determined and described to participants as the leg most likely used to kick a ball [9]. A three-dimensional representation of 9.02 mm of the tibia in the axial direction was obtained from 110 CT slices with an isotropic voxel size of 82 μm [30]. 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 (2.8 min). A reference line was positioned at the tibial endplate with the first CT slice taken from 22.5 mm proximal to the reference line. For follow-up measurements, 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 volumes common to the baseline scans were assessed [30]; only scans with a common region of ≥80% were included in the analyses. On average, 103 ± 5 (dominant leg: 103 ± 5 slices, common region 94 ± 4%; non-dominant leg: 103 ± 5 slices, common region 94 ± 5%) of the original 110 slices were analysed at follow-up. 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 were excluded from the analyses as per the manufacturer visual grading of image quality (13 images, 5%). The methods used to process the data have been previously described [30]. The standard evaluation procedure provided by the manufacturer was used to derive the following

outcome variables: total vBMD (mg HA·cm^3), trabecular vBMD (Tb.vBMD, mg HA·cm^3), cortical vBMD (Ct.vBMD, mg HA·cm^3), trabecular area (Tb.Area, mm^2), trabecular bone volume fraction (Tb.BV/TV, %), number of trabeculae (Tb.N, mm^{-1}), trabecular thickness (Tb.Th, mm), trabecular separation (Tb.Sp, mm), cortical area (Ct.Area, mm^2), cortical thickness (Ct.Th, mm) and cortical perimeter (Ct.Pm, mm). Additionally, detailed analysis of cortical bone was performed using a semi-automated segmentation technique to determine cortical porosity (Ct.Po, %) [31, 32]. Micro-finite element analysis was performed using software provided with the HR-pQCT device, as described previously [33], to calculate the biomechanical properties under uniaxial compression, specifically stiffness (kN·mm^{-1}) and failure load (N). All evaluations were performed by a single investigator to ensure consistency of contouring. The coefficient of variation is ≤ 1.5% for vBMD, ≤ 4.4% for trabecular microarchitecture, ≤ 1.5% for cortical thickness, ≤ 1.5% for cortical and trabecular area, and ≤ 6.2% for cortical porosity [30, 31].

2.6. Biochemical Markers of Bone Metabolism

A venous blood sample was collected either in the morning (0900 to 1100 h) after breakfast (0600 to 0700 h), or early afternoon (1300 to 1500 h) after lunch (1200 to 1300h). Follow-up measurements were taken at the same time of day. Venous blood was withdrawn from a vein in the antecubital fossa using a 21g BD Vacutainer® flashback needle, and collected in serum and EDTA BD Vacutainer® tubes (Becton Dickinson, USA). Blood samples were centrifuged at 2000 rpm (751 g) and 1°C for 10 min before serum and plasma were separated into 1.5mL eppendorfs (Eppendorf, Cambridge, UK) and stored at −80°C until analysis. EDTA plasma samples were analysed for parathyroid hormone (PTH), procollagen type 1 N-terminal propeptide (P1NP) and c-telopeptide cross-links of type 1 collagen (βCTX) by electro-chemiluminescence immunoassay (ECLIA) on the COBAS c601 (Roche Diagnostics,

Mannheim, Germany) platform. Serum samples were analysed for albumin-adjusted calcium (ACa) (Roche) and total 25(OH)D by liquid chromatography tandem mass spectrometry (LC-MS/MS) [34]. 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.

2.7. Statistical Analysis

Data are reported as mean ± standard deviation in the text and tables. All data were analysed using SPSS (v.24, SPSS Inc., USA) and initially checked for normality. All outcomes measured at baseline were compared between those lost to follow-up and the final sample with independent sample t-tests or Mann-Whitney U tests for non-parametric data. High-resolution peripheral quantitative computed tomography outcomes were analysed with 2 × 2 (leg [dominant vs non-dominant] × time [week 1 vs week 13]) repeated-measures ANOVAs. In the event of a significant interaction: i) baseline and follow-up data were compared using paired sample t-tests for each limb independently, and; ii) dominant and non-dominant legs were compared using paired-sample t-tests for each timepoint independently. Areal BMD and markers of bone metabolism were assessed with paired-sample t-tests; non-parametric data (P1NP, total 25(OH)D and ACa) were tested with a Wilcoxon signed rank test. Statistical significance was accepted at $P \leq 0.05$. Effect sizes were calculated using partial eta squared (η_p^2) for ANOVAs and Cohen's d_z for paired-sample t-tests.

3. Results

Forty-five participants (45%) were lost to follow-up due to withdrawal from training for medical reasons (n = 5), withdrawal from training for non-medical reasons (voluntary withdrawal, poor course performance or disciplinary reasons, n = 12), withdrawal from the

study (n = 2) or unavailable at time of follow-up (due to the demands of the course or for unknown reasons, n = 26). Valid HR-pQCT measurements were unavailable for at least one scan on 12 participants (poor image quality, n = 7; insufficient common region, n = 5); complete HR-pQCT data sets were available for 43 men (mean ± SD, age 21 ± 3 years, height 1.76 ± 0.06 m, body mass 76.5 ± 9.4 kg). From these 43 participants, cortical porosity and micro-finite element analysis was successfully completed for 36 participants; baseline and follow-up DXA scans were available for 39 participants, and; baseline and follow-up blood samples were available for 42 participants. There was no difference in those lost to follow-up and the final sample for demographic ($P \geq 0.394$), bone metabolic ($P \geq 0.261$), areal BMD ($P \geq 0.153$) or HR-pQCT outcomes (dominant, $P \geq 0.169$; non-dominant, $P \geq 0.066$). Baseline measures were taken throughout the year (January, n = 1; March, n = 7; April, n = 8; June, n = 10; November, n = 17).

3.1. Areal Bone Mineral Density

Training increased areal BMD for total body ($P = 0.031$, $d_z = 0.36$) and arms ($P = 0.001$, $d_z = 0.57$), but not legs ($P = 0.094$, $d_z = 0.27$) and trunk ($P = 0.338$, $d_z = 0.16$) (Table 1; Figure 1).

Table 1. Total body and regional areal bone mineral density during week 1 and week 13 of British Army infantry training. Data are mean ± SD.

	Pre-Training	*Post-Training*	*Mean Change (95% CI)*
aBMD ($g \cdot cm^2$)			
Total Body	1.22 ± 0.09	1.24 ± 0.08*	0.02 (0.00, 0.03)
Arms	0.80 ± 0.06	0.84 ± 0.09*	0.04 (0.02, 0.06)
Legs	1.32 ± 0.10	1.35 ± 0.10	0.03 (−0.01, 0.07)
Trunk	1.07 ± 0.10	1.09 ± 0.10	0.02 (−0.02, 0.05)

BMD, bone mineral density

*P < 0.05 vs pre-training

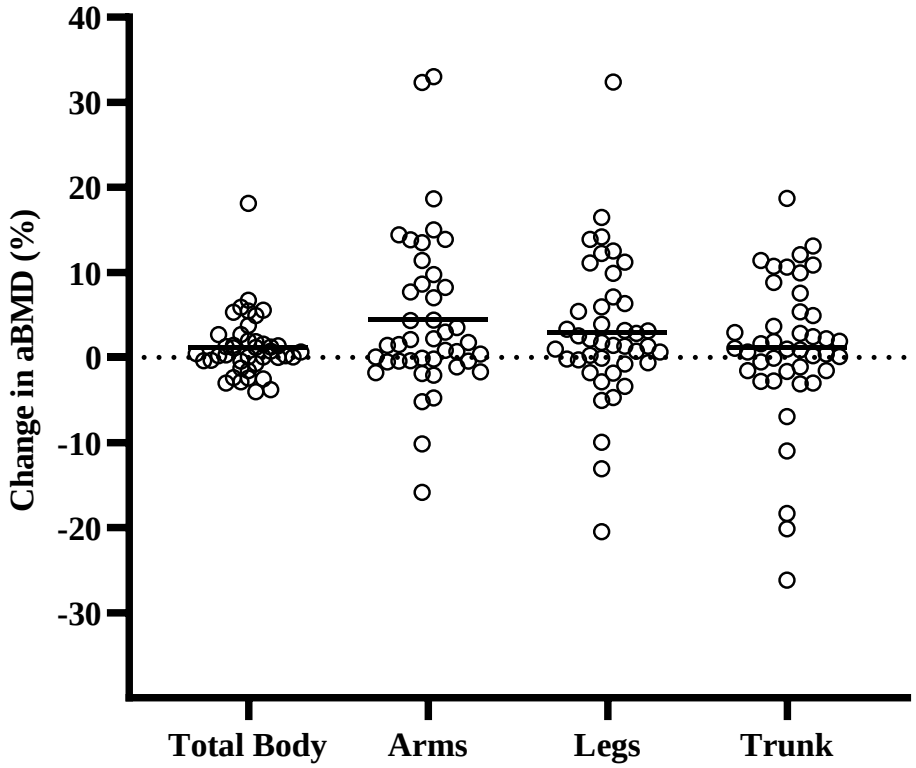

Figure 1. Change (%) in areal bone mineral density in response to British Army infantry training. Horizontal bars represent the mean value; individual participants are represented by open circles.

aBMD, areal bone mineral density

*P < 0.05 pre-training vs post-training

An example 3D reconstruction of the distal tibia is illustrated in Figure 2. Tibial vBMD, geometry, microarchitecture and mechanical strength for the dominant and non-dominant legs are displayed in Table 2, with the relative changes shown in Figures 3 to 6.

3.2. Volumetric Bone Mineral Density

Training increased total vBMD, Tb.vBMD and Ct.vBMD (main effect of time, all $P \leq 0.001$, $\eta_p^2 \geq 0.222$), with no leg × time interactions (all $P \geq 0.072$, $\eta_p^2 \leq 0.075$). There were no differences between legs for total vBMD and Tb.vBMD (main effect of leg, both $P \geq 0.187$,

$\eta_p^2 \leq 0.041$), but Ct.vBMD was greater in the non-dominant compared to the dominant leg (main effect of leg, $P = 0.011$, $\eta_p^2 = 0.146$).

3.3. Geometry

Training increased Tb.BV/TV, Ct.Area and Ct.Th (main effect of time, $P < 0.001$, $\eta_p^2 \geq 0.339$) and reduced Tb.Area (main effect of time, $P < 0.001$, $\eta_p^2 = 0.460$), with no leg × time interactions (all $P \geq 0.077$, $\eta_p^2 \leq 0.073$). Training had no effect on Ct.Pm (main effects of time, $P = 0.092$, $\eta_p^2 = 0.068$), with no leg × time interaction ($P = 0.311$, $\eta_p^2 = 0.025$). There were no differences between legs for Tb.BV/TV, Tb.Area, Ct.Th or Ct.Pm (main effect of leg, both $P \geq 0.117$, $\eta_p^2 \leq 0.058$), but Ct.Area was greater in the non-dominant compared to the dominant leg (main effect of leg, $P = 0.016$, $\eta_p^2 = 0.131$).

3.4. Microarchitecture

Training had no effect on Tb.N, Tb.Th, Tb.Sp and Ct.Po (main effects of time, all $P \geq 0.210$, $\eta_p^2 \leq 0.037$), with no leg × time interaction (all $P \geq 0.447$, $\eta_p^2 \leq 0.014$) and no difference between legs (main effects of leg, all $P \geq 0.143$, $\eta_p^2 \leq 0.053$).

3.5. Mechanical Strength

Training had no effect on stiffness and failure load (main effects of time, both $P \geq 0.188$, $\eta_p^2 \leq 0.049$) with no leg × time interaction ($P \geq 0.429$, $\eta_p^2 \leq 0.018$). Stiffness and failure load were higher in the non-dominant leg compared to the dominant leg (main effects of leg, both $P \leq 0.006$, $\eta_p^2 \leq 0.202$).

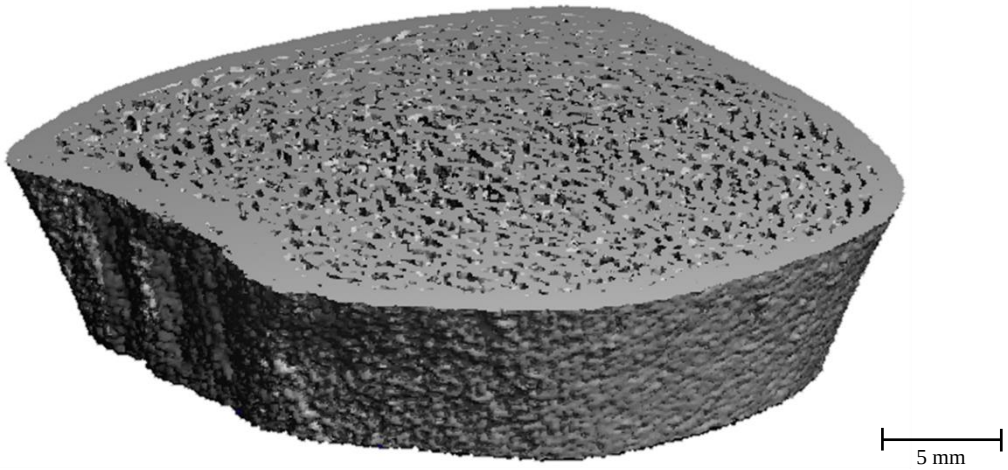

Figure 2. Three-dimensional construction of the dominant distal tibia from a randomly selected participant at the pre-training timepoint.

Table 2. Bone volumetric density, geometry, microarchitecture and biomechanical properties of the distal tibia of the dominant and non-dominant leg during week 1 and week 13 of British Army infantry training. Data are mean ± SD.

	Dominant			Non-Dominant		
	Pre-Training	*Post-Training*	*Mean Change (95% CI)*	*Pre-Training*	*Post-Training*	*Mean Change (95% CI)*
Total Bone						
vBMD (mg HA/cm^3)	345 ± 39	351 ± 41*	6 (4, 8)	347 ± 39	354 ± 40*	7 (4, 10)
Trabecular Bone						
Tb.vBMD (mg HA/cm^3)	229 ± 25	232 ± 25*	3 (2, 4)	229 ± 25	233 ± 25*	4 (2, 6)
Tb.Area (mm^2)	700 ± 121	697 ± 122*	−3 (−5, −2)	706 ± 121	703 ± 122*	−3 (−4, −2)
Tb.BV/TV (%)	19.1 ± 2.1	19.3 ± 2.1*	0.2 (0.1, 0.4)	19.0 ± 2.1	19.4 ± 2.1*	0.4 (0.2, 0.5)
Tb.N (mm^{-1})	2.16 ± 0.29	2.20 ± 0.30	0.05 (−0.04, 0.12)	2.15 ± 0.27	2.17 ± 0.28	0.02 (−0.04, 0.09)
Tb.Th (μm)	90 ± 13	89 ± 11	−1 (−4, 3)	90 ± 12	90 ± 11	1 (−2, 3)
Tb.Sp (μm)	383 ± 60	375 ± 60	−8 (−22, 6)	383 ± 57	378 ± 57	−5 (−17, 6)
Cortical Bone						
Ct.vBMD (mg HA/cm^3)	883 ± 29	888 ± 26*	5 (1, 9)	888 ± 28†	896 ± 26*†	8 (4, 13)
Ct.Area (mm^2)	146 ± 25	150 ± 26*	4 (2, 6)	149 ± 26†	153 ± 27*†	4 (3, 5)
Ct.Th (mm)	1.28 ± 0.25	1.32 ± 0.26*	0.04 (0.02, 0.06)	1.30 ± 0.27	1.35 ± 0.26*	0.05 (0.02, 0.08)
Ct.Pm (mm)	115 ± 8	115 ± 8	0 (−1, 0)	115 ± 8	115 ± 8	0 (0, 0)
Ct.Po (%)	4.8 ± 1.7	4.7 ± 1.4	−0.1 (−0.4, 0.2)	4.6 ± 1.5	4.5 ± 1.6	−0.1 (−0.3, 0.1)

Stiffness (kN/mm)	284.7 ± 33.3	286.3 ± 30.1	1.6 (−4.4, 7.7)	288.6 ± 33.1†	292.4 ± 30.5†	3.7 (−1.0, 8.5)
Failure Load (kN)	14.21 ± 1.58	14.30 ± 1.48	0.09 (−0.16, 0.35)	14.39 ± 1.61†	14.57 ± 1.49†	0.17 (−0.02, 0.37)

Ct.Area, cortical area; Ct,Pm, cortical perimeter; Ct.Po, cortical porosity; Ct.Th, cortical thickness; Ct.vBMD; cortical volumetric bone mineral density; mg HA/cm^3, milligram of hydroxyapatite per cubic centimetre; Tb.Area, trabecular area; Tb.BV/TV, trabecular volume to total bone volume ratio; Tb.N, number of trabeculae; Tb.Sp, trabecular separation; Tb.Th, trabecular thickness; Tb.vBMD, trabecular volumetric bone mineral density; vBMD, volumetric bone mineral density.

*$P < 0.05$ vs pre-training; †$P < 0.05$ vs dominant leg at the same timepoint

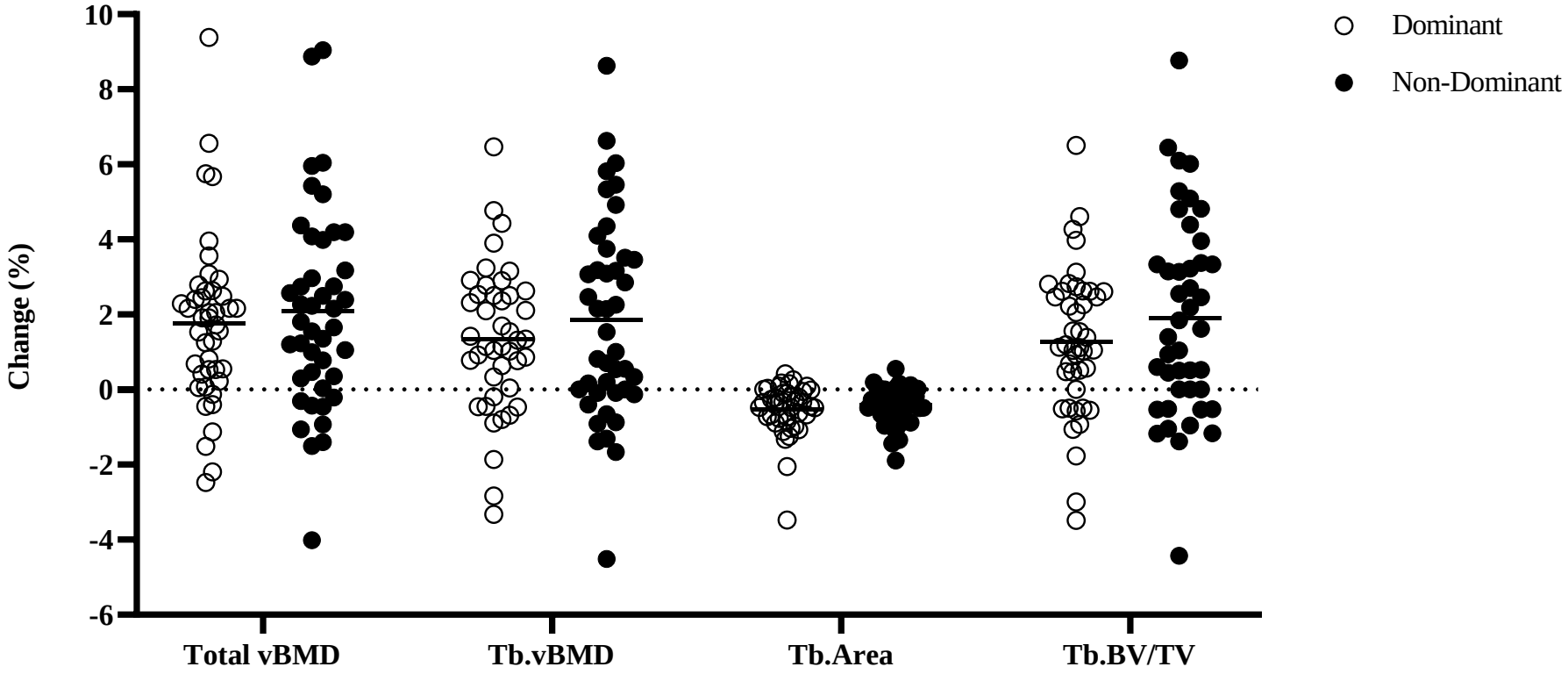

Figure 3. Change (%) in total and trabecular volumetric bone mineral density, and trabecular geometry (area and volume) of the distal tibia in response to British Army infantry training. Horizontal bar represents the mean value; individual participants are represented by circles.

Total vBMD; total volumetric bone mineral density; Tb.vBMD, trabecular volumetric bone mineral density; Tb.Area, trabecular area; Tb.BV/TV, trabecular volume to total bone volume ratio.

*P < 0.05 pre-training vs post-training

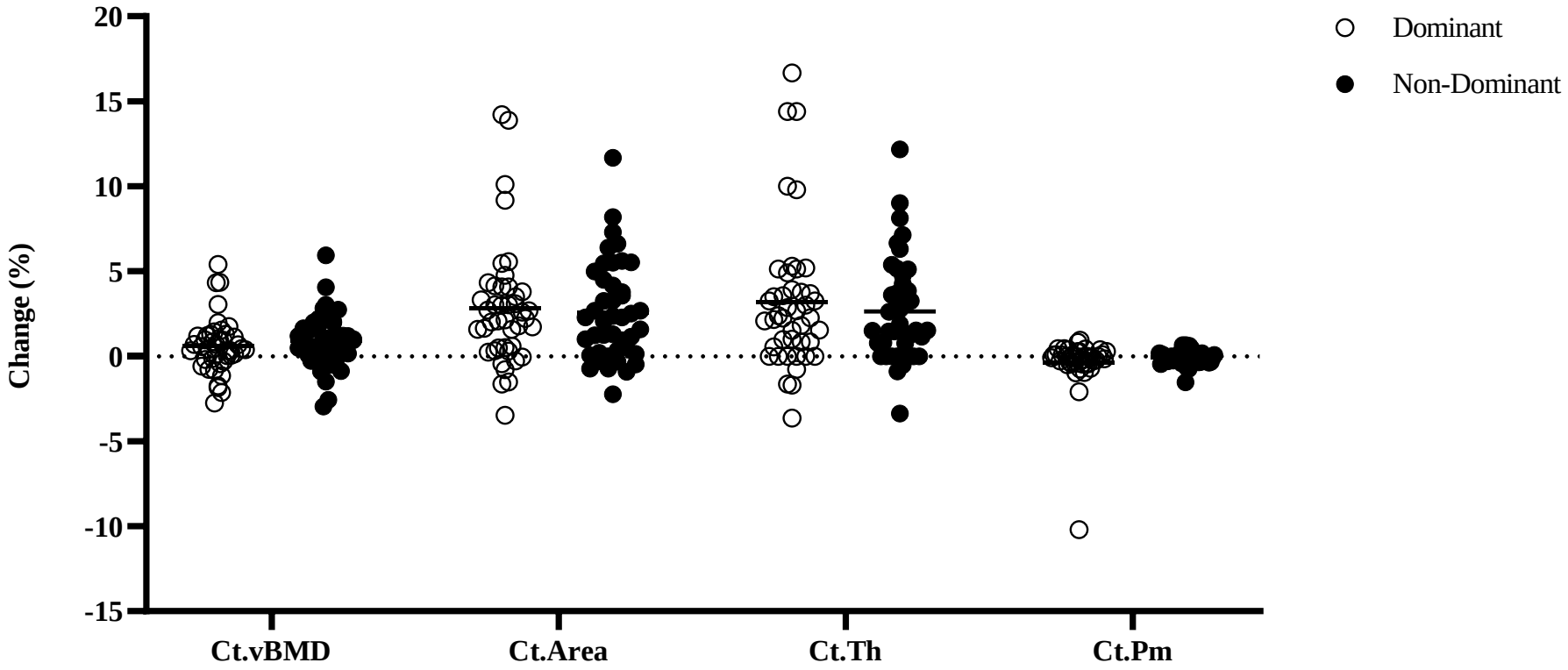

Figure 4. Change (%) in cortical volumetric bone mineral density and geometry (area, thickness and perimeter) of the distal tibia in response to British Army infantry training. Horizontal bar represents the mean value; individual participants are represented by circles. Cortical thickness and cortical perimeter data were removed from the graph for one participant to improve clarity.

Ct.vBMD, cortical volumetric bone mineral density; Ct.Area, cortical area; Ct.Th, cortical thickness; Ct.Pm, cortical perimeter

*P < 0.05 pre-training vs post-training

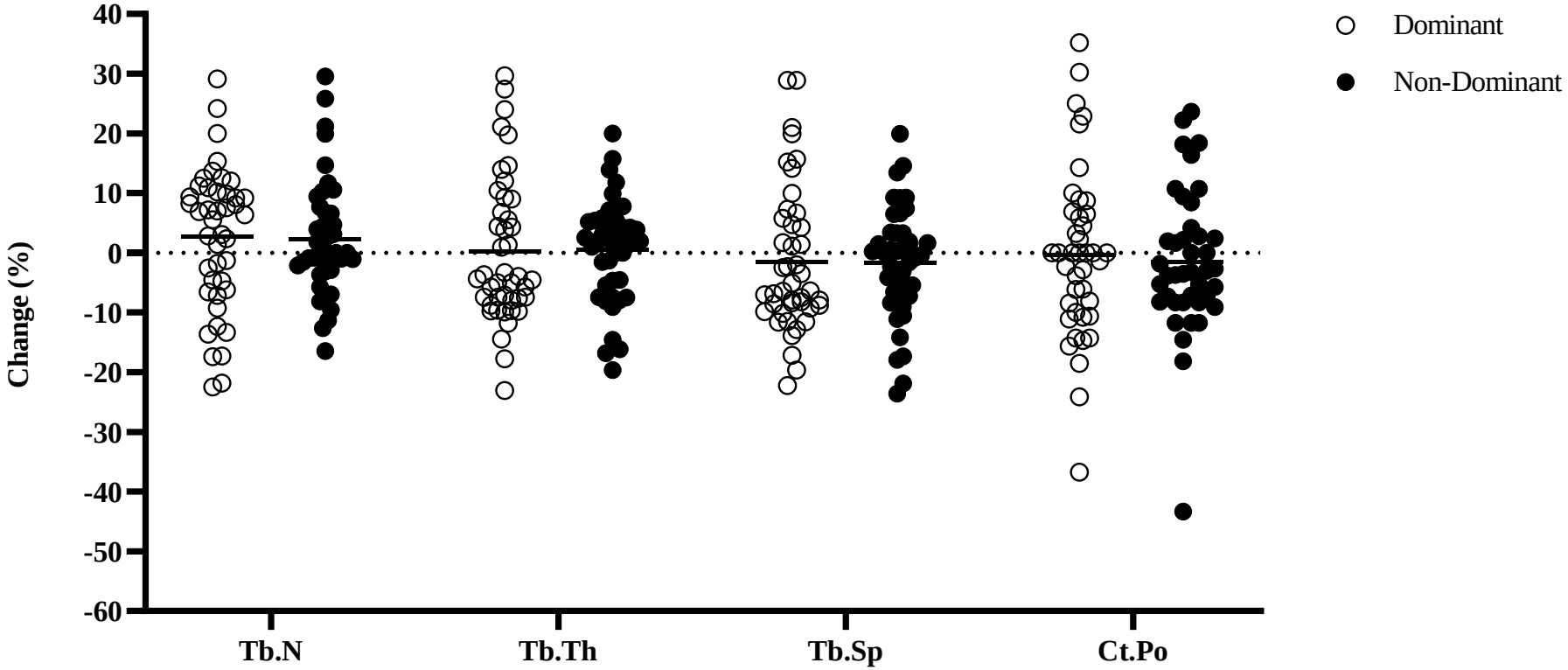

Figure 5. Change (%) in trabecular microarchitecture and cortical porosity of the distal tibia in response to British Army infantry training. Horizontal bar represents the mean value; individual participants are represented by circles.

Tb.N, number of trabeculae; Tb.Th, trabecular thickness; Tb.Sp, trabecular seperation; Ct.Po, cortical porosity

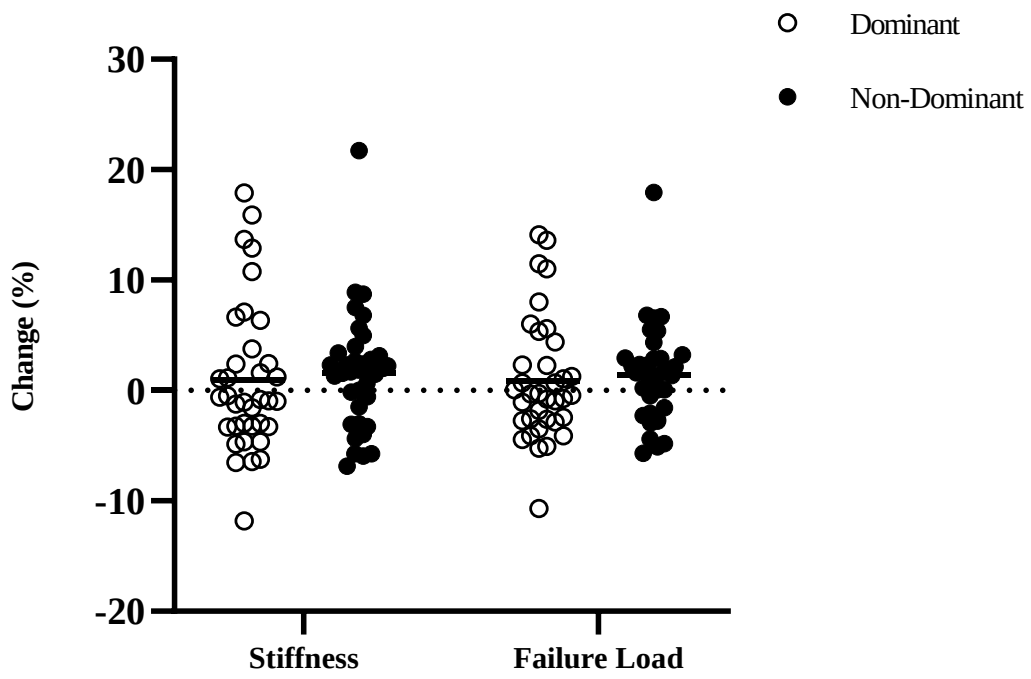

Figure 6. Change (%) in stiffness and failure load of the distal tibia in response to British Army infantry training. Horizontal bar represents the mean value; individual participants are represented by circles.

3.6. Biochemical Markers of Bone Metabolism

βCTX was lower ($P < 0.001$, $d_z = -0.66$) and total 25(OH)D was higher ($P = 0.029$) at week 13 compared with week 1. PTH ($P = 0.605$, $d_z = 0.08$), P1NP ($P = 0.385$) and ACa ($P = 0.254$) were not different between timepoints (Table 3).

Table 3. Changes in biochemical markers of bone metabolism. Data are mean ± SD.

	Pre-Training	*Post-Training*	*Mean Change (95% CI)*
P1NP ($\mu g \cdot l^{-1}$)	99.1 ± 39.9	94.3 ± 31.1	−4.8 (−13.2, 3.6)
βCTX ($\mu g \cdot l^{-1}$)	0.52 ± 0.17	0.41 ± 0.16*	−0.11 (−0.16, −0.06)
Total 25(OH)D ($nmol \cdot l^{-1}$)	50.3 ± 27.9	59.7 ± 29.2*	9.4 (2.3, 16.5)
ACa ($mmol \cdot l^{-1}$)	2.37 ± 0.07	2.39 ± 0.10	0.02 (−0.01, 0.06)
PTH ($pg \cdot ml^{-1}$)	3.6 ± 1.3	3.8 ± 1.3	0.1 (−0.3, 0.5)

P1NP, procollagen 1 N-terminal propeptide; βCTX, C-telopeptide cross-links of type 1 collagen; ACa, albumin-adjusted calcium; PTH, parathyroid hormone.

*P < 0.05 vs pre-training

4. Discussion

This study reports the adaptations of the tibia to a short period (13 weeks) of British Army infantry basic military training. Basic military training is physically (and mentally) arduous and consists of prolonged periods of weight-bearing activity [35-37], indicated by the high incidence of lower limb injuries [10]. We demonstrated increases in density (trabecular and cortical bone) and adaptations to geometry (increases in trabecular volume, cortical thickness and cortical area, and reductions in trabecular area) of the distal tibia in men undergoing basic military training. We found no evidence of changes in trabecular or cortical (porosity) microarchitecture, or estimated mechanical strength. No difference in adaptation between the dominant and non-dominant tibia supports the use of either limb in assessing the effect of exercise interventions. Increased arm aBMD and decreased bone resorption (βCTX) were observed at week 13. The bilateral measurement protocol, and concurrent assessment of whole-body aBMD and bone metabolic markers, provides a novel assessment of skeletal macro- and microstructure adaptations to arduous exercise. These data confirm our previous findings that

anabolic bone changes occur with relatively short periods of unaccustomed exercise, but are likely achieved at forces close to fracture threshold in some individuals [7].

4.1. Volumetric Bone Mineral Density

We observed bilateral increases in trabecular vBMD (~2%), which was similar for both limbs. The lack of significant change in legs aBMD, despite a similar magnitude of increase (2.8%), suggests DXA is less sensitive than HR-pQCT in detecting subtle lower limb adaptations following exercise training. An increase in trabecular vBMD is recognised as an early adaptation to mechanical loading [7, 38, 39]. Previous pQCT studies have demonstrated increases in distal tibia trabecular vBMD following 8 weeks aerobic training [38], 12 weeks soccer training [39] and 8 to 10 weeks of basic military training [7, 8]. In a recent HR-pQCT study, trabecular vBMD at the distal tibia increased in female recruits (2%) following 8 weeks US Army basic military training [9]. An increase in the volume and frequency of irregular and high magnitude tibial impacts likely contributes to the osteogenic nature of military training [7, 8, 40]. Exercise training is osteogenic at skeletal sites where the mechanical stress is greatest [15] and basic military training involves prolonged periods of locomotion, often whilst carrying heavy loads [35, 37], and high tibial impacts during activities like drill [41, 42]. During human locomotion, the tibia experiences high bending, torsion and compression stresses [43, 44], which increase in proportion to speed [43]. The distal tibia is predominantly exposed to compressive forces [44] and an increase in trabecular vBMD is considered important for shock absorption to resist these stresses [7]. Military training also involves a high volume of upper body exercise [35], which as a result of increased muscle action, likely explains the large increase in arms aBMD (5.2%).

Cortical vBMD increased in both the dominant (0.6%) and non-dominant (0.9%) leg. Exercise intervention studies are most consistently associated with an increase in trabecular, but not cortical vBMD. Cortical vBMD at the tibia diaphysis (38% and 66% sites), measured by pQCT, did not change following 8 weeks aerobic or resistance training in women [38], but increased (14%, 38% and 66% sites) following 12 weeks soccer training [39] and 10 weeks military training [7] in men. In contrast, cortical vBMD of the distal tibia (−0.3%), measured by HR-pQCT, decreased in female US Army recruits following 8 weeks basic military training [9]. The authors postulated this reduction in cortical vBMD was due to the unmineralised nature of new bone, or the lag between bone formation with bone resorption, supported by an increase in bone resorption (βCTX) and unchanged bone formation (P1NP). The longer training duration in our study may have allowed new cortical bone to mineralise, indicated by a reduction in bone resorption (βCTX) and unchanged bone formation (P1NP), supporting a net increase in bone formation; a sex difference in the skeletal adaptations to mechanical loading may also explain differences between studies. These observations suggest that military training results in intracortical remodelling, an important process in the pathogenesis of stress fractures due to a temporary increase in porosity from the removal of fatigue damage [2]. Increased cortical porosity temporarily reduces mechanical strength [17, 22], and contributes to the increased fragility with aging [22, 45], but we did not observe a change in cortical porosity, in agreement with others [9]. The greater cortical vBMD in the non-dominant leg is likely due to its supporting actions, consistent with athlete data [46], and may explain the greater failure load in this leg. An increase in cortical vBMD, however, which increases stiffness, does not necessarily confer a mechanical advantage, with changes in geometry also important contributors to bone strength [7].

4.2. Geometry

The increase in cortical area, as a result of the thickening of the cortex, confirms our previous pQCT findings at diaphyseal sites following military training [7], and corroborates recent HR-pQCT data following US Army basic training [9]. Reductions in trabecular area and unchanged cortical perimeter, consistent with previous HR-pQCT studies of basic military training [9], reflect an increase in cortical thickness due to endocortical contraction, not periosteal expansion, which might explain why we observed no change in bone strength (stiffness or failure load). The ability of bone to increase its cross-sectional area is critical to resisting axial loading during weight bearing activity, and confers a structural advantage as the tibial cortex is placed further from the neutral axis to increase resistance to bending [7, 39]. This biomechanical response is responsible for increased strength, where only modest improvements in vBMD are observed [7]. The distal tibia is pre-dominantly trabecular bone, and the forces are mainly compressive [44]. Changes to tibial geometry may be less important in increasing strength compared to the tibial diaphysis where bending forces are more prominent [7], and trabecular microarchitecture makes an important contribution to the increases in strength at the distal tibia following mechanical loading [9].

4.3. Microarchitecture

Trabecular microarchitecture (trabecular number, thickness and separation) and cortical porosity remained unchanged following training. Animal studies show that the increase in bone strength induced with running training is mediated by changes in trabecular microarchitecture in addition to changes in density and geometry [28]. Few studies have examined the human tibial microstructure in response to mechanical loading. Cross-sectional HR-pQCT studies have reported no difference in trabecular microarchitecture between athletes and controls [27], higher trabecular number and lower trabecular separation in soccer players or alpine skiers compared with controls or swimmers [25], and higher cortical porosity in athletes compared to

controls [26]. The increase in mechanical strength following US Army basic training was accompanied by an increase in trabecular thickness and number, a reduction in trabecular separation, and unchanged cortical porosity [9]. Our study had a smaller sample size and used lower resolution imaging than Hughes et al. [9] (voxel size of 82 vs 61 μm), which may have prevented us detecting subtle changes in trabecular microarchitecture. The dense trabecular network is important for absorbing, and distributing to the cortex, the applied mechanical stresses [21]. The network of trabeculae have an ansiotropic distribution aligned parallel to the mechanical stress axis, and therefore, trabecular microarchitecture is conditioned by the imposed mechanical strains [19] and an important contributor to bone strength [4, 6, 16, 19]. Our data suggest short-term mechanical loading does not change the trabecular microarchitecture or estimated mechanical strength in men.

4.4. Limitations

This study has several limitations. Due to the high level of attrition during infantry training, we were only able to follow-up those individuals who completed training and our data are subject to survivor bias. We were also limited to measuring the distal tibia with our HR-pQCT model, whereas the diaphysis, subject to high bending stresses, is more clinically relevant in recruits. The interpretation of our data is limited by our pre-training vs post-training design and we are unable to comment on the temporal changes in bone. Finally, we were unable to include a comparator control group, however, this is typical of observational mechanical loading studies [7, 9, 39], and we therefore do not think this affects the interpretation of the data.

4.5. Conclusion

A short period of unaccustomed basic military training (13 weeks) increased density and altered geometry of the distal tibia in male military recruits. Bone microarchitecture and *estimated*

bone strength did not change with training. The osteogenic effects of basic military training are likely due to an increase in unaccustomed dynamic and high-impact loading.

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Competing Interests

The authors have no competing interests to declare.

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