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Changes in tibial bone microarchitecture in female recruits in response to 8 weeks of U.S. Army Basic Combat Training☆

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ABSTRACT

Background: U.S. Army Basic Combat Training (BCT) is a physically-demanding program at the start of military service. Whereas animal studies have shown that increased mechanical loading rapidly alters bone structure, there is limited evidence of changes in bone density and structure in humans exposed to a brief period of unaccustomed physical activity.

Purpose: We aimed to characterize changes in tibial bone density and microarchitecture and serum-based biochemical markers of bone metabolism in female recruits as a result of 8 weeks of BCT.

Methods: We collected high-resolution peripheral quantitative computed tomographic images of the distal tibial metaphysis and diaphysis (4% and 30% of tibia length from the distal growth plate, respectively) and serum markers of bone metabolism before and after BCT. Linear mixed models were used to estimate the mean difference for each outcome from pre- to post-BCT, while controlling for race/ethnicity, age, and body mass index.

Results: 91 female BCT recruits volunteered and completed this observational study (age = 21.5 ± 3.3 yrs). At the distal tibial metaphysis, cortical thickness, trabecular thickness, trabecular number, bone volume/total volume, and total and trabecular volumetric bone density (vBMD) increased significantly by 1–2% (all $p < 0.05$) over the BCT period, whereas trabecular separation, cortical tissue mineral density (TMD), and cortical vBMD decreased significantly by 0.3–1.0% (all $p < 0.05$). At the tibial diaphysis, cortical vBMD and cortical TMD decreased significantly (both −0.7%, $p < 0.001$). Bone strength, estimated by micro finite element analysis, increased by 2.5% and 0.7% at the distal tibial metaphysis and diaphysis, respectively (both $p < 0.05$). Among the biochemical markers of bone metabolism, sclerostin decreased (−5.7%), whereas bone alkaline phosphatase, C-telopeptide cross-links of type 1 collagen, tartrate-resistance acid phosphatase, and 25(OH)D increased by 10–28% (all $p < 0.05$).

Conclusion: BCT leads to improvements in trabecular bone microarchitecture and increases in serum bone formation markers indicative of new bone formation, as well as increases in serum bone resorption markers and decreases in cortical vBMD consistent with intracortical remodeling. Together, these results demonstrate specific changes in trabecular and cortical bone density and microarchitecture following 8 weeks of unaccustomed physical activity in women.

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1. Introduction

Stress fractures are common and costly skeletal injuries that occur in the lower extremities of endurance athletes and military personnel. Female athletes and military service members are at greater risk of stress fracture than their male counterparts [1,2]. Stress fractures occur during periods of heightened physical activity, such as Basic Combat Training (BCT), when Army recruits undergo 8–12 weeks of activities that include running, calisthenics, and marching [3]. During BCT, up to 5% of men and 20% of women sustain a stress fracture [4,5]. The most common stress fracture sites are the tibia, fibula, and metatarsals, followed by the femur and pelvis [6].

There are multiple factors affecting stress fracture risk [1,7], but notably the pathophysiology that underlies stress fracture is not well understood. In simple terms, stress fractures result when the transient deleterious effects of bone remodeling outpace the protective effects of adaptive bone formation [8]. Thus, understanding the magnitude and extent of these bone changes during BCT may lead to new insights into stress fracture etiology. Although animal studies have demonstrated that novel physical activity or mechanical loading is associated with changes in bone density, macrostructure, and microstructure, primarily due to *de novo* bone formation on existing surfaces [9,10], there are limited reports of analogous changes in humans. Increased volumetric bone mineral density (vBMD) in military recruits and civilians has been reported following 8–13 weeks of military training [11] and aerobic and resistance training [12,13]. However, few studies have reported specific changes in bone microstructure in response to novel physical activity.

Assessing changes in bone microarchitecture, such as trabecular thickness, number, and separation, may reveal skeletal adaptations that occur in response to unaccustomed physical activity. However, the resolution required to measure changes in bone microarchitecture using non-invasive imaging became available only recently. Specifically, with a voxel size of 61 μm, the second generation high-resolution pQCT (HR-pQCT) scanner enables assessment of changes in bone microarchitecture independently of changes in bone density [14]. Accordingly, the purpose of this prospective, longitudinal study was to assess changes in bone metabolism, density and microarchitecture following 8 weeks of BCT in female U.S. Army recruits. We hypothesized that BCT would produce changes in trabecular and cortical bone microarchitecture and changes in bone turnover markers indicative of adaptive bone formation.

2. Methods

2.1. Volunteers

Female U.S. Army recruits from Fort Jackson, South Carolina were eligible to participate in this study if they were between the ages of 17 and 42; were not pregnant or breastfeeding; and had no self-reported history of an endocrine disorder, bone-modifying disorder, amenorrhea, kidney disease, renal calculi, or glucocorticoid drug prescription within two years of the study. Recruits were briefed on the voluntary nature of the study, without command staff influence, and with an ombudsperson present. Consistent with the BCT environment, the recruits were provided three meals a day in the dining facility. Study volunteers were also provided with one, ~130-calorie placebo snack bar with 20 mg and 0.7 IU of incidental calcium and vitamin D per day, respectively, for consumption between meals.

Volunteers completed baseline data collection during the first week of BCT immediately prior to starting training, and follow-up data collection 8 weeks later at the end of the BCT training period. Recruits underwent physical and military specific training during BCT, including road marches carrying heavy loads, running, resistance exercises such as push-ups and sit-ups, hand-to-hand combat training, and weapons training. While not directly measured in this study, the amount of time spent in various activities and the energy expenditure associated with general BCT have been published elsewhere [3,15].

This study was approved by the Human Use Review Committee at the U.S. Army Research Institute of Environmental Medicine (USARIEM) in Natick, MA, and by the Human Research Protection Office at the U.S. Army Medical Research and Materiel Command (USAMRMC) in Fort Detrick, MD. The study was conducted at Fort Jackson, SC, between the months of April and June 2015. Volunteers participated in this study after providing informed written voluntary consent. Investigators adhered to U.S. Army Regulation 70–25 and USAMRMC Regulation 70–25 on the participation of volunteers in research.

2.2. Anthropometrics

Standing height was measured to the nearest cm at baseline only using a stadiometer (Creative Health Products, Plymouth, MI), while body mass was measured to the nearest 0.1 kg at baseline and follow-up using a calibrated digital scale (Belfour Scales, Saukville, WI). Height and body mass were measured with volunteers in physical training uniforms (athletic shorts and t-shirt) and without shoes. Body composition was assessed at baseline and follow-up using skinfold measurements at the abdomen, triceps, and suprailiac regions in accordance with the American College of Sports Medicine Guidelines for Exercise Testing and Prescription [16]. Skinfold thicknesses were converted to an estimated percent body fat using Jackson and Pollock's 3-site method [17]. In order to minimize between-tester measurement difference in skinfolds from baseline to follow-up, volunteers had their skinfold measurements collected by the same technician at both time points.

2.3. Lifestyle, physical activity, and dietary background

Self-reported demographic, physical activity, and lifestyle characteristics were assessed *via* survey at baseline. Habitual dietary intake was assessed using a validated, semi-quantitative Block 2005 Food Frequency Questionnaire (NutritionQuest, Berkeley, CA) at baseline and follow-up. At baseline, participants were asked to answer questions regarding foods consumed and their quantities during the 3 months prior to entering BCT. Questionnaires were self-administered under the supervision of Registered Dietitians and intake data were analyzed by NutritionQuest utilizing USDA's Food and Nutrient Database for Dietary Studies v.1.0 [18].

2.4. Blood collection and analysis

Fasting blood samples were collected from the antecubital vein at the beginning and end of BCT. Blood was separated for serum and plasma, frozen, and shipped to USARIEM and Pennington Biomedical Research Center (PBRC, Baton Rouge, LA) for biochemical assays. A small amount of heparinized whole blood was analyzed on-site for time-sensitive markers, such as ionized calcium (iCa), using a portable iSTAT® analyzer and Chem8+ cartridges (Abbott Laboratories, Abbott Park, IL). Enzyme-linked immunosorbent assays (ELISA) were performed using a Dynex DS-2 immunoassay system for: bone alkaline phosphatase (BAP, Quidel), osteocalcin (OCN, ALPCO), osteoprotegerin (OPG, ALPCO), tartrate-resistant acid phosphatase 5b (TRAP5b, Quidel), C-telopeptide cross-links of type I collagen (CTX, Immunodiagnostic Systems), and procollagen I N-terminal propeptide (P1NP, MyBioSource). Sclerostin and soluble RANK ligand (sRANKL) were measured by multiplexing on a Luminex MAGPIX system using kits from EMD Millipore. Intact parathyroid hormone (PTH) was assayed on a Siemens Immulite 2000 system. Inter-assay coefficients of variation (CV) were as follows: iCa 0.67%, BAP 2.0%, OCN 7.2%, OPG 5.4%, TRAP5b 5.0%, CTX 4.7%, P1NP 6.9%, sclerostin 12.2%, sRANKL 10.3%, and PTH 3.9%. All samples were run in duplicate, and samples were re-assayed in the event of poor agreement between the replicates.

We measured 25-hydroxyvitamin D (25(OH)D) using radioimmunoassay (Diasorin Inc., Stillwater, MN) at the Pennington Biomedical Research Center (PBRC) clinical chemistry laboratory, which is accredited by the College of American Pathologists and routinely participates in inter-laboratory standardization testing. The inter-assay CV for 25(OH)D was 6.7%.

2.5. High-resolution peripheral quantitative computed tomography (HR-pQCT)

We used HR-pQCT (XTremeCT II, Scanco Medical, Brüttisellen, Switzerland) scans to assess bone density, microarchitecture and strength at the distal metaphyseal and diaphyseal regions of the tibia. The XtremeCT II utilizes a 61 μm nominal isotropic voxel size, X-ray tube voltage of 68 kVp, and X-ray tube current of 1.47 mA, and obtains 168 parallel images, creating an image stack of approximately 10.25 mm in length. Quality control scans were completed daily using the manufacturer's phantom. HR-pQCT scans were obtained at baseline and follow-up at 4% and 30% of tibia length from the distal end of the non-dominant leg. Leg dominance was explained by study staff as the leg primarily used to kick a ball and was self-determined by participants. If the participant had a history of fracture in the non-dominant leg, the contralateral leg was scanned. Limb length was measured by palpating bony landmarks and using a flexible tape measure to measure the distance from the distal edge of the medial malleolus to the tibial plateau. Percentage of limb length was used as an index in order to effectively scan the same relative region of interest on all participants. For both scan locations, a scout view was performed and the percent distance was measured from the distal tibial plateau reference line to the center slice of the ~1 cm stack. Images were graded on a movement artifact scale of 1–5 [19], with 1 being no movement artifact and 5 being severe movement artifact. Participants were rescanned if the image showed grade 4 or 5 movement artifact. Of the total 384 scans performed at both sites, pre- and post-BCT, 8.2% were repeated due to movement. Scans were only included in the analysis if ranked at a grade 3 or below [19]. Regions of interest at the periosteal and endosteal surface were created following manufacturer procedures [20,21] and then manually checked to ensure proper placement. Where necessary, regions of interest were manually edited. Two-dimensional image registration based on cross-sectional area was used to align pre- and post-BCT scans at the 4% tibial length (metaphyseal) site only. The registration was not used at the 30% tibial length (diaphyseal) site to facilitate detection of changes in cross-sectional area at the diaphysis during BCT.

2.6. Bone microstructural measurements and finite element analysis

The following variables were measured at the distal metaphyseal site: total vBMD and cross-sectional area; trabecular vBMD, thickness, separation, number, and bone volume/total volume (BV/TV); and cortical vBMD, porosity, thickness and tissue mineral density (TMD). At the diaphysis, the following measurements were assessed: total cross-sectional area, periosteal perimeter, medullary area, and cortical vBMD, thickness, porosity, and TMD. We also used micro finite element analysis (μFEA; Scanco Medical FE software version 1.13) to estimate stiffness and failure load at the distal metaphyseal and diaphyseal sites, as previously reported [22]. In brief, each voxel was assigned a modulus of 10 GPa and Poisson's ratio of 0.3. We applied uniaxial compression and estimated failure load as the load when 2% of the elements exceeded 0.7% strain [23].

2.7. Short term precision study

To establish the short term precision for HR-pQCT variables in our laboratory we performed triplicate measurements of 15 subjects at both the distal metaphyseal and diaphyseal sites, with repositioning between each scan. The mean coefficients of variation (CV) for measurements at the 4% tibia site ranged from 0.44% to 0.61% for density measurements and from 0.68% to 1.55% for measurements of trabecular microstructure. The mean CV for cortical thickness was 1.85%. The mean CVs for measurements at the 30% tibia site ranged from 0.15% to 0.32% for measurements of area and from 0.12% to 5.23% for measurements of cortical morphology. The mean CV for cortical density was 0.12%.

2.8. Statistical analysis

Data are presented as mean ± standard deviation (SD) or mean and 95% confidence interval (CI). To estimate the mean difference and its corresponding 95% CI in each microarchitectural bone measurement and each blood biomarker from pre- to post-BCT, we used a linear mixed model for each outcome of interest. We included a random intercept for each study participant to account for within individual correlation between the pre- and post-BCT training measurements. Covariates evaluated in the statistical model were decided *a priori*, and included race/ethnicity (White Hispanic, White Non-Hispanic, Black Hispanic, Black Non-Hispanic, Other), age at baseline (yrs; continuous), and BMI at baseline (kg/m^2; continuous). We also tested for potential effect modification by race/ethnicity by including an interaction term in the model. We performed additional analyses to evaluate baseline predictors of change in trabecular vBMD, cortical vBMD, and BV/TV at the distal metaphyseal site. To do so, the change in trabecular vBMD, cortical vBMD, and BV/TV were dichotomized at the median, and the participants from the lower 50th percentile were compared to the participants from the upper 50th percentile to evaluate differences in the following baseline covariates: trabecular vBMD, cortical vBMD, BV/TV, age, BMI, weight, dietary calcium, 25OHD, race/ethnicity (White, Non-White), prior oral contraceptive use, physical activity over the 3 months prior to BCT (≤2 day exercise per week, ≥3 days exercise per week) and the change in the following covariates during training: weight, BMI, sclerostin, BAP, TRAP5b and CTX. Comparisons were conducted using *t*-tests for continuous predictor variables and chi-square tests for dichotomous predictor variables. All analyses were conducted using SAS software (version 9.3; SAS Institute, Inc. Cary, NC). Statistical significance was set at $p < 0.05$.

3. Results

3.1. Volunteer characteristics

Of the 99 volunteers who completed baseline data collection, 91 completed the follow-up visit at the end of BCT. Those who did not complete the study either left the training unit where the research was conducted (n = 4), left BCT entirely (n = 3), or left for unknown medical reasons (n = 1). The population was primarily composed of Black Non-Hispanic and White Non-Hispanic women (Table 1). On average, participants had BMI values that were normal (BMI within 18.5–24.9 kg/m^2) or overweight (BMI > 25.0 kg/m^2). Consistent with Army weight standards, no participant was categorized as obese. Approximately 50% of participants reported participating in physical activity 3 to 5 days per week within the 3 months prior to BCT. Less than a quarter of participants reported current hormonal contraceptive use or recent cigarette smoking. Body weight and BMI did not change during BCT [weight: 63.0 ± 9.5 kg to 63.2 ± 8.6 kg, p = 0.57, and BMI: 23.8 ± 2.8 kg/m^2 to 23.9 ± 2.4 kg/m^2, p = 0.58]. However, percent body fat decreased by 2.7 ± 2.8% during BCT [Baseline: 24.1 ± 4.3%; Follow-up: 21.4 ± 3.6%, p < 0.001].

3.2. Bone density, microarchitecture, and μFEA-derived estimates of bone strength

Eight weeks of BCT led to significant changes in bone density, microarchitecture and strength. At the metaphyseal region of the distal

Table 1
Baseline demographic characteristics.

Baseline characteristics	Mean ± SD or n (%)
Age (yrs)	21.5 ± 3.3
Race/ethnicity (n,%)	
White Hispanic	12 (13.2%)
White Non-Hispanic	28 (30.8%)
Black Hispanic	3 (3.3%)
Black Non-Hispanic	34 (37.4%)
Other	14 (15.4%)
Height (cm)	162.4 ± 7.2
Weight (kg)	63.0 ± 9.5
BMI n (%)	
< 18.5 kg/m^2	4 (4.4%)
18.5–24.9 kg/m^2	45 (49.5%)
25–29.9 kg/m^2	42 (46.2%)
> 30 kg/m^2	0 (0)
Body fat (%)	24.1 ± 4.3
Total intake (kcal/day)[a]	1964 ± 882
Dietary calcium (mg/day)[a]	798 ± 390
Meeting RDA for calcium (n, %)[b]	23 (29.1%)
Dietary vitamin D (IU/day)[a]	146 ± 118
Meeting RDA for vitamin D (n, %)[b]	1 (1.3%)
Physical activity n (%)	
Low, 0–2 days/wk	31 (34.1%)
Moderate, 3–5 days/wk	46 (50.1%)
High, 6+ days/wk	14 (15.4%)
Current contraceptive use (n, %)	17 (18.9%)
Current smoker (n, %)[c]	20 (22.0%)

[a] Dietary intake information was excluded if implausible energy intake was reported (< 300 kcal or > 4500 kcal, n = 13 pre, n = 1 post) as previously reported [24].

[b] RDA = Recommended daily allowance (calcium = 1300 mg/day < 19 years of age ≥ 1000 mg/day; vitamin D = 600 IU/day).

[c] Current smoker = reported smoking within past 30 days.

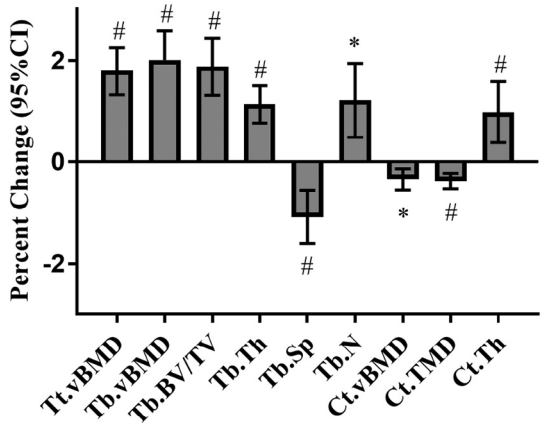

Fig. 1. Mean percent change (± 95% CI) from baseline in bone properties at the distal tibial metaphysis (4% site) following 8 weeks of Basic Combat Training. Results are adjusted for race, ethnicity, baseline age, and baseline BMI. Tt.vBMD = total volumetric bone mineral density; Tb.vBMD = trabecular volumetric bone mineral density; Tb.BV/TV = trabecular bone volume/total volume; Tb.Th = trabecular thickness; Tb.Sp = trabecular separation; Tb.N = trabecular number; Ct.vBMD = cortical volumetric bone mineral density; Ct.TMD = cortical tissue mineral density; Ct.Th = cortical thickness. Significantly different from baseline, *p < 0.05, #p < 0.001.

tibia (Fig. 1), mean (95% CI) trabecular thickness [1.13% (0.76, 1.50); p < 0.001], trabecular number [1.21% (0.48, 1.94); p < 0.05], trabecular bone volume/total volume [1.87% (1.31, 2.43); p < 0.001], trabecular vBMD [2.01% (1.44, 2.58); p < 0.001], and cortical thickness [0.98% (0.38, 1.58); p < 0.001] all increased significantly, whereas trabecular separation [−1.09% (−1.61, −0.56); p < 0.001], cortical vBMD [−0.34% (−0.55, −0.14); p < 0.05] and cortical TMD

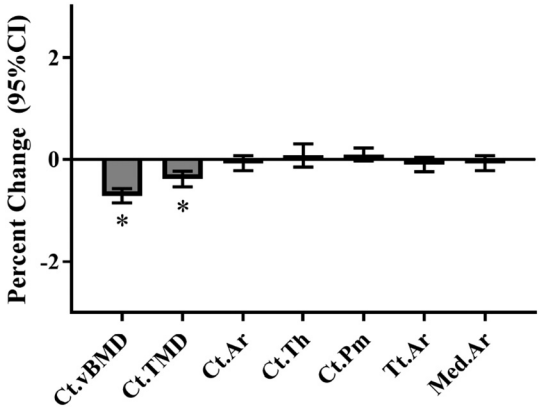

Fig. 2. Mean percent change (± 95% CI) from baseline in bone properties at the tibial diaphysis (30% site) following 8 weeks of Basic Combat Training. Results are adjusted for race, ethnicity, baseline age, and baseline BMI. Ct.vBMD = cortical volumetric bone mineral density; Ct.TMD = cortical tissue mineral density; Ct.Ar = cortical area; Ct.Th = cortical thickness; Ct.Pm = cortical periosteal perimeter; Tt.Ar = total cross-sectional area; Med.Ar = medullary area. Significantly different from baseline, *p < 0.001.

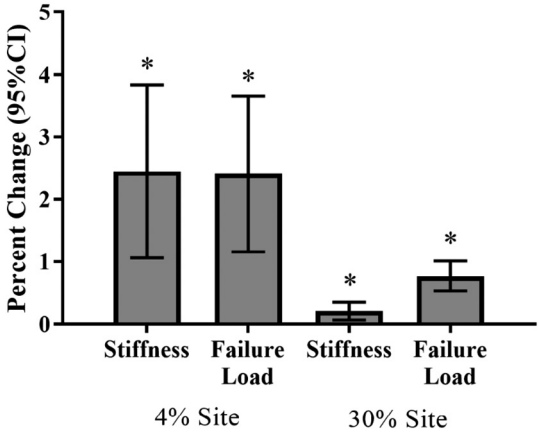

Fig. 3. Mean percent change (± 95% CI) from baseline in stiffness and failure load at the distal tibial metaphysis and diaphysis following 8 weeks of Basic Combat Training. Significantly different from baseline, *p < 0.01.

[−0.38% (−0.53, −0.23); p < 0.001] decreased significantly. Cortical porosity did not change. Absolute changes are reported in Supplemental Table 1. Representative images of changes in bone microarchitecture and visual overlays of the distal tibial metaphysis in a top and median responder, pre- and post-BCT, are depicted in Fig. 4 and Supplemental Fig. 1, respectively. At the diaphyseal site, both cortical vBMD [−0.71% (−0.86, −0.57); p < 0.001] and cortical TMD [−0.72% (−0.88, −0.57); p < 0.001] decreased slightly (Fig. 2). There were no changes in total area, cortical perimeter, medullary area, cortical thickness, and cortical porosity at the diaphysis (all p > 0.05) following BCT (Supplemental Table 2). μFEA-derived estimates of failure load and stiffness at the distal tibial metaphysis [2.41% (1.16, 3.65) and 2.45% (1.06, 3.84), respectively] and diaphysis [0.77% (0.53, 1.01) and 0.21% (0.07, 0.35), respectively] increased significantly (Fig. 3, p < 0.01 for all). The changes in bone density, microarchitecture and strength did not differ by race/ethnicity.

3.3. Circulating biomarkers of bone metabolism

BCT led to significant changes in circulating biomarkers of bone metabolism (Table 2). In particular, serum levels of sclerostin, a negative regulator of bone formation, decreased on average by 5.7% (p = 0.02). Among the serum biochemical markers of bone formation,

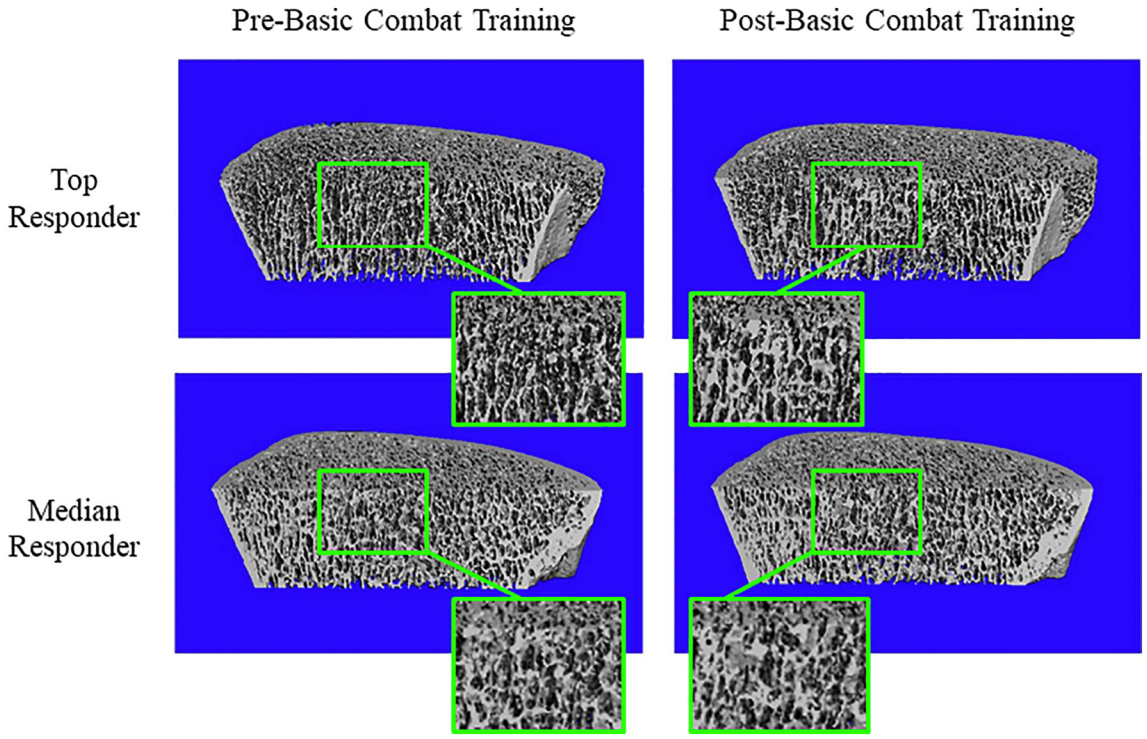

Fig. 4. Three-dimensional reconstruction of the pre- and post-Basic Combat Training distal tibia scans of the top and median responder. The top responder was an 18 year old, Non-Hispanic White Soldier whose bone volume/total volume (BV/TV) change was +10.50%. The median responder shown here was a 27 year old, Non-Hispanic Black Soldier whose BV/TV change was +1.0%. Note in the enlarged regions that trabecular thickness and trabecular separation are visually increased and decreased, respectively, following Basic Combat Training.

BAP increased on average by 26.2% ($p < 0.0001$) while P1NP was unchanged ($p = 0.53$). Biochemical markers of bone resorption also increased, including TRAP5b (19.1%, $p < 0.0001$) and CTX (10%, $p = 0.02$). Biochemical markers of osteoclastic differentiation, including sRANKL and OPG, did not change during BCT ($p = 0.51$ and $p = 0.26$, respectively). 25(OH)D increased significantly (28%, $p < 0.0001$), whereas PTH was stable ($p = 0.71$) during BCT. Table 2 reports unadjusted values for changes in biochemical markers of bone metabolism as no statistically significant changes were observed when adjusting for age, race/ethnicity, and baseline BMI.

3.4. Predictors of change

Individuals with the greatest increase in trabecular vBMD (3.72 ± 3.17%) had a lower baseline trabecular vBMD ($p < 0.001$) and were more likely to exercise < 2 days a week, 30 days prior to BCT ($p = 0.05$) than those individuals with the smallest increase in trabecular vBMD (0.28 ± 0.50%). Similarly, individuals with the greatest increases in trabecular BV/TV (3.36 ± 3.04%) had significantly lower baseline BV/TV ($p = 0.003$) compared to those with the smallest change in BV/TV (0.03 ± 0.62%). Further, individuals with the greatest decline in cortical vBMD (−1.67 ± 0.56%) were more likely to be vitamin D deficient at baseline (25OHD < 20 ng/ml) than individuals with the least decline in cortical vBMD (0.36 ± 0.91%, $p = 0.04$). We did not observe any other relationships between potential predictors and changes in trabecular or cortical density or microstructure.

4. Discussion

In this study, 8 weeks of BCT in young women led to changes in bone density and microarchitecture at the distal tibial metaphysis and changes in density at the tibial diaphysis, as assessed by high-resolution peripheral quantitative computed tomography (HR-pQCT). Specifically,

Table 2
Changes in serum biochemical markers of bone metabolism following 8 weeks of BCT.

Biochemical marker	Pre mean (SD)	Post mean (SD)	Absolute change (95% CI)	p-Value
Sclerostin (pg/ml)	955 (548)	898 (438)	−54 (−102, −7)	0.02
BAP (U/l)	20.7 (6.5)	26.2 (7.6)	5.4 (4.4, 6.4)	< 0.0001
P1NP (pg/l)	28.7 (25.4)	30.6 (29.4)	1.51 (−3.31, 6.33)	0.54
OCN (ng/ml)	8.43 (2.41)	8.67 (2.56)	0.22 (−0.13, 0.58)	0.21
TRAP5b (U/l)	2.57 (0.88)	3.08 (1.08)	0.49 (0.34, 0.63)	< 0.0001
CTX (ng/ml)	0.50 (0.22)	0.55 (0.20)	0.05 (0.01, 0.09)	0.02
sRANKL (pg/ml)	24.1 (22.1)	23.2 (23.0)	−0.79 (−3.16, 1.58)	0.51
OPG (pg/ml)	81.2 (33.4)	84.4 (28.5)	3.1 (−2.4, 8.6)	0.26
25(OH)D (ng/ml)	20.4 (7.8)	26.1 (5.6)	5.8 (4.6, 6.9)	< 0.0001
Ionized calcium (nmol/l)	1.22 (0.04)	1.22 (0.06)	0.01 (−0.003, 0.02)	0.14
Parathyroid hormone (pg/ml)	36.1 (14.9)	36.7 (15.3)	0.55 (−2.31, 3.40)	0.70

BAP = bone alkaline phosphatase; P1NP = procollagen I N-terminal propeptide; OCN = osteocalcin; TRAP5b = tartrate-resistant acid phosphatase; CTX = C-telopeptide cross-links of type I collagen; sRANKL = soluble RANK ligand; OPG = osteoprotegerin; 25(OH)D = 25-hydroxyvitamin D.

at the distal tibial metaphysis, trabecular BV/TV and thickness increased while trabecular separation decreased. Cortical vBMD and TMD declined at both the metaphyseal and diaphyseal sites. These changes in bone density and microstructure suggest that the skeletal response to BCT includes both new bone formation and, potentially, intracortical remodeling. These interpretations of heightened bone formation and remodeling are supported by significant increases in circulating concentrations of biomarkers of both bone formation and resorption during BCT, as also previously reported in other studies [25,26]. To our knowledge, this is one of the first studies to demonstrate specific changes in trabecular and cortical bone density and microarchitecture in women during a relatively short period (8 weeks) of unaccustomed physical activity.

Notably, the magnitude of the observed increases in trabecular and total vBMD at the distal tibial metaphysis are consistent with prior reports that used standard pQCT to assess the response to physical activity interventions and, as such, strengthen and extend upon the existing body of literature. For example, trabecular vBMD increased by 0.8–1.2% in the distal tibia following 13 weeks of aerobic and resistance training in 20-year-old women [12]. Similarly, trabecular vBMD increased by ~1.3% at the distal tibia in 20-year-old women following 8 weeks of aerobic training, whereas no change occurred in the non-exercise control and anaerobic exercise groups over the 8 weeks [13]. Likewise, in male and female Army recruits, total vBMD of the distal tibia increased by ~2% following 8 weeks of BCT, with slightly more favorable increases (~3%) reported in recruits administered daily supplemental calcium and vitamin D [11]. These studies suggest that anabolic changes in the bones of young adults occur relatively quickly in response to unaccustomed physical activity, as previously suggested by animal studies [27–29]. Our observations that lower baseline values of trabecular vBMD and BV/TV were associated with greater changes in trabecular vBMD and BV/TV, respectively, during BCT are also consistent with the notion that bones are undergoing adaptive changes in response to magnitude of perceived mechanical loading. These findings are in alignment with the mechanostat theory of bone functional adaptation that posits that the weakest bones will experience the greatest strains with mechanical loading and will therefore likely experience the greatest magnitude of bone adaptation [30].

Unique to this study was the ability to measure specific changes in trabecular and cortical bone microarchitecture. Accordingly, the changes we observed in trabecular bone microarchitecture following BCT at the distal metaphyseal region, including an increase in trabecular thickness and number and a decrease in trabecular separation, are consistent with animal studies of mechanical loading [9,10] and suggest that new bone has been formed on trabecular surfaces. This new bone formation on the trabecular surfaces may be partially accounted for by the filling in of existing resorption cavities. However, given the short timeframe of BCT, increased trabecular thickness and decreased trabecular separation most likely reflect *de novo* bone deposition *via* the process of bone modeling rather than bone remodeling. The mechanical significance of this new bone formation on the trabecular microstructures, as suggested by our findings, is unclear. When we used μFEA to estimate failure load at the distal tibial metaphysis, we saw a modest, but significant, 2.5% increase in estimated failure load. Although smaller in magnitude, these findings are in alignment with the doubling of bone strength observed when new bone formation occurred on the periosteal surface of the cortical midshaft in rodents following 5 weeks of novel mechanical loading [27].

In addition to changes in bone microarchitecture suggestive of new bone formation, we also observed declines in cortical vBMD and TMD at both the metaphyseal and diaphyseal sites. These cortical changes may be reflective of the undermineralized nature of newly formed bone. When new bone matrix, or osteoid, is deposited, this bone tissue can take more than a year to become as mineralized as its neighboring, older bone tissue, thus decreasing the overall average vBMD and TMD [31]. An alternative or potentially concurrent physiological response that could reduce cortical vBMD and TMD is increased bone remodeling. Intracortical remodeling has been posited to intensify during periods of heightened physical activity in an effort to replace fatigue-damaged bone [32,33]. Fatigue damage in bone following mechanical loading has been shown to elicit apoptosis of osteocytes, the primary mechanosensitive cells in bones, which is followed by pro-osteoclastic signaling and subsequent bone remodeling [33]. As there is a temporal lag between osteoclastic bone resorption and osteoblastic bone formation and subsequent mineralization, the average vBMD and TMD could decline accordingly. We did not capture a significant change in cortical porosity at the diaphyseal site. However, we did observe increases in markers of bone resorption that support the notion that increased remodeling may have occurred during BCT.

Should increases in bone remodeling occur, as suggested by increases in markers of bone resorption, it can be initiated not only by mechanical stimuli but also by PTH in response to decreases in circulating ionized calcium and subsequent secondary hyperparathyroidism [34]. Although increases in resting concentrations of circulating PTH have been reported previously in response to BCT [11], PTH was unchanged in the current study. Increases in PTH in response to acute bouts of exercise are well documented in the literature, and recent reports suggest that this acute increase in PTH is due to both decreases in ionized calcium and increases in phosphorus [35]. As blood samples were only obtained at baseline (prior to the start of training) and 8 weeks later, it is possible that exercise-induced changes in circulating PTH concentrations may have occurred during the 8 weeks but were not detected. Given the known PTH response to exercise, its relatively short half-life, and the observed elevation in turnover markers, it is likely that acute and transient PTH changes occurred during the 8 weeks. However, this possibility could not be directly tested given the experimental design. In addition, the rise in circulating 25OHD observed in the current study could have prevented elevations in PTH through negative feedback of the PTH-1alpha hydroxylase axis. The increases in 25OHD observed in the current study were likely due to a combination of cutaneous synthesis as well as release from endogenous fat stores [11], as volunteers experienced favorable shifts in body composition as a result of training. Since dietary intake was low and supplementation was not provided during BCT, changes in vitamin D status are unlikely related to intake.

Although seemingly small in magnitude, the changes in trabecular and cortical bone density and microstructure we observed in this study are notable when compared to previously reported changes in the same parameters following treatment with osteoporosis therapies [36–40]. For example, the decline in cortical vBMD of 0.3–0.7% in the tibia in the current study after only 8 weeks of BCT, although somewhat smaller in magnitude, is analogous to a 1.6% decline in tibial cortical vBMD following one year of treatment with teriparatide, a known stimulator of bone remodeling [40]. In the same study, one year of treatment with the potent anti-remodeling agent denosumab led to approximately a 2% increase in total and trabecular vBMD at the distal tibia. Here, we report a ~2% increase in total and trabecular vBMD of the distal tibia after only 8-weeks of BCT. Clearly the mechanisms of action of the two osteoporosis therapies differ and are likely distinct from the changes in bone metabolism that are induced by the mechanical loading generated during BCT. However, comparing these observations from osteoporosis therapy trials to the changes in bone microarchitecture in the current study confirm the potent stimulus provided by the BCT environment.

To our knowledge, this is the first study to report a reduction in circulating sclerostin (a negative regulator of bone formation) in response to BCT. Results of studies of chronic exercise on circulating sclerostin have been variable, with reports of a 7% decrease in sclerostin after a 12-month resistance training or jump intervention in men [41], but no change after a 12-month exercise intervention in postmenopausal women [42]. Alternatively, one study reported a 38% decrease in serum sclerostin after a 16-week resistance training

intervention in elderly women; however, after 1 year of follow-up, participants remained physically active and sclerostin levels were no longer different from baseline [43]. In another study in premenopausal women, circulating sclerostin decreased by 33% after 8 weeks of a physical activity intervention, consistent with the response seen in the present study [44]. It is not known whether this reduction in sclerostin is necessary to facilitate the observed adaptive bone formation. However, as sclerostin expression is inhibited by mechanical loading [45,46], it is reasonable to postulate that this signaling pathway was involved in the adaptive bone formation process in recruits during BCT.

Our study had a number of strengths. First, we enrolled a diverse sample of female Soldiers undergoing novel military training. Second, use of HR-pQCT imaging allowed us to observe changes in cortical and trabecular bone microarchitecture in response to a brief period of physical activity. Moreover, use of the second generation HR-pQCT device in particular allowed for assessment of trabecular bone microarchitecture independently of trabecular bone density. Notably, our observations reinforce the tenet that it is possible to detect small changes in bone microarchitecture with this technique, as we demonstrated using longitudinal study design. Our study also had several limitations, including the lack of a control group not exposed to BCT. Thus, one could question whether the amount of bone formation observed in this study could be due to normal accumulation of bone mineral in young adult women. However, prior work has demonstrated no appreciable changes in areal bone mineral density by DXA over the course of a year in young adult women [47], and no changes in vBMD of the distal tibia after 8 weeks in non-exercising young adult women using pQCT [13], making this possibility unlikely. Another limitation is that we collected serum samples at the beginning and at the end of the training period only, and therefore this study may not have captured acute and/or transient changes in bone metabolism that occurred with the imposition of novel physical activity. Finally, the sample size was not robust enough to evaluate or to identify factors that explain the individual variability in the skeletal response to heightened physical activity. Larger trials are needed to address the relationship between changes in bone microarchitecture and skeletal injury.

In conclusion, in this prospective observational study, we used state-of-the-art high resolution imaging to demonstrate specific changes in trabecular and cortical bone microarchitecture indicative of adaptive bone formation, and potentially, intracortical remodeling, in women following Basic Combat Training. Changes in serum biochemical markers of bone metabolism support the simultaneous occurrence of adaptive bone formation and intracortical remodeling, although neither process was directly measured. These findings provide evidence that changes in bone microarchitecture over a relatively brief period of time can be captured when physical training is combined with high resolution bone imaging.

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Declarations of interest

None.

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Appendix A. Supplementary data

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