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ORIGINAL RESEARCH

Regional Changes in Density and Microarchitecture in the Ultradistal Tibia of Female Recruits After U.S. Army Basic Combat Training

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Abstract

Musculoskeletal injuries, such as stress fracture, are responsible for over 10-million lost-duty days among U.S. Army Soldiers. During Basic Combat Training (BCT), an 8- to 10-week program that transforms civilians into Soldiers, women are four times more likely than men to sustain a stress fracture. In this work, we performed high-resolution peripheral quantitative computed tomography scans on the ultradistal tibia of 90 female recruits [age = 21.5 ± 3.3 (mean ± standard deviation) years] before the start of BCT and after 8 weeks into BCT. Then, we divided the scanned bone volume into four sectors—lateral, posterior, medial, and anterior—and computed the bone density and microarchitectural parameters in each of the four sectors pre- and post-BCT. We used linear mixed models to estimate the mean difference for bone density and microarchitectural parameters, while controlling for age, race, and pre-BCT body mass index. Our results revealed that the total volumetric bone mineral density, trabecular volumetric bone mineral density, and trabecular thickness increased ($p < 0.05$) in each of the four sectors. In addition, cortical thickness and trabecular bone volume/total volume increased in both medial and posterior sectors ($p < 0.05$). Overall, six and five out of nine parameters improved in the medial and posterior sectors, respectively, after BCT. In conclusion, the heightened physical activity during BCT led to the most beneficial bone adaptation in the medial and posterior sectors of the ultradistal tibia, which is indicative of higher loading in these sectors during activities performed in the course of BCT.

Keywords High-resolution peripheral quantitative computed tomography · Bone · Stress fracture in women · Exercise · Bone adaptation

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Introduction

Musculoskeletal injuries resulting from basic and advanced training are often cited as the single greatest medical impediment to Warfighter readiness [1, 2]. One such musculoskeletal injury, stress fracture, is a common overuse injury among the military population. From 2009 to 2012, there were 31,758 reported incidents of stress fracture among active U.S. Service members [3], with the incidence rate being 18 times higher in recruits who underwent Basic Combat Training (BCT) than in experienced military personnel [4]. Importantly, female recruits are more susceptible to stress fracture during BCT than are their male counterparts, with one study showing that males sustain 19.3 injuries for every 1000 recruits whereas females sustain 79.9 [5]. The greater risk of stress fracture in female recruits (and women in general) is often attributed to their lower bone density and microarchitectural parameters when compared to those of men [6]. During BCT, an 8- to 10-week training program, recruits perform physical activities, such as running, marching with load-carriage, and calisthenics [7]. Previous studies have shown that the sudden onset of these activities over a relatively brief period increases the bone density of the tibia [8, 9]. Our recent study of cross-sectional changes of the ultradistal tibia in female recruits who underwent BCT also showed increases in bone density and microarchitecture indicative of new bone formation [10].

The changes observed in bone as a result of initial military training [9] or training interventions [8] can be attributed to adaptation of the bone to external physical loading, in agreement with theories of bone functional adaptation [11]. Because the loads acting on the bone during these activities are likely not uniformly distributed across the tibia, the bone may undergo region-specific adaptation. For example, during running, the anterior region of the tibia is subjected to tensile forces, while the medial and posterior sectors are subjected to compressive forces [12]. An investigation of exercise-induced changes in bone density among 57 female volunteers 20.1 ± 1.6 (mean ± standard deviation [SD]) years of age, using peripheral quantitative computed tomography (pQCT) images of the distal tibia, revealed region-specific changes in bone density [8]. Specifically, these participants showed considerable increases in bone density only in the medial-posterior and medial-anterior sectors of the distal tibia. Similarly, an investigation of changes in the distal tibial properties of 90 male infantry recruits 21.0 ± 3.0 (mean ± SD) years of age who underwent 13 weeks of initial military training, showed that trabecular density increased in every sector except the posterior sector, with the most beneficial change occurring in the medial-posterior sector [9]. However, given the limited resolution of pQCT images, the authors could not document changes in microarchitectural parameters at the distal tibia. A pQCT study on male collegiate athletes who performed repetitive and forceful unilateral jumping showed that the tibial shaft of the jump leg, when compared to that of the non-jump leg, showed greater improvements in cortical parameters of the medial and posterior sides [13]. From the pQCT studies above, it can be concluded that physical activities (e.g., marching, running, and jumping) improve bone properties, with the greatest improvement occurring in the medial-posterior or medial-anterior sector. In a recent study using high-resolution pQCT (HR-pQCT) images, enhanced bone properties in the medial sector of young, healthy adults were also attributed to loading of the bone due to physical activities, such as walking and running [14].

In this study, we extended our previous cross-sectional HR-pQCT analyses of the ultradistal tibia in female recruits who underwent 8 weeks of BCT [10], and performed regional analyses by dividing the cross section into four sectors (lateral, posterior, medial, and anterior). Specifically, we quantified regional changes in bone density and microarchitectural parameters following BCT. We hypothesized that when recruits perform training-specific physical activities (e.g., marching, running, and jumping) during BCT, the greatest changes in bone density and microarchitectural parameters would occur in the medial and posterior sectors of the ultradistal tibia, because of the asymmetric loading of the bone resulting from these activities.

Materials and Methods

Study Protocol

We used data from a previously reported study to determine the regional changes in bone density and microarchitectural parameters following BCT [10]. Briefly, between the months of April and June 2015, we enrolled 99 female U.S. Army recruits from Fort Jackson, SC, as volunteers for the study. We collected the data from the participants during the first week of BCT, prior to the start of training (i.e., pre-BCT) and 8 weeks after commencement of training (i.e., post-BCT). For each participant, we measured their standing height using a stadiometer and body mass using a calibrated digital scale. Participants completed a questionnaire, which surveyed self-reported demographic information, physical activity prior to BCT, and lifestyle characteristics. This study was approved by the Human Research Protection Office at the U.S. Army Medical Research and Materiel Command (USAMRMC), Fort Detrick, MD, and by the Human Use Review Committee at the U.S. Army Research Institute of Environmental Medicine, Natick, MA. Participants in this study provided informed written consent. Investigators adhered to U.S. Army Regulation 70-25 and USAMRMC Regulation 70-25 on the participation of volunteers in research.

Participant Characteristics

We collected pre-BCT data from 99 participants and, of these, post-BCT data were collected from 91 participants. Among the eight participants who did not finish the follow-up study, four left the training unit, three stopped BCT entirely, and one withdrew for unknown medical reasons. We excluded one subject from our study because of image artifacts. The average age of the remaining 90 participants was 21.5 ± 3.3 (mean ± SD) years. The study population consisted of 39 white women (43%), 37 black women (41%), and 14 participants who were either Asian or had a multi-racial background (16%). All of the subjects in our study were physically active before they started BCT, as 30 (33%), 46 (51%), and 14 (16%) performed physical activities twice, three to five times, and more than six times per week, respectively. The pre-BCT body mass indices (BMIs) were less than 18.5 kg/m^2, between 18.5 and 24.9 kg/m^2, and between 25.0 and 29.9 kg/m^2 for 4, 45, and 41 subjects, respectively. The sample size for this study was based on a cross-sectional study performed by Schnackenburg et al. on female athletes with ($n = 19$) and without ($n = 19$) a stress fracture [15]. We assumed that the changes in trabecular density for the female recruits undergoing BCT (with a stress fracture incidence rate of 8% [5]) would be less than those observed in the study by Schnackenburg et al. where 50% of the subjects had a stress fracture. By considering the changes in trabecular bone density in female recruits to be one-half of those observed in the female athletes, we found that it would require between 54 and 92 female recruits, both before and after BCT, to observe differences in means ranging from 25 to 6%, respectively, at 75% power with a significance level of 15%.

HR-pQCT Imaging

We acquired HR-pQCT images (XtremeCTII; Scanco Medical AG, Brüttisellen, Switzerland) of the non-dominant leg of the participants. For participants with a history of fracture in the non-dominant leg, we scanned the contralateral leg. To ensure the quality and reproducibility of images, we performed daily scans using the manufacturer's phantom. Three technicians acquired all scans for this study.

We measured the tibial length by palpating bony landmarks and using a flexible tape measure to determine the distance between the distal edge of the medial malleolus and the tibial plateau. We scanned the ultradistal metaphyseal region of the tibia, at a distance of 4% of the length of the tibia from its ultradistal end pre- and post-BCT. For each subject, we collected 168 image slices corresponding to a scan length of 10.25 mm at an isotropic voxel size of 61 µm. We assigned a grade between 1 and 5 to represent a movement artifact [16]. Images with no movement were assigned a value of 1, whereas those with severe movements were assigned a value of 5. The participants were re-scanned in real time or excluded from analysis if their images were graded as 4 or 5.

Following the recommendations of the International Society for Clinical Densitometry [17] on short-term precision assessment, we used triplicate scans from 15 participants to calculate the in vivo precision of HR-pQCT-derived parameters. For these participants, we determined the percentage coefficient of variation (%CV) and least significant change (LSC) at the 80% and 95% confidence intervals [18] for the density and microarchitectural parameters in each of the four sectors and the entire cross section (Table 1).

Regional Analyses of Density and Microarchitectural Parameters

We performed regional analyses of bone density and microarchitectural parameters of the images, using a previously developed approach [14, 15, 19, 20]. Two technicians performed segmentations of the bone. Briefly, we identified the periosteal and endosteal surfaces of the bone by following the manufacturer's procedures, evaluated the surfaces for accuracy, and manually edited the surfaces whenever needed. Next, for each subject, we identified an image from the middle of the stack of the acquired HR-pQCT images. Then, we identified the centroids of the tibia and fibula using image-processing tools in MATLAB (The MathWorks, Inc., Natick, MA), and divided the cross-sectional volume into

Table 1 Percentage coefficient of variation (%CV) and least significant change at 80% (LSC^{80}) and 95% (LSC^{95}) confidence intervals for density and microarchitectural parameters on each of the four sectors and the entire cross section. We used triplicate scans of the ultradistal tibia from 15 subjects to compute these values

	Lateral			Posterior			Medial			Anterior			Cross section		
	%CV	LSC^{80}	LSC^{95}	%CV	LSC^{80}	LSC^{95}	%CV	LSC^{80}	LSC^{95}	%CV	LSC^{80}	LSC^{95}	%CV	LSC^{80}	LSC^{95}
Volumetric bone mineral density (mgHA/cm^3)															
Tt.vBMD	0.76	1.14	1.74	1.08	1.88	2.88	1.33	2.10	3.22	0.83	2.14	3.28	0.68	1.93	2.95
Tb.vBMD	1.02	1.94	2.98	0.59	0.94	1.44	1.25	2.23	3.41	0.70	1.50	2.29	0.49	1.18	1.80
Ct.vBMD	0.68	1.41	2.16	0.90	2.03	3.11	1.26	4.51	6.91	0.67	1.48	2.27	0.67	1.56	2.39
Trabecular microarchitectural parameters															
Tb.BV/TV	1.02	1.88	2.88	0.78	1.37	2.10	1.39	2.46	3.76	1.21	2.58	3.94	0.65	1.51	2.30
Tb.N (1/mm)	1.49	3.58	5.47	1.67	3.73	5.71	1.71	3.77	5.77	0.95	2.35	3.60	1.30	2.82	4.32
Tb.Th (mm)	0.62	1.48	2.26	0.61	1.10	1.68	1.09	2.27	3.48	0.90	1.91	2.93	0.76	1.53	2.34
Tb.Sp (mm)	1.15	2.63	4.03	1.30	2.78	4.26	1.45	3.29	5.03	0.72	1.69	2.59	0.93	1.96	3.00
Cortical microarchitectural parameters															
Ct.Po (%)	0.10	0.24	0.37	0.20	0.56	0.86	0.17	0.40	0.62	0.22	0.51	0.79	0.07	0.16	0.24
Ct.Th (mm)	1.83	4.38	6.71	1.97	4.37	6.68	2.43	6.21	9.50	2.22	4.82	7.37	1.68	3.99	6.10

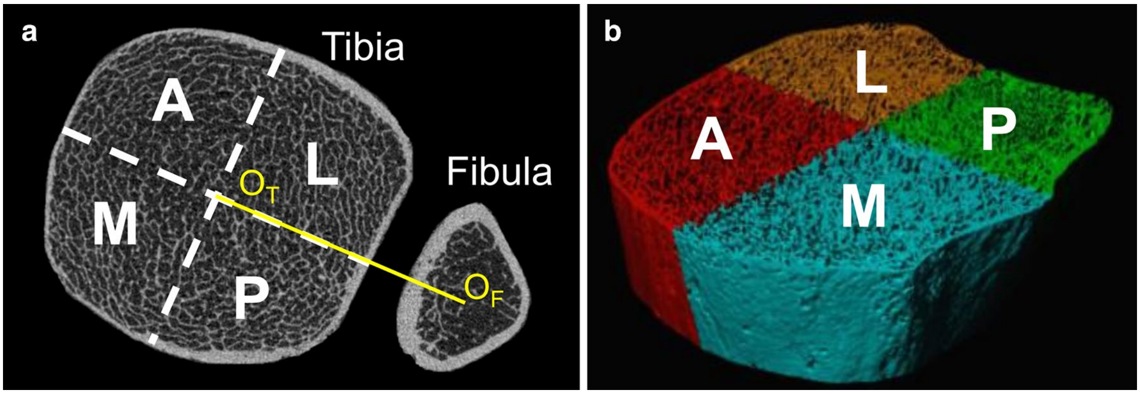

Fig. 1 **a** Definition of sectors for regional analyses on a pre-BCT image. First, we identified the centroids of the tibia (OT) and fibula (OF). Next, we connected the centroids with a line (yellow line). Using this line as a reference, we divided the cross section into lateral (L), posterior (P), medial (M), and anterior (A) sectors. For both pre- and post-BCT conditions, we performed the scans at an ultradistal metaphyseal region of the tibia, at a distance of 4% of the length of the tibia from its ultradistal end. We used the percentage of the length of the tibia from its ultradistal end as an indicator to scan the same region of interest. **b** We used the definition of sectors on a slice as a reference and divided the tibial region of interest into lateral, posterior, medial, and anterior sectors. Then, we computed the density and microarchitectural parameters of the bone in each these four sectors

four sectors—lateral, posterior, medial, and anterior—using a customized script in Image Processing Language (IPL v5.42; Scanco Medical AG) (Fig. 1a). By dividing the cross-sectional volume into four sectors using the line connecting the centroids of the tibia and fibula for all scans, we accounted for any positioning error due to rotation of the limb between scans for the same subject. Finally, we determined the density and microarchitectural parameters in each sector volume for 90 participants (Fig. 1b), pre- and post-BCT, using a customized script in IPL v5.42. Specifically, we measured bone density parameters, such as total volumetric bone mineral density (Tt.vBMD, mgHA/cm^3), trabecular volumetric bone mineral density (Tb.vBMD, mgHA/cm^3), and cortical volumetric bone mineral density (Ct.vBMD, mgHA/cm^3). We also measured microarchitectural parameters, such as trabecular bone volume fraction (Tb.BV/TV), trabecular number (Tb.N, 1/mm), trabecular thickness (Tb.Th, mm), trabecular separation (Tb.Sp, mm), cortical porosity (Ct.Po, %), and cortical thickness (Ct.Th, mm). In our study, we did not perform the cross-sectional-area method of registration, which is the default registration scheme in Image Processing Language v5.42, to account for differences in the cross-sectional area. This method becomes inappropriate when changes in cross-sectional area are due to periosteal bone deposition, as observed during BCT. Rather, we relied on the manufacturer's guidelines to reduce repositioning error due to axial misplacement during the follow-up scan [21].

Statistical Analysis

We determined regional differences using linear mixed-effects analysis for the density and microarchitectural parameters between (1) sectors for pre- and post-BCT images with sector considered as a fixed effect and (2) pre- and post-BCT for each sector with time considered as a fixed effect. We accounted for the within-subject variation in our analysis by assigning a random intercept to each of the density and microarchitectural parameters. We considered age, race, and pre-BCT BMI as covariates in our analyses. We used a likelihood-ratio test to compute the statistical significance of a given characteristic, i.e., by comparing the linear mixed-effects model including the characteristic to a model without the characteristic (a null model). Subsequently, for pairwise comparisons, we performed a post hoc Tukey test. We used the Holm–Bonferroni correction to account for errors that arise from multiple comparisons. We represent all data as means ± one standard error of the mean (SE), unless otherwise noted. We used a criterion of $p < 0.05$ to test for statistical significance, and performed all analyses in the statistical package R [22, 23].

Results

Regional- and Cross-Sectional Analyses Pre- and Post-BCT

Analyses of pre-BCT bone density and microarchitectural parameters between sectors (Table 2) revealed that Tt.vBMD was lowest in the anterior sector. Whereas Tb.vBMD was highest in the medial sector and lowest in the anterior sector, Ct.vBMD showed the opposite pattern, being highest in the anterior sector and lowest in the medial sector. Similar to Tb.vBMD, Tb.BV/TV and Tb.Th were lowest in the anterior sector and highest in the medial sector. Accordingly, Tb.Sp

Table 2 Regional measures of density and microarchitectural parameters of the ultradistal tibia before Basic Combat Training (pre-BCT) for all participants ($n = 90$)

	Lateral	Posterior	Medial	Anterior	Cross section
Volumetric bone mineral density (mgHA/cm^3)					
Tt.vBMD	263.48 ± 4.00	262.92 ± 4.34	258.23 ± 4.37	$219.77 \pm 3.78^{\dagger}$	$246.16 \pm 3.64^{L,P,M,A}$
Tb.vBMD	199.27 ± 3.40	202.57 ± 3.68	$216.82 \pm 3.98^{\dagger}$	$169.69 \pm 3.72^{\dagger}$	$195.98 \pm 3.20^{P,M,A}$
Ct.vBMD	$854.85 \pm 5.52^{*}$	$840.59 \pm 6.03^{\ddagger}$	$826.91 \pm 5.57^{\ddagger}$	$865.33 \pm 5.53^{*}$	$849.86 \pm 4.95^{P,M,A}$
Trabecular microarchitectural parameters					
Tb.BV/TV	0.292 ± 0.005	0.297 ± 0.005	$0.317 \pm 0.006^{\dagger}$	$0.247 \pm 0.006^{\dagger}$	$0.287 \pm 0.005^{P,M,A}$
Tb.N (1/mm)	$1.665 \pm 0.018^{\dagger}$	1.717 ± 0.020	1.706 ± 0.017	$1.562 \pm 0.018^{\dagger}$	$1.651 \pm 0.017^{P,M,A}$
Tb.Th (mm)	0.234 ± 0.002	0.233 ± 0.002	$0.241 \pm 0.002^{\dagger}$	$0.219 \pm 0.002^{\dagger}$	$0.233 \pm 0.002^{M,A}$
Tb.Sp (mm)	$0.552 \pm 0.007^{\ddagger}$	$0.537 \pm 0.007^{*}$	$0.525 \pm 0.006^{*}$	$0.600 \pm 0.008^{\dagger}$	$0.558 \pm 0.006^{P,M,A}$
Cortical microarchitectural parameters					
Ct.Po (%)	0.012 ± 0.001	0.011 ± 0.001	0.014 ± 0.001	0.013 ± 0.001	$0.009 \pm 0.001^{L,M,A}$
Ct.Th (mm)	$0.814 \pm 0.020^{\dagger}$	0.705 ± 0.018	$0.585 \pm 0.013^{\dagger}$	0.704 ± 0.015	$0.716 \pm 0.015^{L,M}$

The data are presented as mean ± one standard error of the mean

$^{*}p < 0.05$, $^{\ddagger}p < 0.01$, and $^{\dagger}p < 0.001$: indicate a statistically significant difference of one sector from each of the other three sectors after adjusting for age, race, and pre-BCT body mass index (BMI), and performing the Holm-Bonferroni correction. $^{L,P,M,A}p < 0.05$: indicates a statistically significant difference between the cross-sectional value and the value of a particular sector (*L* lateral, *P* posterior, *M* medial, *A* anterior), after adjusting for age, race, and pre-BCT BMI, and performing the Holm-Bonferroni correction

Table 3 Regional measures of density and microarchitectural parameters of the ultradistal tibia after Basic Combat Training (post-BCT) for all participants ($n = 90$)

	Lateral	Posterior	Medial	Anterior	Cross section
Volumetric bone mineral density (mgHA/cm^3)					
Tt.vBMD	265.43 ± 3.80	267.47 ± 4.19	263.77 ± 3.81	$222.51 \pm 3.49^{\dagger}$	$250.13 \pm 3.32^{L,P,M,A}$
Tb.vBMD	200.62 ± 3.33	204.53 ± 3.59	$220.39 \pm 3.45^{\dagger}$	$171.73 \pm 3.50^{\dagger}$	$198.51 \pm 2.94^{M,A}$
Ct.vBMD	852.63 ± 5.23	844.42 ± 5.84	$833.90 \pm 5.41^{*}$	$864.50 \pm 5.20^{*}$	$850.82 \pm 4.71^{M,A}$
Trabecular microarchitectural parameters					
Tb.BV/TV	0.294 ± 0.005	0.299 ± 0.005	$0.322 \pm 0.005^{\dagger}$	$0.249 \pm 0.006^{\dagger}$	$0.290 \pm 0.005^{P,M,A}$
Tb.N (1/mm)	$1.681 \pm 0.019^{*}$	1.729 ± 0.020	1.711 ± 0.017	$1.570 \pm 0.018^{\dagger}$	$1.662 \pm 0.017^{P,M,A}$
Tb.Th (mm)	0.235 ± 0.002	0.234 ± 0.002	$0.245 \pm 0.002^{\dagger}$	$0.221 \pm 0.002^{\dagger}$	$0.235 \pm 0.002^{M,A}$
Tb.Sp (mm)	$0.548 \pm 0.007^{*}$	$0.534 \pm 0.007^{*}$	$0.523 \pm 0.006^{*}$	$0.597 \pm 0.008^{\dagger}$	$0.555 \pm 0.006^{P,M,A}$
Cortical microarchitectural parameters					
Ct.Po (%)	0.013 ± 0.001	0.011 ± 0.001	0.013 ± 0.001	0.014 ± 0.001	$0.009 \pm 0.001^{L,M,A}$
Ct.Th (mm)	$0.819 \pm 0.020^{\dagger}$	0.730 ± 0.018	$0.606 \pm 0.013^{\dagger}$	0.710 ± 0.015	$0.729 \pm 0.015^{L,M}$

The data are presented as mean ± one standard error of the mean

$^{*}p < 0.05$, $^{\ddagger}p < 0.01$, and $^{\dagger}p < 0.001$: indicate a statistically significant difference of one sector from each of the other three sectors after adjusting for age, race, and pre-BCT body mass index (BMI), and performing the Holm-Bonferroni correction. $^{L,P,M,A}p < 0.05$: indicates a statistically significant difference between the cross-sectional value and the value of a particular sector (*L* lateral, *P* posterior, *M* medial, *A* anterior), after adjusting for age, race, and pre-BCT BMI, and performing the Holm-Bonferroni correction

was lowest in the medial sector and highest in the anterior sector. Similar to other trabecular parameters, Tb.N was lowest in the anterior sector. Ct.Po did not differ between sectors, while Ct.Th was lowest in the medial sector and highest in the lateral sector. Analyses of the post-BCT bone parameters between sectors showed a pattern similar to that of the pre-BCT bone parameters (Table 3).

We compared the cross-sectional pre-BCT density and microarchitectural parameters with those of each sector (Table 2, Online Resource 1). For Tt.vBMD and Tb.vBMD, the cross-sectional value was lower than the values of the posterior and medial sectors and higher than the value of the anterior sector. Conversely, the cross-sectional Ct.vBMD was higher than the posterior- and medial-sector values, but lower than the anterior-sector value. Among the trabecular microarchitectural parameters, for Tb.BV/TV, Tb.N, and Tb.Th, the cross-sectional value was higher than the anterior-sector value, while for Tb.Sp, it was lower than the anterior-sector value. Among the cortical parameters, for Ct.Po and Ct.Th, the cross-sectional value was lower than the lateral-sector value. With the exception of Tb.vBMD and Ct.vBMD, the differences in the density and microarchitectural parameters between the cross-sectional and sector-specific values of the post-BCT data were similar to those of the pre-BCT data (Table 3, Online Resource 1).

Changes in Bone Density and Microarchitectural Parameters Post-BCT

Following 8 weeks of BCT, we observed significant region-specific changes in bone density and microarchitectural parameters when compared to the pre-BCT data (Fig. 2, Online Resource 2). Among the density parameters, Tt.vBMD and Tb.vBMD increased (significantly in each one of the four sectors (Fig. 2a and b). In contrast, Ct.vBMD increased only in the medial sector (Fig. 2c). Among the microarchitectural parameters, Tb.BV/TV increased in the posterior and medial sectors (Fig. 2d). Whereas Tb.N did not significantly change in any sector (Fig. 2e), Tb.Th increased in each one of the four sectors (Fig. 2f). Tb.Sp decreased in the lateral sector (Fig. 2g). Conversely, Ct.Po increased in the lateral sector (Fig. 2h). Finally, Ct.Th increased in the posterior and medial sectors (Fig. 2i). Overall, the medial sector exhibited beneficial adaptations of the bone in six out of nine bone parameters. For the entire cross section, Tt.vBMD, Tb.vBMD, Tb.BV/TV, Tb.Th, and Ct.Th increased after 8 weeks of BCT (Fig. 2). We did not observe any change in Ct.vBMD, Tb.N, Tb.Sp, or Ct.Po post-BCT.

From our analyses, we found that a number of individuals showed changes in density and microarchitectural parameters greater than that of the LSC. For example, the change in Tt.vBMD was greater than the LSC^{80} in 49, 42, 52, and 35 participants for the lateral, posterior, medial, and anterior sectors, respectively, while 39 recruits showed a greater change than the LSC^{80} value for the entire cross section. Similarly, the change in Tt.vBMD was greater than the LSC^{95} in 32, 25, 32, and 21 participants for the lateral, posterior, medial, and anterior sectors, respectively, while 21 recruits showed a greater change than the LSC^{95} value for the entire cross section.

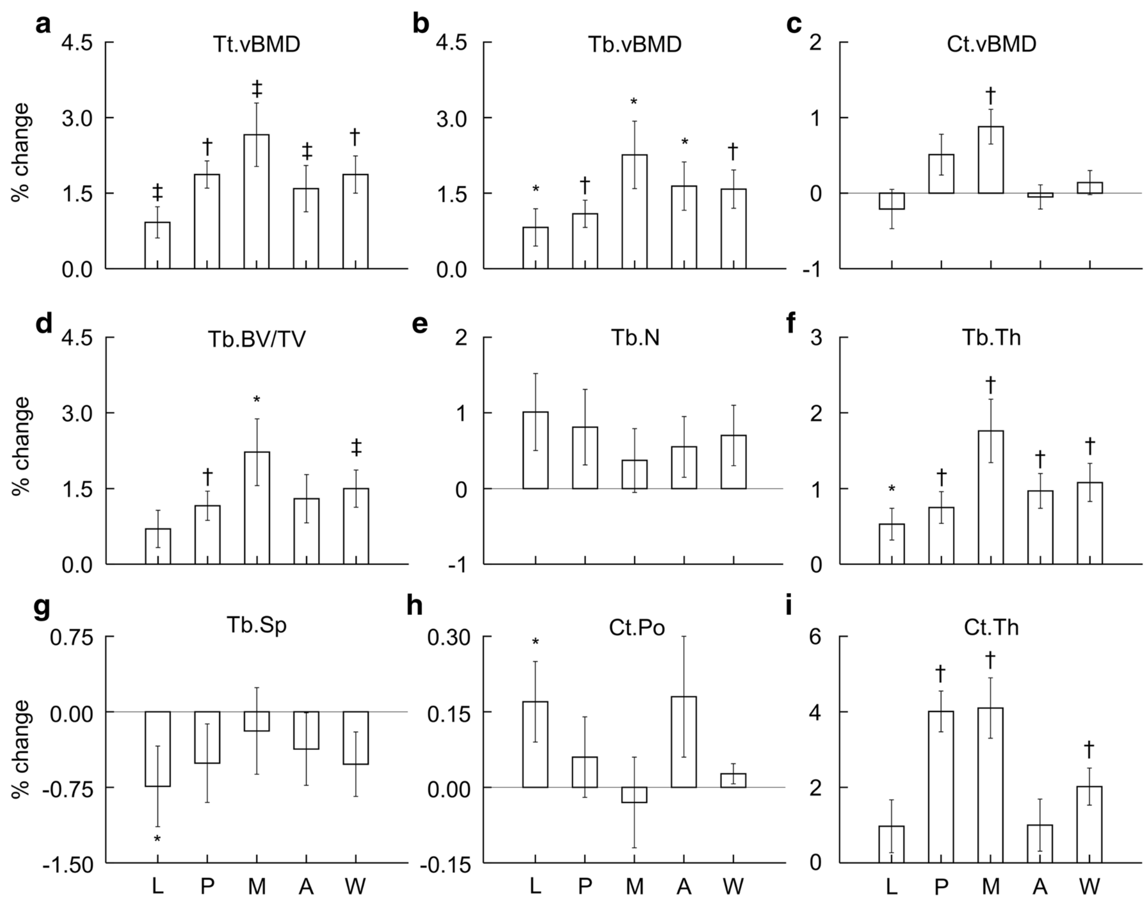

Fig. 2 Percentage change of density and microarchitectural parameters between pre- and post-BCT measurements in each sector and cross section for all participants ($n=90$). Statistically significant differences between pre- and post-BCT measurements after adjusting for age, race, and pre-BCT body mass index, and performing the Holm-Bonferroni correction, are denoted by $^{*}p<0.05$, $^{\ddagger}p<0.01$, and $^{\dagger}p<0.001$. The bar height and vertical line length represent the mean and one standard error of the mean, respectively. *A* anterior, *L* lateral, *M* medial, *P* posterior, *C* cross section

Next, for the parameters that showed improvement in more than one sector (i.e., Tt.vBMD, Tb.vBMD, Tb.Th, Tb.BV/TV, and Ct.Th; Fig. 2), we analyzed between-sector differences in the degree of change to identify the sector with the best improvement after BCT. Our analysis showed that, although Tt.vBMD, Tb.vBMD, and Tb.Th increased in each one of the four sectors, the increases of Tt.vBMD and Tb.vBMD in the medial sector were higher than the increase in the lateral sector by 1.74% and 1.44%, respectively, while the improvements in the medial, anterior, and posterior sectors did not significantly differ. In contrast, the improvement of Tb.Th in the medial sector was different from that in the other three sectors, with the greatest difference observed between the lateral and medial sectors (1.23%). For Tb.BV/TV and CT.Th, there was a significant increase in the posterior and medial sectors. However, we did not observe any differences between them.

Discussion

We previously performed cross-sectional analyses of HR-pQCT scans acquired from the ultradistal tibia of female recruits before and after 8 weeks of BCT, and found significant changes in bone density and microarchitectural parameters [10]. In this study, we performed regional analyses on the same cohort, by dividing the cross section of the bone into four sectors—lateral, posterior, medial, and anterior—and quantifying the changes in bone parameters due to BCT. We investigated the differences in the density and microarchitectural parameters between the entire cross section and individual sectors, as well as between sectors, pre- and post-BCT (Tables 2 and 3). In support of our hypothesis, we observed significant improvements in the majority of the density and microarchitectural parameters within the posterior and medial sectors post-BCT.

Comparison of the average cross-sectional parameters with the values for each sector revealed that most of the cross-sectional values were significantly different from at least one of the sectors in both pre- and post-BCT data (Tables 2 and 3). Specifically, with the exception of Ct.Th, the average values of the cross-sectional trabecular and cortical parameters were significantly different from their corresponding values in the anterior and medial sectors. Although, Ct.vBMD, Ct.Po, and Tb.Sp changed in at least one sector post-BCT, the cross-sectional results did not show any change. These results show that cross-sectional analysis, which averages the bone properties over the entire cross section, can obscure regional variations, in agreement with other studies [14, 15].

In the between-sector analyses, we observed that the anterior sector had inferior trabecular bone parameters when compared to other sectors (Table 2). At the same time, with the exception of Ct.Po, the medial sector had inferior cortical bone parameters when compared to the other sectors (Table 2). These results are in agreement with previous micro-computed tomography (μCT) and HR-pQCT investigations of the tibia [14, 24]. Lai et al. performed regional analyses of μCT data from 20 cadaveric tibiae and showed that Tb.N, Tb.Th, and bone volume fraction in the anterior and posterior sectors of the distal tibia are lower than those in the other sectors [24]. In a cohort of young healthy participants (101 women and 84 men; age range, 18–30 years), using HR-pQCT images of the distal tibia, Unnikrishnan et al. [14] observed a similar trend in the cortical and trabecular bone parameters for different races and sexes. Similar to our results, they found that Ct.Th and Ct.vBMD are lower in the medial sector, while Tb.vBMD, Tb.Th, and Tb.N are lower in the anterior sector. They attributed these regional differences to the adaptation of the bone in response to habitual asymmetric loading during normal daily activities, such as walking. Interestingly, in our study, although the bone parameters changed after 8 weeks of BCT, these changes did not affect the relative between-sector differences for each parameter. For example, trabecular bone parameters were still inferior in the anterior sector (Table 3), while cortical bone parameters (except for Ct.Po) were inferior in the medial sector (Table 3) post-BCT.

A number of animal and human studies have shown that physical training induces changes in tibial bone density, microarchitecture, and geometry [25–28]. While using HR-pQCT to investigate differences in bone quality among alpine skiers (high impact), soccer players (moderate impact), swimmers (low impact), and control participants, Schipilow et al. reported considerable differences in bone properties [29]. They showed that compared to swimmers, alpine skiers and soccer players have significantly higher bone density, cortical thickness, and failure load. Finally, a pQCT study conducted in the distal tibia of young male recruits [$n = 90$; age, 21 ± 3 years (mean $\pm$ SD)] who underwent 13 weeks of initial military training also reported a cross-sectional increase of 0.92% in the Tb.vBMD [9]. In our cohort of female recruits, who likely underwent low-impact and high-impact physical activities during BCT [7], we also observed improvements in most bone density and microarchitectural parameters post-BCT. For example, Tt.vBMD, Tb.vBMD, and Tb.Th significantly increased in all sectors of the bone post-BCT, while Tb.BV/TV and Ct.Th increased in the posterior and medial sectors. One negative bone outcome is the increase of Ct.Po in the lateral sector after BCT. This increase may have occurred due to periosteal apposition or incomplete intra-cortical remodeling during the 8-week study period.

Among the different sectors, the medial and posterior sectors had the greatest number of parameters that improved favorably post-BCT (six and five parameters for the medial and posterior sectors, respectively, out of the nine analyzed). The favorable increase might be indicative of the adaptive response of the bone to asymmetric loading of the tibia resulting from the physical activities performed during BCT. For example, it is widely accepted that during running, the posterior sector of the tibia is subjected to compressive loading, while the anterior sector is subjected to tensile loading [12]. Moreover, using an integrated musculoskeletal finite element model, Xu et al. reported high stress on the medial side and high cumulative tibial stress on the medial-posterior side in the proximal tibia and tibial shaft in a healthy, young woman walking with a load of 10, 20, or 30% of body weight [30]. The high stress and high cumulative tibial stress are attributed to the interaction of the semitendinosus muscle and gastrocnemius, soleus, and tibialis posterior muscles, respectively, with the tibia. While we cannot determine a direct link between the changes in the bone parameters and stress fracture from our study because we lack knowledge about stress fracture in these recruits, we believe that the increased likelihood of its occurrence might be due to inadequate adaptation of the bone to the additional loads on these sectors.

Evans et al. [8], while investigating exercise-induced changes in the tibias of young women, suggested that participants starting an exercise program with lower initial bone density are likely to show greater increases after the training regimen. The results of our previous study of cross-sectional analyses in female recruits are also consistent with this assertion [10]. However, among sectors, the greatest changes did not occur in the sector that had the lowest pre-BCT value. For example, the greatest changes in Tt.vBMD and Tb.vBMD occurred in the medial sector and not in the anterior sector, which had the lowest pre-BCT value. Similarly, the percentage increase in Tb.Th was greater in the medial sector when compared to all other sectors. The percentage increase in Ct.Th of the medial sector with the lowest pre-BCT value was no different from that of the posterior sector, which had a higher pre-BCT value than did the medial sector. However, Ct.vBMD increased significantly only in the medial sector, which incidentally had the lowest pre-BCT value of Ct.vBMD. From these results, we can conclude that between-sector changes in the bone due to BCT might be influenced more by asymmetric loading acting on the tibia during BCT than by their pre-BCT value.

Our study has a few limitations. First, we did not have a control group (i.e., a separate group that did not undergo BCT) to make comparisons between BCT-induced changes and changes in non-trained controls. However, a pQCT study with a short-term training intervention showed an increase in regional trabecular density akin to our study, even though the control group did not exhibit a significant change in any of the bone parameters [8]. Second, we divided the bone volume into four sectors, aligning each sector with the anatomical directions. With this approach, we could not investigate bone parameters in subsectors, such as the medial-posterior sector, a frequent site of stress fracture [31]. Nevertheless, we used a consistent approach to divide the sectors in the pre- and post-BCT images of the tibia, which enabled us to make accurate sector-wise comparisons within participants longitudinally.

Conclusion

Our study reinforces the concept that for young and healthy participants, physical training to which they are unaccustomed can induce a bone formation response that leads to changes in bone density and microarchitecture. Our analyses showed that the bone formation response is regionally heterogeneous, with the medial and posterior sectors showing the most beneficial changes in bone parameters during BCT. Our findings suggest that these sectors undergo higher loading than other sectors during activities performed in the course of BCT.

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Compliance with Ethical Standards

Conflict of interest The authors declare no competing interests. The opinions and assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the U.S. Army, the U.S. Department of Defense, or The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc.

Ethical Approval This study was approved by the Human Research Protection Office at the U.S. Army Medical Research and Materiel Command (USAMRMC), Fort Detrick, MD, and by the Human Use Review Committee at the U.S. Army Research Institute of Environmental Medicine, Natick, MA. Investigators adhered to U.S. Army Regulation 70-25 and USAMRMC Regulation 70-25 on the participation of volunteers in research.

Informed Consent Participants in this study provided informed written consent.

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