Bone 112 (2018) 194–201

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Full Length Article

# Regional variation of bone density, microarchitectural parameters, and elastic moduli in the ultradistal tibia of young black and white men and women

Ginu Unnikrishnan[a], Chun Xu[a], Kristin L. Popp[b,c], Julie M. Hughes[d], Amy Yuan[b], Katelyn I. Guerriere[d], Signe Caksa[b], Kathryn E. Ackerman[b,e], Mary L. Bouxsein[b,f,g], Jaques Reifman[a,*]

[a] *Department of Defense Biotechnology High Performance Computing Software Applications Institute, Telemedicine and Advanced Technology Research Center, United States Army Medical Research and Materiel Command, MCMR-TT, 504 Scott Street, Fort Detrick, MD 21702, USA*
[b] *Endocrine Unit, Massachusetts General Hospital, 50 Blossom Street, THR-1051, Boston, MA 02114, USA*
[c] *Harvard Medical School, 25 Shattuck Street, Boston, MA 02115, USA*
[d] *Military Performance Division, United States Army Research Institute of Environmental Medicine, 10 General Greene Ave, Natick, MA 01760, USA*
[e] *Division of Sports Medicine, Boston Children's Hospital, Harvard Medical School, 319 Longwood Avenue, Boston, MA 02115, USA*
[f] *Center for Advanced Orthopedic Studies, Beth Israel Deaconess Medical Center, Harvard Medical School, One Overland Street, Boston, MA 02215, USA*
[g] *Department of Orthopedic Surgery, Harvard Medical School, Boston, MA 02215, USA*

ARTICLE INFO

*Keywords:*
Bone adaptation
High-resolution peripheral quantitative computed tomography
Micro-finite element analyses
Stress fracture

ABSTRACT

Whole-bone analyses can obscure regional heterogeneities in bone characteristics. Quantifying these heterogeneities might improve our understanding of the etiology of injuries, such as lower-extremity stress fractures. Here, we performed regional analyses of high-resolution peripheral quantitative computed tomography images of the ultradistal tibia in young, healthy subjects (age range, 18 to 30 years). We quantified bone characteristics across four regional sectors of the tibia for the following datasets: white women ($n = 50$), black women ($n = 51$), white men ($n = 50$), black men ($n = 34$), and all subjects ($n = 185$). After controlling for potentially confounding variables, we observed statistically significant variations in most of the characteristics across sectors ($p < 0.05$). Most of the bone characteristics followed a similar trend for all datasets but with different magnitudes. Regardless of race or sex, the anterior sector had the lowest trabecular and total volumetric bone mineral density and highest trabecular separation ($p < 0.001$), while cortical thickness was lowest in the medial sector ($p < 0.05$). Accordingly, the anterior sector also had the lowest elastic modulus in the anterior-posterior and superior-inferior directions ($p < 0.001$). In all sectors, the mean anisotropy was ~3, suggesting cross-sector similarity in the ratios of loading in these directions. In addition, the bone characteristics from regional and whole-bone analyses differed in all datasets ($p < 0.05$). Our findings on the heterogeneous nature of bone microarchitecture in the ultradistal tibia may reflect an adaptation of the bone to habitual loading conditions.

## 1. Introduction

Lower-extremity stress fractures, caused by repeated loading to the bone, are common overuse injuries among Service members and athletes [1–4]. Military recruits who participate in basic combat training are at particularly high risk of this injury because of the sudden increase in physical activities, such as marching, running, and repetitive jumping [1]. Risk factors for stress fracture include, among others, race, sex, age, and body mass index [5–7]. In military recruits, whites compared to blacks, and women compared to men, are at higher risk of this injury [5,7]. Our recent study of the ultradistal tibia in young, healthy subjects, using whole-bone analyses of high-resolution peripheral

*Abbreviations:* μCT, Micro-computed tomography; μFE, Micro-finite element; Ct.Po, Cortical porosity; Ct.Th, Cortical thickness; Ct.vBMD, Cortical volumetric bone mineral density; $E_1$, Medial-lateral elastic modulus; $E_2$, Anterior-posterior elastic modulus; $E_3$, Superior-inferior elastic modulus; HR-pQCT, High-resolution peripheral quantitative computed tomography; SD, Standard deviation; Tb.N, Trabecular number; Tb.Sp, Trabecular separation; Tb.Th, Trabecular thickness; Tb.vBMD, Trabecular volumetric bone mineral density; Tt.vBMD, Total volumetric bone mineral density

* Corresponding author at: Department of Defense Biotechnology High Performance Computing Software Applications Institute, Telemedicine and Advanced Technology Research Center, U.S. Army Medical Research and Materiel Command, MCMR-TT, 504 Scott Street, Fort Detrick, MD 21702-5012, USA.
*E-mail address:* jaques.reifman.civ@mail.mil (J. Reifman).

https://doi.org/10.1016/j.bone.2018.05.004
Received 7 January 2018; Received in revised form 11 April 2018; Accepted 2 May 2018
Available online 03 May 2018
8756-3282/ Published by Elsevier Inc.

quantitative computed tomography (HR-pQCT) images, showed that whites compared to blacks, and women compared to men, have inferior bone-health related characteristics, which might explain the higher risk of fractures in these groups [8].

Conventional whole-bone analyses of the HR-pQCT images, which averages bone characteristics over the entire cross section, while important in the field of bone biomechanics, can obscure regional variation in these characteristics [9–11]. For example, although whole-bone values of density and microarchitecture of cortical and trabecular bones between stress-fractured and non-stress-fractured women athletes (n = 19; age range, 18 to 45 years) showed no significant differences, regional analyses revealed a significant difference between the two groups in the posterior sector [10].

Few studies have investigated regional variation of density and microarchitectural parameters of the tibia, especially for subjects with an age range similar to that of military recruits [9,11–14]. Using pQCT images of the ultradistal tibia, Evans et al. reported regional variation in cortical and trabecular bone mineral density of young military recruits (n = 128; 108 women and 20 men; age range, 18 to 21 years) [12]. However, owing to the limited resolution of the pQCT images, the authors were not able to evaluate the microarchitectural parameters at the tibia. Regional variations of both density and microarchitectural parameters of ultradistal trabecular bone were quantified by Sode et al. using HR-pQCT images of healthy men and women (n = 146; 93 women and 53 men; age range, 20 to 78 years; 46% Asians and 46% whites) [11]. However, this study recruited only 30 subjects (17 women and 13 men) younger than 29 years, and the analyses were not stratified based on race.

In this study, we performed regional analyses of HR-pQCT images of the ultradistal tibia collected from a larger cohort of young, healthy subjects with an age range similar to that of military recruits. We also stratified the subjects based on race and sex to identify differences in bone characteristics that might be correlated with the reported differences in the risk of stress fracture between blacks and whites as well as between men and women. Specifically, we quantified the variation in density measurements, microarchitectural parameters, and mechanical properties for the following datasets: white women, black women, white men, black men, and all subjects. We hypothesized that for each of the four stratified datasets, we would observe significant regional variation in bone characteristics.

## 2. Materials and methods

### 2.1. Subjects and imaging protocol

Briefly, as previously reported [8], we enrolled 185 young, healthy subjects: 50 white women, 51 black women, 50 white men, and 34 black men, all between the ages of 18 to 30 years and having a body mass index between 18 and 30 kg/m$^2$ (Table 1). To determine the sample size, we conducted a power analysis using the method of Chow et al. [15], under the assumption that racial/ethnic differences in bone characteristics of young, healthy women are equal to those in post-menopausal women [16]. We calculated that between 25 and 52 women subjects in the black and white groups would be needed to achieve 75% power to detect differences in group means ranging between 10% and 7%, respectively, with a significance level of 15%.

All subjects completed a questionnaire, which surveyed, among other items, socio-economic status, health, bone fracture history, and recent physical activity [e.g., type, frequency (hours-per-week), duration (months-per-year and years-per-life)]. For each subject, we measured height using a wall-mounted stadiometer, body mass using a calibrated electronic scale, and tibial length, defined as the distance from the medial tibial plateau to the distal edge of the medial malleolus, using an anthropometric tape. Further details on subject enrollment, exclusion criteria, and imaging protocols are described in our earlier study [8]. We received approval for the study from the Human Research Protection Office at the U.S. Army Medical Research and Materiel Command (Ft. Detrick, Maryland) and the Institutional Review Board of Partners Health Care (Boston, Massachusetts). Prior to study participation, we obtained written consent from each subject.

We obtained HR-pQCT images (XtremeCT; Scanco Medical AG, Brüttisellen, Switzerland) of the tibia of the non-dominant leg of each subject. We scanned the contralateral leg for subjects with a history of leg or ankle fracture. Using an isotropic voxel resolution of 82 μm, we collected 110 image slices (corresponding to 9.02 mm) at an ultradistal tibial site (i.e., 4% of the tibial length proximal to the distal end of the tibia). The short-term precision for HR-pQCT measurements, due to repositioning of the tibia, ranged from 0.2 to 1.7% for density parameters, from 0.7 to 8.6% for microarchitectural parameters, and from 2.1 to 4.8% for stiffness obtained by micro-finite element (μFE) analyses.

### 2.2. Regional analyses of bone density and microarchitectural parameters

We performed regional analyses of bone density and microarchitectural parameters in three steps. In the first step, we used a semi-automated segmentation procedure in Image Processing Language (IPL v5.08b; Scanco Medical AG) to identify the periosteal and endocortical surface contours. We inspected the periosteal and endocortical surfaces for accuracy, and modified the surfaces whenever necessary. We then determined whole-bone and cortical-bone volumes from the periosteal and endocortical surface contours, respectively, and subtracted the cortical-bone volume from the whole-bone volume to obtain the trabecular-bone volume.

In the second step, we divided the whole bone into four sectors, similar to previously published studies on regional analyses of tibial bone [9–11]. First, we extracted an image at the center of the HR-pQCT scan of each subject and identified the centroids of the tibia and fibula, using image-processing tools in MATLAB (The MathWorks, Inc., Natick, MA). Then, with a line connecting the centroids as a reference, we divided the whole-bone volume into lateral, posterior, medial, and anterior sectors, using a customized script in IPL (Fig. 1a).

In the third and final step, we determined bone density and microarchitectural parameters for the whole bone and each sector, using

**Table 1**
Characteristics of 185 young, healthy subjects (101 women and 84 men) considered for the study [8]. Subjects ranged from 18 to 30 years of age, with a body mass index between 18 and 30 kg/m$^2$. The data are presented as mean ± one standard deviation.

| | White women | Black women | White men | Black men | Total |
|---|---|---|---|---|---|
| | (n = 50) | (n = 51) | (n = 50) | (n = 34) | (n = 185) |
| Age (years) | 24.5 ± 2.9 | 22.2 ± 3.2 | 24.9 ± 3.2 | 24.3 ± 3.6 | 24.4 ± 3.4 |
| Height (m) | 1.65 ± 0.11 | 1.66 ± 0.08 | 1.80 ± 0.08 | 1.78 ± 0.07 | 1.72 ± 0.10 |
| Mass (kg) | 63.4 ± 9.6 | 64.4 ± 10.2 | 78.5 ± 11.5 | 78.2 ± 11.4 | 70.5 ± 12.7 |
| Body mass index (kg/m$^2$) | 23.3 ± 3.2 | 23.3 ± 2.5 | 24.2 ± 2.9 | 24.9 ± 3.4 | 23.9 ± 3.0 |
| Tibial length (cm) | 36.8 ± 2.4 | 37.8 ± 2.9 | 40.8 ± 2.9 | 41.3 ± 3.1 | 39.0 ± 3.4 |
| Physical activity (h/wk) | 4.9 ± 4.3 | 2.3 ± 2.8 | 5.9 ± 5.4 | 5.1 ± 8.6 | 4.5 ± 5.5 |

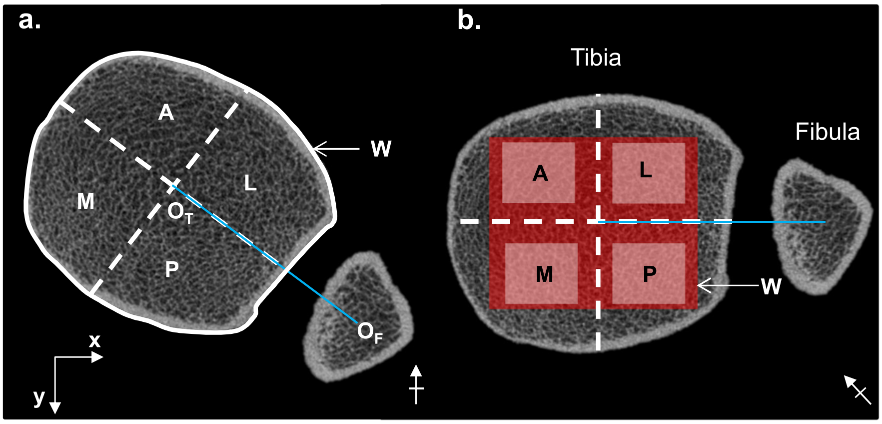


**Fig. 1.** Methodology for regional analyses of bone microstructure and mechanical properties.
a) Definition of four sectors (L, P, M, A) for regional analyses of bone density and microarchitectural parameters on a representative high-resolution peripheral quantitative computed tomography (HR-pQCT) image of a tibia. Using a line connecting the centroids of the tibia ($O_T$) and fibula ($O_F$) as a reference line, the whole bone was divided into the sectors. b) Representation of subvolumes of trabecular bone extracted from a whole bone (W) and sectors (L, P, M, A) for calculation of regional mechanical properties. The subvolumes were determined after aligning the reference line with the x-axis of the HR-pQCT image. A, Anterior; L, Lateral; M, Medial; P, Posterior; W, Whole bone.

Scanco analysis software v5.08b (Scanco Medical AG). Specifically, we determined total volumetric bone mineral density (Tt.vBMD; mgHA/cm$^3$), cortical volumetric bone mineral density (Ct.vBMD; mgHA/cm$^3$), cortical thickness (Ct.Th; mm), cortical porosity (Ct.Po; %), trabecular volumetric bone mineral density (Tb.vBMD; mgHA/cm$^3$), and trabecular number (Tb.N; 1/mm). In addition, we derived trabecular separation (Tb.Sp; mm) and trabecular thickness (Tb.Th; mm) from Tb.vBMD and Tb.N [17].

### 2.3. Regional analyses of mechanical properties of trabecular subvolumes

We determined the elastic modulus of trabecular subvolumes in the medial-lateral ($E_1$; GPa), anterior-posterior ($E_2$; GPa), and superior-inferior ($E_3$; GPa) directions using μFE analyses (Scanco FE software v1.16; Scanco Medical AG). For the whole bone and each sector, we extracted the subvolumes after aligning the line connecting the centroids of the tibia and fibula with the x-axis of the HR-pQCT image (Fig. 1b). All trabecular subvolumes had rectangular cross sections, and the dimensions were varied depending on the size of the tibia. The dimension of the subvolume in the superior-inferior direction was fixed at 9.02 mm (corresponding to 110 images) for all subjects. We determined the dimension in the anterior-posterior and medial-lateral directions using an iterative algorithm. First, using image-processing tools in MATLAB, we created an initial square cross-section with an edge-length of 6.56 mm (i.e., 80 pixels) in the trabecular bone of the lateral, posterior, medial, and anterior sectors. The nearest edges of the cross-section were 0.82 mm (i.e., 10 pixels) away from the anterior-posterior and medial-lateral axes. Second, we increased the edge-lengths of the cross-section in all sectors in the anterior-posterior and medial-lateral directions until the rectangular cross-sections captured the maximum possible trabecular region in each sector without intersecting the sector boundaries. Finally, using IPL, we extracted the subvolume of the rectangular cross-section from the trabecular bone region and separately performed uniaxial compression tests in the medial-lateral, anterior-posterior, and superior-inferior directions. We assigned each element of the μFE model with a linear-elastic material property (a Young's modulus of 10 GPa and Poisson's ratio of 0.3), and determined the anisotropy of the trabecular subvolumes by dividing $E_3$ by the smaller of $E_1$ and $E_2$.

### 2.4. Statistical analysis

Using R and lme4 [18,19], we performed a linear mixed-effects analysis to determine the variation of each density measure, microarchitectural parameter, and mechanical property across the four sectors and with the whole bone. We considered height, body mass, age, and physical activity as covariates and sector as a repeated measure. We assigned a random intercept to the bone characteristic (i.e., density, microarchitectural parameter, or mechanical property) of each individual to account for the within-subject dependence. To determine the statistical significance of a given characteristic, we used a likelihood-ratio test—by comparing the linear mixed-effects model with the bone characteristic to a model without the characteristic (i.e., a null model). We then performed a Tukey test on the linear mixed-effects model for pairwise comparisons, where errors due to multiple comparisons were accounted for, using the Holm-Bonferroni correction. We performed the linear mixed-effects and analyses separately for each of the following datasets: white women ($n = 50$), black women ($n = 51$), white men ($n = 50$), black men ($n = 34$), and all subjects ($n = 185$). All data are presented as mean ± one standard deviation (SD), where we used a criterion of $p < 0.05$ after the Holm-Bonferroni correction to test for statistical significance. We separately performed between-race and between-sex comparisons of the bone characteristics in each sector, using analysis of covariance after adjusting for the height, body mass, age, and physical activity.

## 3. Results

### 3.1. Regional analyses of bone density and microarchitectural parameters

We observed statistically significant regional variations in all bone density measurements (except Ct.vBMD) and microarchitectural parameters for white women, black women, white men, and black men, after adjusting for height, body mass, age, and physical activity, as well as accounting for within-subject dependence (Figs. 2 and 3). Specifically, the anterior sector had the lowest Tt.vBMD and Tb.vBMD (Figs. 2a and 3a, respectively, $p < 0.001$), as well as the highest Tb.Sp (Fig. 3b, $p < 0.001$). The medial sector had the lowest Ct.Th (Fig. 2c, $p < 0.05$) and highest Tb.Th (Fig. 3c, $p < 0.001$). Ct.vBMD (Fig. 2b) showed no regional differences in black men, but was lower in the medial sector compared to the lateral sector in white men ($p < 0.05$), and lowest in the medial sector in women ($p < 0.001$). Ct.Po (Fig. 2d) was lowest in the anterior sector in men ($p < 0.05$), highest in the medial sector in black women ($p < 0.05$), and higher in the lateral sector when compared to the anterior and posterior sectors in white women ($p < 0.05$). With the exception of Ct.Po, variations in bone density and architectural parameters across all sectors mostly followed a similar trend for all datasets but with different magnitudes.

The mean whole-bone value in black and white men and women, when compared with the anterior sector, was higher for Tt.vBMD (9%, $p < 0.001$), Tb.vBMD (6%, $p < 0.001$), and Tb.N (3%, $p < 0.001$), and lower for Tb.Sp (5%, $p < 0.001$). Similarly, the mean whole-bone value of Ct.Th was higher than the Ct.Th of the medial sector in all four datasets (13%, $p < 0.001$). We did not observe any differences between whole-bone and regional analyses for Ct.vBMD in men or for Ct.Po in white women.

Between-race and between-sex comparisons of the density and microarchitectural parameters showed that whites, compared to blacks,

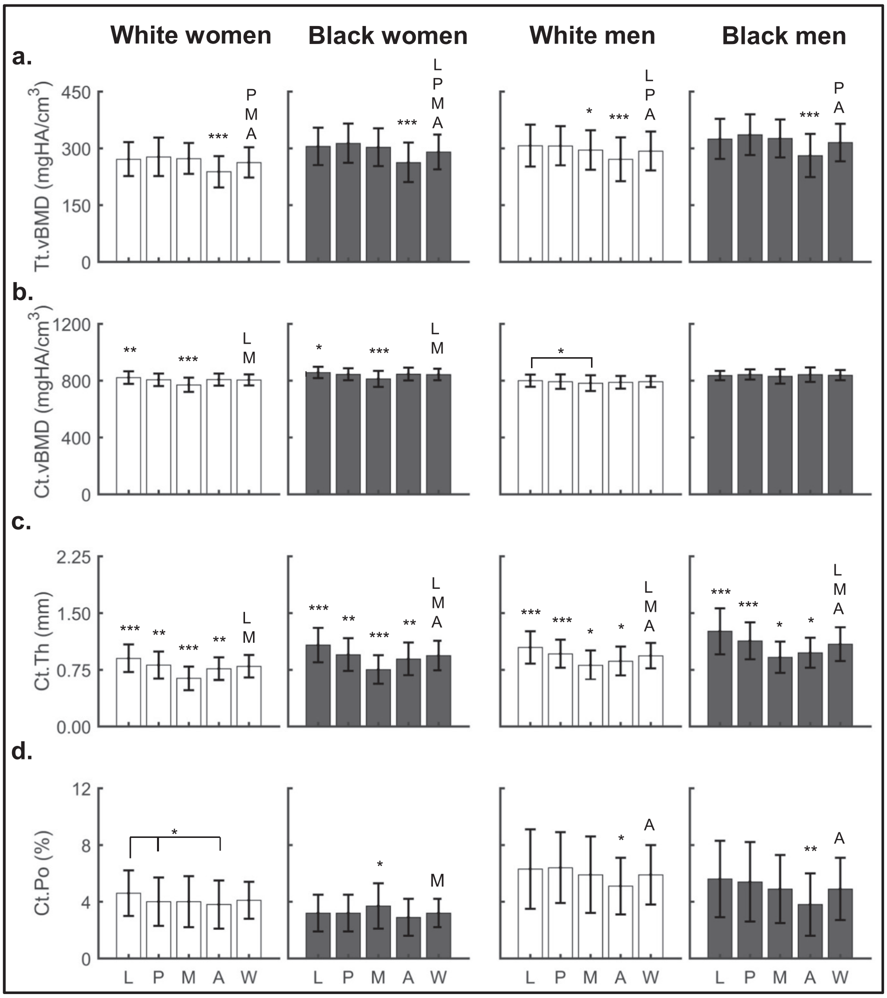

**Fig. 2.** Comparisons of density measurements and microarchitectural parameters of cortical bone across datasets. Panels from top to bottom show a) total volumetric bone mineral density (Tt.vBMD), b) cortical volumetric bone mineral density (Ct.vBMD), c) cortical thickness (Ct.Th), and d) cortical porosity (Ct.Po) obtained from analyses of regional and whole bone for white women (n = 50), black women (n = 51), white men (n = 50), and black men (n = 34). Data are expressed as mean ± one standard deviation. In this and all subsequent figures, statistically significant differences between one sector and each of the other sectors, after adjusting for height, body mass, age, and physical activity and after the Holm-Bonferroni correction, are denoted by $^{***}p < 0.001$, $^{**}p < 0.01$, and $^{*}p < 0.05$. A statistically significant difference (after the aforementioned adjustments) between the whole-bone value and the value of a particular sector is denoted by a superscript letter ($^{L,P,M,A}p < 0.05$). Brackets above the bars with two notches indicate a significant difference between a single pair of sectors. Brackets with three or more notches indicate significant differences between one sector (long notch) and the other sectors (short notches). A, Anterior; L, Lateral; M, Medial; P, Posterior; W, Whole bone.

had lower Ct.vBMD, Ct.Th, and Tb.Th ($p < 0.01$) in all sectors, higher Ct.Po and Tb.N in the lateral and anterior sectors ($p < 0.01$), and lower Tb.vBMD in the medial sector ($p < 0.01$). Similarly, women, compared to men, had lower Tt.vBMD, Ct.Th, Tb.vBMD, and Tb.Th in all sectors ($p < 0.01$) and lower Ct.Po in the lateral, posterior, and medial sectors ($p < 0.05$).

For the entire cohort (n = 185), we found statistically significant regional variations in all bone density measurements and microarchitectural parameters (Table 2). Compared to other sectors, the anterior sector had the lowest Tt.vBMD, Ct.Po, Tb.vBMD, Tb.Th, and Tb.N as well as the highest Tb.Sp ($p < 0.01$). Ct.vBMD and Ct.Th were lowest in the medial sector ($p < 0.001$). The whole-bone values of density and microarchitectural parameters were significantly different from those of at least one sector (Table 2, $p < 0.05$).

### 3.2. Regional analyses of mechanical properties of trabecular subvolumes

The medial-lateral ($E_1$), anterior-posterior ($E_2$), and superior-inferior ($E_3$) elastic moduli for trabecular subvolumes (Fig. 4a–c), after adjusting for covariates and within-subject dependence, were significantly lower in the anterior sector than in other sectors for white women and black men and women ($p < 0.001$). All moduli, except for $E_1$ in men, followed a similar trend across sectors. In contrast to elastic moduli, we observed statistically significant regional variations in anisotropy in white men only (Fig. 4d, $p < 0.05$).

When compared to whole bone, the anterior sector had the lowest elastic moduli in black and white women (10% lower for $E_1$, 25% for $E_2$, and 23% for $E_3$; $p < 0.001$). In men, $E_2$ (11%, $p < 0.001$) and $E_3$ (8%, $p < 0.001$) were lowest in the anterior sector when compared to the whole bone. Between-race and between-sex comparisons of the elastic moduli, showed that women had lower $E_1$ and $E_3$ in the lateral and anterior sectors ($p < 0.05$), when compared to men. However, we did not see any difference in the elastic moduli between blacks and whites.

For the entire cohort, the anterior sector had the lowest elastic moduli ($E_1$, $E_2$, and $E_3$) when compared to other sectors (Table 2, $p < 0.001$). However, we did not observe any difference in anisotropy between sectors. For each mechanical property, the value for the whole bone was significantly different from that of at least one sector ($p < 0.05$).

## 4. Discussion

In our previous study of whole-bone analyses of the ultradistal tibia in young, healthy subjects [n = 185; age (mean ± one SD), 24.4 ± 3.4 years], we observed that bone characteristics from HR-pQCT images (i.e., density, microarchitectural parameters, and mechanical properties, such as whole-bone stiffness and failure load) were more favorable for men than for women, and for blacks than for whites [8]. In the present study, we performed regional analyses for the same subjects (Table 1) to quantify the variation in bone characteristics. Specifically, we analyzed the density, microarchitecture, and mechanical properties of trabecular subvolumes for the following datasets: white women (n = 50), black women (n = 51), white men (n = 50), black men (n = 34), and all subjects (n = 185). For each dataset, we observed statistically significant regional variations in bone

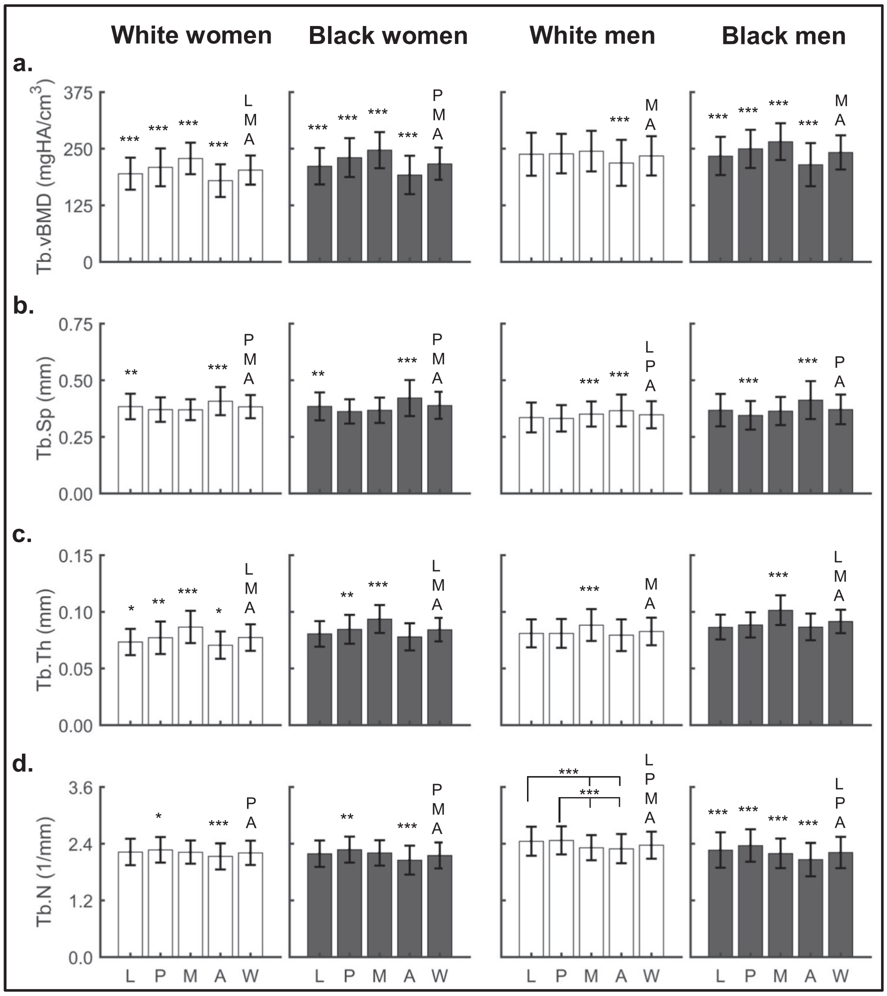


**Fig. 3.** Comparisons of density measurements and microarchitectural parameters of trabecular bone across datasets. Panels from top to bottom show a) trabecular volumetric bone mineral density (Tb.vBMD), b) trabecular separation (Tb.Sp), c) trabecular thickness (Tb.Th), and d) trabecular number (Tb.N) obtained from analyses of regional and whole bone for white women (n = 50), black women (n = 51), white men (n = 50), and black men (n = 34). Data are expressed as mean ± one standard deviation.

**Table 2**
Regional and whole-bone measures of density, microarchitectural parameters, and mechanical properties of the ultradistal tibia from all subjects (n = 185). The data are presented as mean ± one standard deviation.

| | Lateral | Posterior | Medial | Anterior | Whole bone |
|---|---|---|---|---|---|
| Density and microarchitectural parameters | | | | | |
| Tt.vBMD (mgHA/cm$^3$) | 300 ± 54 | 306 ± 55* | 297 ± 51 | 262 ± 54*** | 288 ± $50^{L,P,M,A}$ |
| Ct.vBMD (mgHA/cm$^3$) | 828 ± 46** | 820 ± 50 | 796 ± 58*** | 819 ± 51 | 818 ± $45^{L,M}$ |
| Ct.Th (mm) | 1.05 ± 0.26*** | 0.95 ± 0.23*** | 0.77 ± 0.21*** | 0.87 ± 0.20*** | 0.93 ± $0.21^{L,P,M,A}$ |
| Ct.Po (%) | 4.8 ± 2.4 | 4.7 ± 2.4 | 4.6 ± 2.3 | 3.9 ± 1.9*** | 4.4 ± $2.0^{L,A}$ |
| Tb.vBMD (mgHA/cm$^3$) | 218 ± 45*** | 230 ± 45*** | 245 ± 42*** | 200 ± 47*** | 222 ± $40^{L,P,M,A}$ |
| Tb.Th (mm) | 0.080 ± 0.012** | 0.082 ± 0.013*** | 0.092 ± 0.014*** | 0.078 ± 0.014** | 0.083 ± $0.012^{L,M,A}$ |
| Tb.Sp (mm) | 0.368 ± 0.066 | 0.353 ± 0.058*** | 0.363 ± 0.054 | 0.401 ± 0.076*** | 0.372 ± $0.060^{P,M,A}$ |
| Tb.N (1/mm) | 2.28 ± 0.32*** | 2.34 ± 0.30*** | 2.24 ± 0.27*** | 2.14 ± 0.32*** | 2.23 ± $0.29^{L,P,A}$ |
| Mechanical properties of trabecular subvolumes | | | | | |
| $E_1$ (GPa) | 0.99 ± 0.48 | 1.01 ± 0.44 | 1.07 ± 0.42* | 0.82 ± 0.44*** | 0.87 ± $0.39^{L,P,M,A}$ |
| $E_2$ (GPa) | 1.22 ± 0.54*** | 1.37 ± 0.55*** | 1.50 ± 0.53*** | 0.83 ± 0.45*** | 1.03 ± $0.45^{L,P,M,A}$ |
| $E_3$ (GPa) | 2.64 ± 0.72*** | 2.92 ± 0.67** | 3.05 ± 0.59** | 2.10 ± 0.72*** | 2.53 ± $0.56^{L,P,M,A}$ |
| Anisotropy | 3.03 ± 0.84 | 3.21 ± 0.88 | 3.16 ± 0.92 | 3.16 ± 0.85 | 3.24 ± $0.84^{L}$ |

***$p < 0.001$, **$p < 0.01$, *$p < 0.05$: indicates a statistically significant difference of one sector from each of the other sectors, after adjusting for height, body mass, age, and physical activity and after the Holm-Bonferroni correction; $^{L,P,M,A}p < 0.05$: indicates a statistically significant difference between the whole-bone value and the value of a particular sector (L-lateral, P-posterior, M-medial, A-anterior), after adjusting for height, body mass, age, and physical activity and performing the Holm-Bonferroni correction.

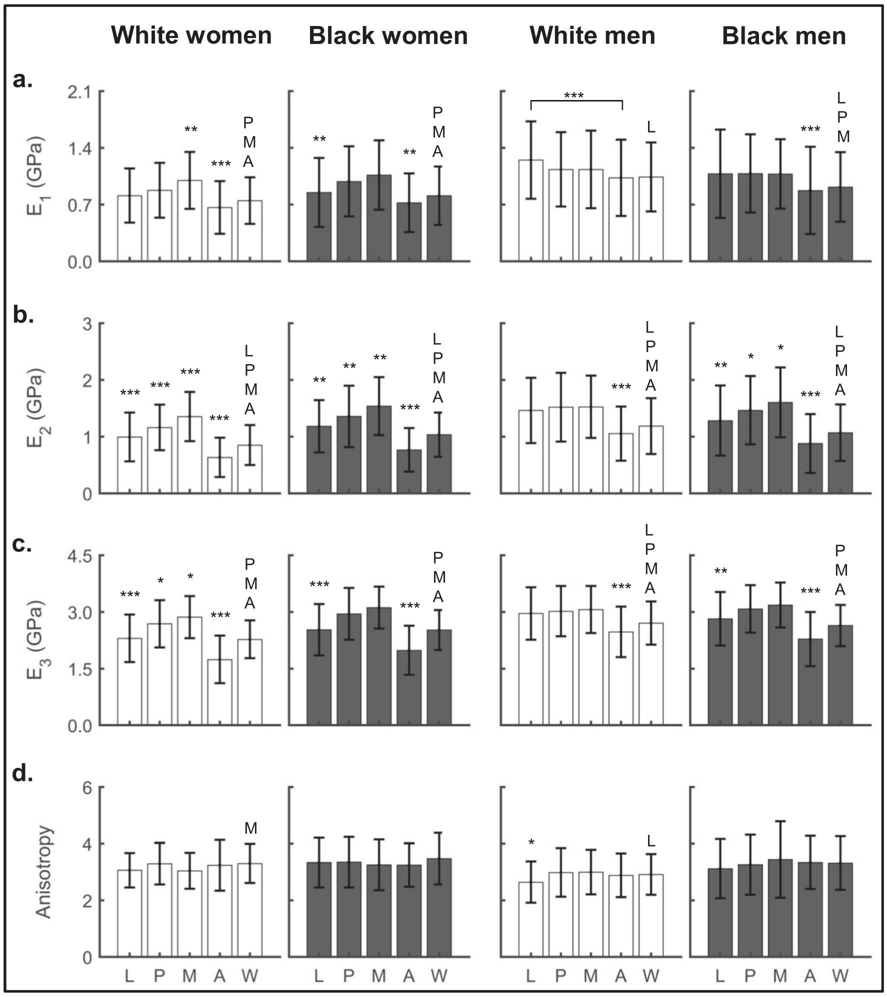


**Fig. 4.** Comparisons of mechanical properties for trabecular bone across datasets. a) Medial-lateral ($E_1$), b) anterior-posterior ($E_2$), and c) superior-inferior ($E_3$) elastic moduli, and d) anisotropy obtained from analyses of regional and whole-bone trabecular subvolumes for white women (n = 50), black women (n = 51), white men (n = 50), and black men (n = 34). Data are expressed as mean ± one standard deviation.

characteristics across sectors. For most characteristics, the cross-sector variation was similar across races and sexes but with different magnitudes (Table 2, Figs. 2 and 3, Supplementary Figs. 1 and 2). For black and white men and women, the anterior sector, when compared to other sectors, had unfavorable values of Tt.vBMD, Tb.vBMD, Tb.Sp, $E_2$, and $E_3$, and the medial sector had the lowest Ct.Th.

The higher elastic moduli in the superior-inferior ($E_3$) direction relative to those in the anterior-posterior ($E_2$) and medial-lateral ($E_1$) directions suggests that the distal tibia is predominantly subjected to loads in the vertical direction (Table 2, Fig. 4a–c, Supplementary Fig. 3a–c). Consistent with the unfavorable trabecular bone density and microarchitecture in the anterior sector, the elastic moduli in the anterior sector were consistently lower than those in other sectors, with the moduli in the anterior sector being at least 20% lower than those in the medial sector. Our simulations also showed that the mean anisotropy (i.e., $E_3$ divided by the smaller of $E_1$ and $E_2$) was ~3, similar to previously published data [20]. The similarity in the anisotropy across sectors indicates that the ratios of loading in mutually perpendicular directions are similar in the four sectors.

As expected, we observed statistically significant differences for most of the bone characteristics between the whole bone and at least one corresponding regional value in all datasets (Figs. 2 and 3, $p < 0.05$). In black and white men and women, the mean values of Tb.vBMD, Tb.Th, $E_2$, and $E_3$ in the medial and anterior sectors were higher and lower, respectively, than the whole-bone value. This indicates that conventional analyses, through the process of averaging over whole bone, can obscure regional variations in bone-health related characteristics.

Previous regional analyses of the ultradistal tibia, using HR-pQCT and micro-CT (μCT) images, revealed differences in cortical and trabecular density and microarchitecture similar to those observed here [9,11,21]. In 145 healthy subjects (92 women and 53 men; age range, 20 to 78 years), regional analyses of HR-pQCT images of the ultradistal tibia showed that, relative to other regions, the lateral sector has the greatest Ct.Th and the anterior sector has the lowest Ct.Po, whereas Ct.vBMD showed no variation [9]. These observations in a population older than ours are generally consistent with our results, except for the difference in variations that we found in Ct.Po and Ct.vBMD in women. For 146 healthy subjects (93 women and 53 men; age range, 20 to 78 years), after dividing the trabecular bone into eight sectors, Sode et al. reported that the inner-anterior and inner-lateral sectors have inferior trabecular parameters, whereas the outer-posterior and outer-medial sectors have superior trabecular parameters [11]. Regional μCT analyses of trabecular cores from anterior and posterior regions of the ultradistal tibia of 20 cadavers (18 men and 2 women; age, 70.8 ± 8.5 years) showed significantly lower Tb.N, Tb.Th, anisotropy, and bone volume fraction, as well as higher Tb.Sp in the anterior region [21]. Our results also showed that, when compared to the posterior sector, the anterior sector had lower Tb.N and Tb.Th, as well as higher Tb.Sp. However, in contrast to our results, a pQCT study of the tibial shafts of 72 healthy postmenopausal women (age range, 47 to 60 years) reported that the posterior region has the highest Ct.vBMD, whereas the anterior cortex has the greatest thickness [22]. This discrepancy could be due to the difference in scan location (tibial shaft vs. ultradistal site).

The regional variations in density, microarchitecture, and elastic moduli could be due to adaptation of the bone, in agreement with Wolff's law, to the spatially varying biomechanical loads during normal daily activities. During walking and running, the posterior and medial regions of the tibia are subjected to predominantly compressive forces, while the anterior region is subjected to tensile forces, with the maximum force occurring on the posterior aspect of the tibia [23–27]. Spatial variations of this biomechanical load during such activities are not dependent on race or sex, which explains the similarity in the regional variation for most of the bone characteristics in all four datasets. Resemblance of the regional variations of vBMD across sexes was previously reported in pre-adolescent subjects (85 girls and 76 boys; age, 12.1 ± 0.5 years) [28] and healthy young Israeli Defense Force recruits (20 men and 108 women; age range, 18 to 21 years) [12].

On performing between-race and between-sex comparisons of the bone characteristics, we observed that whites, compared to blacks, and women, compared to men, had inferior bone density and microarchitectural parameters in most of the sectors. These differences were similar to those of conventional whole-bone analyses [8]. These results, together with the similar cross-sector variations in black and white men and women (Figs. 2 and 3), indicate that race and sex have a greater influence on the magnitude of the bone characteristics than on the pattern of regional variation of these characteristics across the ultradistal tibia.

In this study, we could not determine any causal relationship between bone characteristics and stress fracture because we excluded subjects with a history of stress fracture; nonetheless, a few observations are in order. In the tibia, stress fractures occur most frequently in the posterior-medial region [29]. A previous study found lower bone quality and muscle strength in women athletes with stress fractures (n = 19; age range, 18 to 45 years) than in those without stress fractures [10]. Regional analyses of HR-pQCT images showed that the posterior sector of the subjects with stress fracture had lower Tb.vBMD and cortical area, compared to subjects without stress fracture. Therefore, it was suggested that this sector might not be well adapted to running-related activities in stress-fractured athletes. Our study on healthy subjects does not support the notion that the posterior sector has unfavorable cortical and trabecular parameters, and hence, is at greater risk of stress fracture. In fact, our analyses showed that the medial and anterior sectors had unfavorable cortical and trabecular parameters, respectively. This discrepancy between stress-fractured and healthy subjects could either be due to poor adaptation of the bone in the posterior sector prior to the injury in stress-fractured subjects or due to preferential loss of the bone in this sector after the injury from disuse osteoporosis.

Our study is subject to the following limitations. First, we divided the tibia into four sectors similar to previous studies on regional analyses of HR-pQCT images [9,11]. Although the sectors are aligned along the anatomical directions, this approach is inadequate for evaluating bone characteristics for sub-sectors, such as the posterior-medial sector, which is a frequent site of stress-fracture injury in the tibia [29]. However, our approach is systematic and consistent, which enables comparisons of the results from the regional analyses across different subjects and with reported data in the literature [9,11]. Second, we did not capture the entire trabecular region for the μFE analyses of trabecular subvolume because of technical limitations in obtaining a uniform cross section for determining the elastic moduli. However, because these exclusions were minimal and in peripheral regions of the bone, they are unlikely to alter the conclusions of the study. Finally, we performed all μFE analyses, using uniform material properties as in previous studies [8]. We do not believe that differences in the intrinsic material properties either due to race or sex will markedly affect the results of the regional μFE analyses.

## 5. Conclusion

In conclusion, our study shows that the ultradistal tibia in young, healthy subjects is heterogeneous, with substantial regional variation in bone-health related characteristics, such as bone density, microarchitecture, and elastic moduli. Similar to whole-bone analyses, regional analyses show that blacks and men have better bone characteristics than whites and women, respectively, across the different sectors of the tibia. However, the regional variation of these characteristics shows similar trend in all datasets, indicating that race and sex primarily affect the magnitude of changes but not their patterns. These findings suggest that the heterogeneity in the tibia is an adaptation to the biomechanical loads from normal daily activities, which might be independent of race or sex.

## Funding

We acknowledge support from the United States Department of Defense, Defense Health Program, and Joint Program Committee 5 (W81XWH-15-C-0024) and the National Institutes of Health shared instrumentation grant (S10 RR023405).

## Conflicts 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 United States Army or of the United States Department of Defense. This paper has been approved for public release with unlimited distribution.

## Acknowledgements

We thank Dr. Tatsuya Oyama for editorial assistance.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bone.2018.05.004.

## References

[1] B.A. Springer, A.E. Ross, Musculoskeletal injuries in military women, in: P.J. Dougherty (Ed.), Borden Institute Monograph Series, United States Government Printing Office, 2011.
[2] D.W. Armstrong III, J.-P.H. Rue, J.H. Wilckens, F.J. Frassica, Stress fracture injury in young military men and women, Bone 35 (2004) 806–816.
[3] A. Nattiv, Stress fractures and bone health in track and field athletes, J. Sci. Med. Sport 3 (2000) 268–279.
[4] B.R. Waterman, B. Gun, J.O. Bader, J.D. Orr, J.P.J. Belmont, Epidemiology of lower extremity stress fractures in the United States Military, Mil. Med. 181 (2016) 1308–1313.
[5] L. Bulathsinhala, J.M. Hughes, C.J. McKinnon, J.R. Kardouni, K.I. Guerriere, K.L. Popp, R.W. Matheny, M.L. Bouxsein, Risk of stress fracture varies by race/ethnic origin in a cohort study of 1.3 million US Army Soldiers, J. Bone Miner. Res. 32 (2017) 1546–1553.
[6] T.J. Beck, C.B. Ruff, R.A. Shaffer, K. Betsinger, D.W. Trone, S.K. Brodine, Stress fracture in military recruits: gender differences in muscle and bone susceptibility factors, Bone 27 (2000) 437–444.
[7] N.S. Bell, T.W. Mangione, D. Hemenway, P.J. Amoroso, B.H. Jones, High injury rates among female Army trainees: a function of gender? Am. J. Prev. Med. 18 (2000) 141–146.
[8] K.L. Popp, J.M. Hughes, A. Martinez-Betancourt, M. Scott, V. Turkington, S. Caksa, K.I. Guerriere, K.E. Ackerman, C. Xu, G. Unnikrishnan, J. Reifman, M.L. Bouxsein, Bone mass, microarchitecture and strength are influenced by race/ethnicity in young adult men and women, Bone 103 (2017) 200–208.
[9] G.J. Kazakia, J.A. Nirody, G. Bernstein, M. Sode, A.J. Burghardt, S. Majumdar, Age- and gender-related differences in cortical geometry and microstructure: improved sensitivity by regional analysis, Bone 52 (2013) 623–631.
[10] K.E. Schnackenburg, H.M. Macdonald, R. Ferber, J.P. Wiley, S.K. Boyd, Bone quality and muscle strength in female athletes with lower limb stress fractures,

Med. Sci. Sports Exerc. 43 (2011) 2110–2119.
[11] M. Sode, A.J. Burghardt, G.J. Kazakia, T.M. Link, S. Majumdar, Regional variations of gender-specific and age-related differences in trabecular bone structure of the distal radius and tibia, Bone 46 (2010) 1652–1660.
[12] R.K. Evans, C. Negus, A.J. Antczak, R. Yanovich, E. Israeli, D.S. Moran, Sex differences in parameters of bone strength in new recruits: beyond bone density, Med. Sci. Sports Exerc. 40 (2008) S645–653.
[13] R.M. Izard, W.D. Fraser, C. Negus, C. Sale, J.P. Greeves, Increased density and periosteal expansion of the tibia in young adult men following short-term arduous training, Bone 88 (2016) 13–19.
[14] R.K. Evans, C. Negus, A.J. Centi, B.A. Spiering, W.J. Kraemer, B.C. Nindl, Peripheral QCT sector analysis reveals early exercise-induced increases in tibial bone mineral density, J. Musculoskelet. Neuronal Interact. 12 (2012) 155–164.
[15] S.C. Chow, J. Shao, H. Wang, A note on sample size calculation for mean comparisons based on noncentral t-statistics, J. Biopharm. Stat. 12 (2002) 441–456.
[16] M.S. Putman, E.W. Yu, H. Lee, R.M. Neer, E. Schindler, A.P. Taylor, E. Cheston, M.L. Bouxsein, J.S. Finkelstein, Differences in skeletal microarchitecture and strength in African-American and white women, J. Bone Miner. Res. 28 (2013) 2177–2185.
[17] S. Boutroy, M.L. Bouxsein, F. Munoz, P.D. Delmas, In vivo assessment of trabecular bone microarchitecture by high-resolution peripheral quantitative computed tomography, J. Clin. Endocrinol. Metab. 90 (2005) 6508–6515.
[18] R Core Team, A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, 2013.
[19] D. Bates, M. Maechler, B. Bolker, S. Walker, Fitting linear mixed-effects models using lme4, J. Stat. Softw. 67 (2015) 1–48.
[20] M.C. Hobatho, J.Y. Rho, R.B. Ashman, Anatomical variation of human cancellous bone mechanical properties in vitro, Stud. Health. Technol. Inform. 40 (1997) 157–173.
[21] Y.M. Lai, L. Qin, H.Y. Yeung, K.K.H. Lee, K.M. Chan, Regional differences in trabecular BMD and micro-architecture of weight-bearing bone under habitual gait loading—a pQCT and microCT study in human cadavers, Bone 37 (2005) 274–282.
[22] Y.M. Lai, L. Qin, V.W.Y. Hung, K.M. Chan, Regional differences in cortical bone mineral density in the weight-bearing long bone shaft—a pQCT study, Bone 36 (2005) 465–471.
[23] S.A. Meardon, T.R. Derrick, Effect of step width manipulation on tibial stress during running, J. Biomech. 47 (2014) 2738–2744.
[24] S. Sasimontonkul, B.K. Bay, M.J. Pavol, Bone contact forces on the distal tibia during the stance phase of running, J. Biomech. 40 (2007) 3503–3509.
[25] P.-F. Yang, M. Sanno, B. Ganse, T. Koy, G.-P. Brüggemann, L.P. Müller, J. Rittweger, Torsion and antero-posterior bending in the in vivo human tibia loading regimes during walking and running, PLoS One 9 (2014) e94525.
[26] C. Xu, A. Silder, J. Zhang, J. Hughes, G. Unnikrishnan, J. Reifman, V. Rakesh, An integrated musculoskeletal-finite-element model to evaluate effects of load carriage on the tibia during walking, J. Biomech. Eng. 138 (2016) 101001.
[27] C. Xu, A. Silder, J. Zhang, J. Reifman, G. Unnikrishnan, A cross-sectional study of the effects of load carriage on running characteristics and tibial mechanical stress: implications for stress-fracture injuries in women, BMC Musculoskelet. Disord. 18 (2017) 125.
[28] D.M.L. Cooper, Y. Ahamed, H.M. Macdonald, H.A. McKay, Characterising cortical density in the mid-tibia: intra-individual variation in adolescent girls and boys, Br. J. Sports Med. 42 (2008) 690.
[29] A. Nattiv, G. Kennedy, M.T. Barrack, A. Abdelkerim, M.A. Goolsby, J.C. Arends, L.L. Seeger, Correlation of MRI grading of bone stress injuries with clinical risk factors and return to play: a 5-year prospective study in collegiate track and field athletes, Am. J. Sports Med. 41 (2013) 1930–1941.