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Altered trabecular bone morphology in adolescent and young adult athletes with menstrual dysfunction^{☆,,☆☆,★}

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Abstract

Context—Young amenorrheic athletes (AA) have lower bonemineral density (BMD) and an increased prevalence of fracture compared with eumenorrheic athletes (EA) and non-athletes. Trabecular morphology is a determinant of skeletal strength and may contribute to fracture risk.

Objectives—To determine the variation in trabecular morphology among AA, EA, and non-athletes and to determine the association of trabecular morphology with fracture among AA.

Design and setting—A cross-sectional study performed at an academic clinical research center.

Participants—161 girls and young women aged 14–26 years (97 AA, 32 EA, and 32 non-athletes).

Main outcome measure—We measured volumetric BMD (vBMD) and skeletal microarchitecture using high-resolution peripheral quantitative computed tomography. We evaluated trabecular morphology (plate-like vs. rod-like), orientation, and connectivity by individual trabecula segmentation.

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Results—At the non-weight-bearing distal radius, the groups did not differ for trabecular vBMD. However, plate-like trabecular bone volume fraction (pBV/TV) was lower in AA vs. EA ($p = 0.03$), as were plate number ($p = 0.03$) and connectivity ($p = 0.03$). At the weight-bearing distal tibia, trabecular vBMD was higher in athletes vs. non-athletes ($p=0.05$ for AA and $p=0.009$ for EA vs. non-athletes, respectively). pBV/TV was higher in athletes vs. non-athletes ($p=0.04$ AA and $p=0.005$ EA vs. non-athletes), as were axially-aligned trabeculae, plate number, and connectivity. Among AA, those with a history of recurrent stress fracture had lower pBV/TV, axially-aligned trabeculae, plate number, plate thickness, and connectivity at the distal radius.

Conclusions—Trabecular morphology and alignment differ among AA, EA, and non-athletes. These differences may be associated with increased fracture risk.

Keywords

Bone density; Microarchitecture; Amenorrhea; Athlete

1. Introduction

Weight-bearing athletic activity during childhood and adolescence is a critical factor promoting bone growth and mineral accrual [1]. Increased bone accrual and greater peak bone mass in turn is protective against osteoporosis and fracture risk in later life [2]. However, excessive physical activity can lead to energy imbalance and functional hypothalamic oligo-amenorrhea [3]. Young female athletes are at particular risk; in one recent study, approximately 25% of female high school athletes had menstrual dysfunction [4].

This hypogonadal state seen with excessive exercise can counteract the beneficial effects of weight-bearing exercise, leading to lower bone mineral density (BMD) compared to eumenorrheic athletes, as measured by dual-energy x-ray absorptiometry (DXA). This deficit is particularly evident at the spine, a site composed primarily of trabecular bone [5]. More recently, high-resolution peripheral computed tomography (HR-pQCT) has enabled the investigation of volumetric BMD (vBMD) and skeletal microarchitecture in amenorrheic athletes. These studies have highlighted the competing effects of exercise and hypogonadism on skeletal properties. In particular, at the weight-bearing tibia, trabecular density is similar among athletes and controls, while, at the non-weight-bearing radius, trabecular density is significantly lower in amenorrheic athletes compared to eumenorrheic athletes and non-athletes [6,7].

To further investigate the effects of activity and of hypogonadism on skeletal properties, we employed individual trabecula segmentation (ITS) analysis. This procedure classifies individual trabeculae as plate-like or rod-like and also quantitates axially-aligned trabeculae and trabecular connectivity. This distinction is clinically relevant, as osteoporosis due both to aging and hypogonadism specifically involves loss of plate-like trabeculae [8–10]. Importantly, experimentally-determined bone strength is highly correlated with plate-like bone volume fraction and the density of plate-plate junctions as determined by ITS [11]. In addition, an ITS-based model accurately predicts elastic modulus and yield strength of human trabecular bone [12]. ITS of data acquired by HR-pQCT in post-menopausal women

has been shown to distinguish those with fracture from controls, those with hyperparathyroidism from controls, and those on chronic glucocorticoids from controls [13–16].

We hypothesized that young amenorrheic athletes would have compromised trabecular properties at the radius as compared to eumenorrheic athletes and non-athletes in conjunction with their hypogonadal status. We further hypothesized that this distinction would not be evident at the tibia, where increased mechanical loading associated with weight-bearing physical activity would counter the effects of hypogonadism. Finally, we hypothesized that among amenorrheic athletes, trabecular morphology would be altered in those with a history of recurrent fracture.

2. Methods

2.1. Study design and setting

This was an observational cross-sectional study comparing measures of bone density and microarchitecture across three groups: amenorrheic athletes (AA), eumenorrheic athletes (EA), and non-athletes. Study procedures were conducted at a Clinical Research Center of a tertiary care hospital. This study was approved by the Institutional Review Board of Partners HealthCare. Informed consent was obtained from all subjects ≥ 18 years and from the parents of subjects < 18 years. Informed assent was obtained from subjects < 18 years.

2.2. Study participants

Subject recruitment and eligibility has been previously described [6]. Briefly, subjects were recruited from local pediatrics and sports medicine practices as well as from local high schools and colleges. Targeted advertisements for the AA group sought athletes and dancers with oligoamenorrhea, and subjects were informed in recruitment material that research participation would include bone density testing and nutritional and hormonal evaluation. Enrolled subjects were girls and young women ages 14–26 years with a bone age of at least 14 years. Subjects had a body mass index (BMI) between the 10th–90th percentiles for age, or weight ≥ 85% of ideal body weight. Three subjects in the AA group did not meet weight criteria due to low weight but were enrolled in the study as exceptions due to small skeletal frame, history of stable weight, and absence of eating disorder symptoms as determined by the study psychologist.

Subjects were excluded if they used a medication potentially affecting bone metabolism, including estrogen, progesterone, anabolic steroids, systemic glucocorticoids within the previous 3 months, phenytoin, or phenobarbitone. Subjects were also excluded if they had an illness potentially affecting bone metabolism, including hypo or hyperthyroidism, diabetes mellitus, Cushing's syndrome, cancer, renal disease, celiac disease, and inflammatory bowel disease, as well as conditions which may lead to amenorrhea including pregnancy, polycystic ovarian syndrome, thyroid dysfunction and primary ovarian insufficiency. Subjects were further classified by athlete and menstrual status as detailed below.

2.2.1. Athletes—Athletes participated in at least 4 h of aerobic weight-bearing activity or ran at least 20 miles per week for at least 6 months of the preceding year. Rowers, swimmers and gymnasts were excluded due to proposed differing effects of this type of weight-bearing and impact on bone density vs. leg-dominant activities [17,18]. Non-athletes participated in fewer than 2 h/week of weight-bearing exercise and in no organized team sports.

2.2.2. Menstrual status—Amenorrhea was defined as absence of menses for at least three months within a six month period of oligoamenorrhea, or absence of menarche at 16 years with a bone age of 14 years. Six AA had primary amenorrhea, and causes other than functional hypothalamic amenorrhea were ruled out in these participants. Eumenorrhea was defined as at least nine menses in the previous 12 months. All non-athletes were required to be eumenorrheic.

2.3. Study procedures

All subjects underwent a screening visit including a structured history, a physical exam including anthropometric measurements, and a bone age x-ray. Blood samples were obtained to rule out exclusion criteria. DXA (Hologic 4500, Hologic Inc., Waltham, MA) was used to measure bone mineral content and density at the hip and spine as well as body composition parameters, and Z-scores were generated from race-specific normative databases.

2.4. HR-pQCT and micro-finite element analysis (μ FEA)

Total volumetric density, compartment-specific densities, and microarchitecture of the distal radius and tibia were assessed using HR-pQCT (XtremeCT, Scanco Medical AG, Brüttisellen, Switzerland) as previously described [6,7]. Scans consisting of 110 CT slices were acquired with an isotropic voxel size of 82 μ m. The non-dominant arm or leg was scanned unless there was a prior fracture at that region in which case the contralateral side was scanned. 2D scout views were obtained and used to locate the distal CT slice at 9.5 mm and 22.5 mm from the radius and tibia endplate respectively. We used semiautomated software to segment cortical and trabecular regions, and compartment-specific areas and volumetric bone densities as well as microarchitectural features of trabecular and cortical bone were determined.

μ FEA was performed to estimate the biomechanical properties of the bone in the setting of simulated axial compression, as previously described [19]. Briefly, following image segmentation, each bone voxel of the HR-pQCT distal radius and tibia images was converted to hexahedral finite elements having linear-elastic and isotropic material behavior, with a Young's modulus and Poisson's ratio of 10 GPa and 0.3, respectively. Failure load was estimated by scaling the resultant load from a 1% apparent compressive strain until 2% of all elements reached an effective strain >7000 μ strain.

Same-day reproducibility for repeated measurements is 0.2 to 1.4% for density values, 0.3 to 8.6% for trabecular microarchitecture parameters, 0.6 to 2.4% for cortical microarchitecture parameters, 7.3 to 20.2% for cortical porosity measurements, and 2.1 to 3.0% for μ FEA measures.

HR-pQCT and μ FEA analyses of an earlier smaller subset of this cohort have previously been reported [6,7,20].

2.5. Individual trabecula segmentation

The trabecular bone compartment was extracted from each HR-pQCT image and the entire compartment underwent ITS-based morphological analysis as described [13,21,22]. Complete volumetric decomposition was performed and the following morphological parameters were computed including parameters of *scale*: (plate and rod bone volume fraction (pBV/TV and rBV/TV), plate and rod number density (pTbN and rTbN), plate and rod thickness (pTbTh and rTbTh), plate surface area (pTbS), and rod length (rTb ℓ), *topology*: (plate and rod tissue fraction pBV/BV and rBV/BV), and plate–plate, plate–rod, and rod–rod junction density (P–P JuncD, P–R JuncD, and R–R JuncD)), and *orientation*: (axial bone volume fraction (aBV/TV)).

2.6. Laboratory studies

Calcium and phosphate were measured by standard colorimetric assay and 25-hydroxyvitamin D (25OHD) by chemiluminescent immunoassay (DiaSorin, Stillwater, MN, sensitivity 4 ng/mL, intraassay CV 2.9–5.5%) in real time. Serum for parathyroid hormone (PTH) was stored at -80°C and was measured in a single batch in a subset of 64 subjects (15 non-athletes, 16 EA, 33 AA) by chemiluminescent immunoassay (Roche Diagnostics, Basel, Switzerland, sensitivity 1.2 pg/mL, intraassay CV 1.7–3.6%). Serum for insulin-like growth factor 1 (IGF-1) was stored at -80°C and was measured in a single batch in a subset of 63 subjects (12 non-athletes, 13 EA, 38 AA) by chemiluminescent immunoassay (Immunodiagnosics, Fountain Hills, AZ, sensitivity 1.9 ng/mL, intraassay CV <4%).

2.7. Statistical analysis

We used Stata 12.1 (StataCorp LP, College Station, TX) for all analyses. Clinical and laboratory data are reported as mean $\pm$ standard deviation except as noted. All variables were inspected for normality. Cortical porosity was not normally distributed and was log-transformed. For 2-group comparisons, we used a *t*-test assuming unequal variance and using the Satterthwaite approximation of degrees of freedom. For 3-group comparisons, we performed an overall ANOVA followed by a Tukey–Kramer analysis to assess pair-wise between-group differences while controlling for multiple comparisons. Comparisons with a *p*-value of <0.05 are reported as statistically significant. Multivariate regression modeling was performed by reverse stepwise selection ($p < 0.1$ to leave the model), including all variables found to be significant at $p < 0.05$ in univariate correlations as well as variables thought to be clinically relevant.

3. Results

3.1. Subject characteristics

Table 1 describes key clinical and biochemical characteristics of subjects, including 97 AA, 32 EA, and 32 non-athletes. Groups did not differ for age and height. Age at menarche was higher in AA compared with the other groups. Racial distribution varied between groups. AA had lower average weight and BMI than EA. Lean mass was higher in EA than in the

other groups. Fat mass was lower in AA compared to nonathletes, while percent body fat was lower in both athlete groups vs. non-athletes. Serum calcium was slightly higher in AA vs. controls, consistent with a higher serum 25OHD in AA subjects than in the other groups. Serum PTH levels did not vary among groups.

3.2. Areal BMD

As we have shown in an earlier subset of this cohort, areal BMD Z-scores were higher at the total hip and femoral neck in EA compared with the other groups [5,6] (Table 1). At the lumbar spine, areal BMD was lower in AA compared to EA. Prevalence of previous fracture was higher in AA vs. other groups. As previously shown, this difference was driven by an increased prevalence specifically of stress fractures, with no difference in non-stress fractures [20].

3.3. Standard and extended HR-pQCT analyses

All statistical comparisons of HR-pQCT data among groups were adjusted for age and race. At the non-weight-bearing distal radius, we did not detect significant differences among groups in total, cortical and trabecular vBMD, total and trabecular area, cortical thickness, or any component of trabecular microarchitecture. AA had higher cortical porosity compared with non-athletes with a nonsignificant intermediate value in EA (Table 2). Of note, porosity analyses were limited to subjects ≥ 18 years old ($n = 125$) due to the physiologically higher levels of porosity during adolescence and rapid decline with increasing age [23,24]. Failure load, as calculated by μ FEA, was lower in AA vs. EA, with non-athletes having an intermediate value.

At the weight-bearing distal tibia, trabecular vBMD was higher in EA vs. non-athletes and trended higher in AA vs. non-athletes with no difference in cortical vBMD. This difference was consistent with higher trabecular thickness in athletes vs. non-athletes. Total bone cross-sectional area was higher in EA vs. the other groups; this difference corresponded to both higher trabecular area and higher cortical thickness in EA vs. non-athletes, with intermediate values for both measures in AA. Similar to the radius, cortical porosity at the tibia was higher in athletes vs. non-athletes. Failure load was highest in EA, followed by AA, with the lowest values in non-athletes.

3.4. Individual trabecula segmentation (ITS) analyses

We employed ITS to further investigate variation in trabecular microarchitecture among groups (Table 3). As with HR-pQCT, all statistical analyses were adjusted for age and race. As shown in Fig. 1A, at the distal radius, total BV/TV did not vary among groups, consistent with the similar trabecular vBMD among groups. However, pBV/TV was significantly lower in AA vs. EA. This difference was consistent with significantly fewer trabecular plates in AA vs. EA and corresponded to lower P-P JuncD.

At the distal tibia, again similar to standard HR-pQCT, BV/TV was higher in EA than non-athletes, with a trend towards higher BV/TV in AA vs. non-athletes (Table 3 and Fig. 1B). pBV/TV was higher in both groups of athletes vs. non-athletes, with higher pTbN in both groups and higher pTbTh in AA with a quantitatively similar but nonsignificant increase in

EA. In addition, a significantly higher proportion of trabeculae were axially-aligned in both athlete groups vs. non-athletes. While there were limited significant differences between EA and AA at the tibia, overall, the advantage conferred by athletic activity was numerically lower in the AA vs. EA.

3.5. Association of ITS parameters with fracture history

AA status has previously been reported to be associated with a higher rate of prevalent fracture [20]. We thus investigated whether, among the AA subjects, ITS parameters were associated with fracture history. In particular, we compared subjects with a history of 2 or more stress fractures with those with a history of 1 stress fracture, given that recurrent stress fractures are associated with female athlete triad physiology and are thus of significant clinical importance [25]. Our group has previously shown in this cohort that subjects with recurrent fracture have lower lumbar spine BMD and lower trabecular vBMD at the radius but not at the tibia [20]. As shown in Fig. 2, ITS analysis at the distal radius demonstrated that AA with a history of recurrent stress fracture had significantly lower levels of pBV/TV, aBV/TV, pTbTh, pTbN, and P-P JuncD than those without recurrent stress fracture. We did however observe substantial overlap in these parameters between those with and without recurrent stress fracture. For example, examination of the receiver operating characteristic curve for pBV/TV showed an area under the curve of 0.67, indicating poor discrimination. At the tibia, while ITS parameters were quantitatively less favorable among recurrent fracture subjects, these decreases were much smaller than at the radius and were not statistically significant.

3.6. Associations of plate bone volume fraction with clinical characteristics

Black subjects had higher pBV/TV than non-Black subjects at the distal radius ($14.5 \pm 4.1\%$ vs. $10.7 \pm 4.2\%$, $p = 0.04$) and trended to have higher pBV/TV at the distal tibia ($20.5 \pm 3.0\%$ vs. $18.4 \pm 5.0\%$, $p = 0.09$). After adjustment for group status, these differences persisted ($p=0.04$ at the radius and $p=0.06$ at the tibia). Given these findings, we performed post-hoc analyses of our ITS data in the non-Black ($n = 153$) and in the White ($n = 123$) subjects with results similar to those reported here (data not shown).

As shown in Table 4, when combining all subjects, pBV/TV at the distal radius was negatively associated with height, menarchal age, and 25OHD level, and was positively associated with BMI. At the distal tibia, pBV/TV was positively associated with serum PTH levels. There were no associations of pBV/TV with body composition parameters at either site, nor were there associations with serum IGF-1.

3.7. Regression models of plate bone volume fraction

At both the radius and tibia, we generated regression models including all factors associated with pBV/TV in univariate analyses as well as athletic status and menstrual status (amenorrheic or eumenorrheic). We also included BMI and menarchal age in the tibiamodel as these covariates were found to be associated with total trabecular density in our previous report [6]. At the distal radius, Black race and BMI remained positive independent predictors and height was a negative independent predictor of pBV/TV ($p=0.018$, 0.002 , and 0.009 , respectively, adjusted R^2 for the model = 0.14). At the distal tibia, athletic status,

serum PTH, and BMI were positive independent predictors, and height was a negative independent predictor of pBV/TV ($p = 0.04, 0.05, 0.04, \text{ and } 0.001$, respectively, adjusted R^2 for the model = 0.26).

4. Discussion

This study significantly extends our understanding of the effect of low energy availability and resultant hypogonadism on skeletal physiology in adolescent female athletes. Using ITS, we demonstrate important differences in trabecular morphology at the radius between amenorrheic and eumenorrheic athletes. Specifically, we show that the amenorrheic athletes have decreased pBV/TV, pTbN, and P–P JuncD at the radius. Notably, these differences at the radius were not apparent using HR-pQCT alone, demonstrating the clinical sensitivity of this technique.

Our data also further demonstrate the salutary impact of weight-bearing activity on trabecular morphology at the tibia, since all athletes, independent of menstrual status, had increased pBV/TV and aBV/TV vs. non-athletes. While, due to the unequal group sizes, this study design would have been able to detect a smaller effect size as statistically significant when comparing AAs vs. non-athletes than when comparing EAs vs. non-athletes, the fact that we found most tibia plate-like ITS parameters to differ significantly between EAs and non-athletes indicates that power was adequate for these comparisons. Our data also suggest that AA may derive less benefit from physical activity at the tibia given their quantitatively lower plate-like and axial parameters.

Little is known about the development of trabecular morphology, including its determinants during childhood growth. This report is the first to use ITS in adolescent and young adult athletes, and suggests that physiologic alterations in this period of the lifespan may alter trabecular morphology and confer increased risk of fracture. In a study of 169 post-menopausal women, those with a history of fragility fracture had both lower pBV/TV and rBV/TV as well as lower aBV/TV [13]. More strikingly, in a study of 117 osteopenic but not osteoporotic post-menopausal women, those with a history of fragility fracture, had significantly decreased pTbN, aBV/TV, and P–P JuncD at the radius despite similar areal BMD as measured by DXA [14]. In that report, as in our cohort, differences at the tibia between those with and without prevalent fracture were quantitatively smaller and not statistically significant. It is interesting to note that only 28% of subjects in that study reported a history of wrist fracture. Similarly, in our cohort, though ITS differences in AA subjects with recurrent fracture were most pronounced at the radius, 79% of stress fractures occurred in the lower extremity [20]. This suggests that measured alterations at the distal radius reflect clinically important changes in overall bone strength that may be more subtle at weight-bearing sites but which retain mechanical significance.

The importance of trabecular morphology to bone strength and fracture resistance has been demonstrated in experimental models. For example, in a study of bovine tibiae exposed to compressive strain, accumulated microdamage was proportional to the fraction of trabeculae which were rod-like [26]. In addition, a study of human cadaveric vertebrae demonstrated that the fraction of axially aligned trabeculae was strongly associated with empirically-

determined bone strength [27]. These findings thus suggest that the differences in trabecular morphology and alignment in the AA in our cohort may directly influence their risk of fracture.

While our data do not allow us to determine the cause of altered trabecular morphology in AA, estrogen deficiency likely plays a significant role. Data from ovariectomized rats show a dramatically rapid transition from plate-like to rod-like trabeculae evident within 1 week post-procedure and almost complete within 5 weeks [10]. In post-menopausal women, treatment with hormone replacement therapy partially restores plate-like bone volume fraction [28]. Several other metabolic perturbations in these subjects including elevations in basal cortisol as well as decreases in leptin and bioavailable IGF-1 may also contribute to the skeletal phenotype [29].

The positive correlation of serum PTH with pBV/TV at the tibia is interesting in light of the pleiotropic effects of PTH on BMD and microarchitecture at supraphysiologic levels. Chronic elevation of PTH, as in primary hyperparathyroidism, decreases BMD [30]. In particular, a recent ITS analysis of patients with primary hyperparathyroidism showed that, while both pBV/TV and rBV/TV were reduced in comparison to healthy controls, pBV/TV had a larger decrease, leading to a lower plate/rod ratio [31]. In contrast, intermittent PTH is anabolic to bone, leading to an increase in hip and spine BMD and a decrease in fragility fractures in postmenopausal women [32]. The one study to use ITS to study the effect of teriparatide (intermittent recombinant PTH (1–34)) reported increases in pBV/TV at both the radius and tibia [31]. The effect of normal levels of endogenous PTH is less well-described. A longitudinal study of bone acquisition in adolescent girls showed a positive univariate association of PTH with bone mineral gain, but a negative correlation with change in bone density in a multivariate model [33]. A potential synergy between PTH and mechanical loading has been proposed in animal models and may contribute to our finding of a positive correlation between PTH and plate BV/TV at the tibia but not at the radius [34].

Our study has some limitations. We performed a cross-sectional analysis and thus cannot determine when trabecular morphology diverged among the groups nor can we make causal inferences regarding hormonal or other determinants of the observed differences. Skeletal microarchitecture and trabecular morphology have been shown to differ between Chinese-American and White women as well as between African-American and White women [35,36]. Our cohort had a few Asian subjects who had ancestry from varying regions including East and South Asia. We were thus unable to evaluate any effect of Asian ancestry on trabecular morphology. In addition, while the correlation of ITS analyses from HR-pQCT data at an 82 μm voxel size as compared to the gold standard micro-CT with a 25 μm voxel size is very good for most plate parameters as well as axial BV/TV, it is substantially less well-correlated for rod-like trabecular parameters [22]. Thus, the absence of differences in these parameters among our groups may be due to imprecision of measurement. Finally, it is possible that amenorrheic athletes with a history of fractures or other concerns about bone health may have preferentially enrolled in our study; this may have affected results of inter-group comparisons though not the within-group comparison of predictors of stress fracture. Of note as well, though we observed significant differences in ITS parameters between AAs

with and without recurrent stress fracture, substantial overlap in the distributions of these measures limits their clinical usefulness to distinguish those at high vs. low risk.

In conclusion, using ITS, we demonstrate significant differences in trabecular morphology between amenorrheic athletes, eumenorrheic athletes, and healthy non-athletes, which are not apparent by standard HR-pQCT. Altered trabecular morphology is associated with recurrent stress fracture among the amenorrheic athletes suggesting that these changes have clinical significance. Future investigations to determine whether these changes are reversible by improved energy availability, resumption of menses, or therapeutic intervention will improve our understanding of the long-term skeletal implications of the athletic amenorrheic state.

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Fig. 1.

Trabecular parameters from ITS analysis at the radius (1A) and tibia (1B) shown as percent difference from non-athletic controls $\pm$ SEM. EA — eumenorrheic athletes. AA — amenorrheic athletes. *Different from control at $p < 0.05$. †EA different from AA at $p < 0.05$. BV/TV — bone volume fraction; BV/BV — bone tissue fraction; TbN — trabecular number; TbTh — trabecular thickness; P-P JuncD — plate-plate junction density.

Fig. 2.

Percent difference $\pm$ SEM in trabecular parameter from ITS analysis of amenorrheic athletes with recurrent stress fracture relative to amenorrheic athletes with 1 stress fracture. * $p < 0.05$; ** $p < 0.01$. BV/TV — bone volume fraction; BV/BV — bone tissue fraction; TbN— trabecular number; TbTh — trabecular thickness; P–P JuncD— plate–plate junction density.

Table 1

Clinical characteristics of non-athletic controls (NAC), eumenorrheic athletes (EA), and amenorrheic athletes (AA).

	NAC (n = 32)	EA (n = 32)	AA (n = 97)	ANOVA	NAC vs EA	NAC vs AA	EA vs AA
Age (years)	20.1 ± 2.3	19.1 ± 2.4	19.8 ± 2.4	0.246			
Race (n,%)				<0.001	0.281	<0.001	0.010
American Indian	0 (0)	0 (0)	1 (1)				
Asian	5 (16)	3 (9)	4 (4)				
Black	6 (19)	2 (6)	0 (0)				
White	14 (44)	22 (69)	87 (90)				
Multiple Races	6 (19)	4 (13)	5 (5)				
Unknown	1 (3)	1 (3)	0 (0)				
Age at menarche (years)	12.3 ± 1.3	12.5 ± 1.5	13.2 ± 1.9	<0.001	0.851	<0.001	0.003
Height (cm)	162.2 ± 6.8	164.5 ± 7.6	165.1 ± 6.1	0.094			
Weight (kg)	56.7 ± 7.2	60.8 ± 9.5	56.4 ± 7.7	0.023	0.099	0.979	0.019
BMI (kg/m ²)	21.6 ± 2.5	22.4 ± 2.3	20.6 ± 2.3	<0.001	0.329	0.130	0.001
Lean mass (kg)	39.5 ± 4.5	45.0 ± 6.6	41.9 ± 5.2	<0.001	<0.001	0.074	0.015
Fat mass (kg)	16.8 ± 5.1	15.3 ± 4.2	13.7 ± 4.6	0.003	0.405	0.003	0.184
Percent body fat (%)	28.6 ± 5.9	24.3 ± 4.1	23.5 ± 5.7	<0.001	0.006	<0.001	0.751
Fracture history (n,%)							
Any fracture	5 (16%)	8 (25%)	47 (49%)	<0.001	0.701	0.002	0.034
Stress fracture	0 (0%)	2 (6%)	30 (31%)	<0.001	0.779	<0.001	0.005
2 stress fractures	0 (0%)	2 (6%)	15 (16%)	0.032	0.679	0.035	0.315
Serum calcium (mg/dL)	9.0 ± 0.5	9.1 ± 0.7	9.3 ± 0.4	0.003	0.794	0.007	0.061
Serum phosphate (mg/dL)	4.1 ± 0.5	4.0 ± 0.4	4.0 ± 0.4	0.703			
Serum 25OHD (ng/mL)	23 ± 11	28 ± 12	38 ± 13	<0.001	0.196	<0.001	<0.001
Serum PTH ^a (pg/mL)	33.9 ± 6.2	31.9 ± 10.4	29.0 ± 9.8	0.217			
IGF-1 ^a (ng/mL)	300 ± 66	258 ± 55	247 ± 81	0.096			
FN BMD Z-score	-0.5 ± 0.8	0.4 ± 1.0	-0.1 ± 1.0	<0.001	<0.001	0.111	0.011
TH BMD Z-score	-0.2 ± 0.8	0.8 ± 0.9	0.1 ± 0.9	<0.001	<0.001	0.161	0.001
LS BMD Z-score	-0.5 ± 1.0	0.0 ± 0.9	-0.8 ± 1.2	0.002	0.169	0.367	0.001

Bold: statistically significant at p < 0.05 after Tukey-Kramer adjustment for multiple comparison.

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Measured in a subset of subjects (IGF-1: n = 63; PTH: n = 64). BMI, body mass index; 25OHD, 25-hydroxyvitamin D; FN, femoral neck; TH, total hip; LS, lumbar spine; BMD, bone mineral density.

Table 2

HR-pQCT of non-athletic controls (NAC), eumenorrheic athletes (EA), and amenorrheic athletes (AA).

Measurement	NAC	EA	AA	ANOVA	NAC vs EA	NAC vs AA	EA vs AA
<i>Radius</i>							
Total area (mm ²)	255.5 ± 42.1	279.1 ± 43.8	263.2 ± 44.5	0.137			
Tb area (mm ²)	201.5 ± 44.5	221.5 ± 45.1	211.8 ± 45.2	0.429			
Total vBMD (mg HA/cm ³)	327.2 ± 67.1	312.8 ± 53.6	298.5 ± 55.5	0.269			
Cortical vBMD (mg HA/cm ³)	842.9 ± 74.3	823.8 ± 52.5	814.7 ± 69.4	0.203			
Tb vBMD (mg HA/cm ³)	173.0 ± 35.0	177.7 ± 38.4	164.2 ± 30.5	0.253			
Cortical thickness (mm)	0.79 ± 0.21	0.75 ± 0.17	0.70 ± 0.20	0.294			
Tb number (mm ⁻¹)	2.00 ± 0.24	1.97 ± 0.28	1.96 ± 0.26	0.660			
Tb thickness (mm)	0.072 ± 0.013	0.075 ± 0.013	0.070 ± 0.010	0.144			
Cortical porosity (%) ^a	0.62 ± 0.38	0.83 ± 0.43	0.94 ± 0.60	0.017	0.202	0.012	0.702
Failure load (N)	3965 ± 707	4116 ± 674	3701 ± 691	0.025	0.259	0.747	0.018
<i>Tibia</i>							
Total area (mm ²)	605.8 ± 101.7	702.7 ± 89.1	665.1 ± 108.7	0.002	0.001	0.027	0.167
Tb area (mm ²)	489.8 ± 105.6	572.9 ± 92.6	545.1 ± 107.6	0.013	0.010	0.075	0.368
Total vBMD (mg HA/cm ³)	329.1 ± 61.9	337.2 ± 53.2	328.5 ± 46.5	0.498			
Cortical vBMD (mg HA/cm ³)	890.9 ± 41.3	875.1 ± 35.5	868.4 ± 36.4	0.121			
Tb vBMD (mg HA/cm ³)	190.6 ± 32.2	211.9 ± 34.4	202.9 ± 28.1	0.011	0.009	0.052	0.415
Cortical thickness (mm)	1.20 ± 0.25	1.28 ± 0.24	1.22 ± 0.25	0.207			
Tb number (mm ⁻¹)	1.92 ± 0.24	1.99 ± 0.21	1.90 ± 0.27	0.303			
Tb thickness (mm)	0.083 ± 0.014	0.089 ± 0.013	0.090 ± 0.011	0.013	0.082	0.010	0.904
Cortical porosity (%) ^a	1.21 ± 0.86	2.18 ± 1.29	1.94 ± 0.96	0.007	0.006	0.037	0.381
Failure load (N)	10220 ± 1376	12334 ± 1737	11384 ± 1548	<0.001	<0.001	<0.001	0.019

Tb, Trabecular; vBMD, volumetric bone mineral density; HA, hydroxyapatite.

Bold: statistically significant at p < 0.05 after Tukey-Kramer adjustment for multiple comparison.

^aCortical porosity analyses limited to subjects 18 years old (n = 29 NAC, 24 EA, 72 AA).

Table 3

Individual trabecula segmentation analyses of non-athletic controls (NAC), eumenorrheic athletes (EA), and amenorrheic athletes (AA).

Measurement	NAC	EA	AA	ANOVA	NAC vs EA	NAC vs AA	EA vs AA
<i>Radius</i>							
Total BV/TV	0.285 ± 0.046	0.291 ± 0.050	0.272 ± 0.040	0.158			
Plate BV/TV	0.114 ± 0.049	0.126 ± 0.044	0.102 ± 0.038	0.045	0.268	0.831	0.034
Rod BV/TV	0.171 ± 0.026	0.166 ± 0.028	0.170 ± 0.024	0.534			
Axial BV/TV	0.122 ± 0.033	0.129 ± 0.035	0.115 ± 0.028	0.151			
Plate BV/BV	0.387 ± 0.114	0.421 ± 0.097	0.367 ± 0.096	0.044	0.158	0.980	0.038
Plate Tb number (1/mm)	1.49 ± 0.18	1.54 ± 0.16	1.45 ± 0.14	0.035	0.179	0.922	0.028
Rod Tb number (1/mm)	1.89 ± 0.13	1.87 ± 0.14	1.88 ± 0.13	0.627			
Plate Tb thickness (mm)	0.207 ± 0.007	0.206 ± 0.009	0.204 ± 0.010	0.535			
Rod Tb thickness (mm)	0.214 ± 0.006	0.216 ± 0.007	0.215 ± 0.006	0.528			
Plate Tb surface (mm ²)	0.157 ± 0.011	0.159 ± 0.012	0.156 ± 0.012	0.521			
Rod Tb length (mm)	0.648 ± 0.020	0.644 ± 0.021	0.650 ± 0.020	0.406			
Plate-plate JuncD (1/mm ³)	2.21 ± 0.71	2.41 ± 0.63	2.05 ± 0.55	0.034	0.224	0.845	0.026
<i>Tibia</i>							
Total BV/TV	0.310 ± 0.041	0.336 ± 0.039	0.323 ± 0.037	0.021	0.016	0.128	0.326
Plate BV/TV	0.167 ± 0.053	0.201 ± 0.052	0.186 ± 0.46	0.006	0.005	0.037	0.377
Rod BV/TV	0.144 ± 0.036	0.135 ± 0.032	0.137 ± 0.036	0.349			
Axial BV/TV	0.154 ± 0.036	0.177 ± 0.038	0.167 ± 0.032	0.013	0.012	0.050	0.497
Plate BV/BV	0.529 ± 0.127	0.593 ± 0.108	0.572 ± 0.110	0.019	0.023	0.038	0.755
Plate Tb number (1/mm)	1.59 ± 0.12	1.67 ± 0.09	1.63 ± 0.10	0.002	0.001	0.035	0.148
Rod Tb number (1/mm)	1.75 ± 0.18	1.73 ± 0.16	1.71 ± 0.19	0.492			
Plate Tb thickness (mm)	0.224 ± 0.012	0.229 ± 0.010	0.230 ± 0.012	0.046	0.147	0.038	0.965
Rod Tb thickness (mm)	0.219 ± 0.007	0.215 ± 0.005	0.220 ± 0.007	0.012	0.132	0.822	0.009
Plate Tb surface (mm ²)	0.178 ± 0.024	0.184 ± 0.021	0.184 ± 0.024	0.265			
Rod Tb length (mm)	0.654 ± 0.021	0.649 ± 0.016	0.652 ± 0.023	0.615			
Plate-plate JuncD (1/mm ³)	2.53 ± 0.48	2.89 ± 0.42	2.68 ± 0.47	0.006	0.004	0.102	0.138

BV/TV, bone volume over total medullary compartment volume; BV/BV, bone volume over total medullary bone volume, Tb, trabecular, JuncD, junction density.

Bold: statistically significant at $p < 0.05$ after Tukey-Kramer adjustment for multiple comparison.

Table 4

Correlations of key predictors with plate bone volume fraction (BV/TV) at the radius and tibia.

	Radius plate BV/TV		Tibia plate BV/TV	
	r	p	r	p
Age (years)	0.02	0.765	– 0.01	0.873
Height	–0.23	0.005	– 0.14	0.074
BMI	0.28	<0.001	0.13	0.102
Lean mass	0.10	0.240	0.08	0.293
Fat mass	0.05	0.540	– 0.08	0.291
Age at menarche	–0.24	0.003	– 0.09	0.243
25OH-vitamin D	–0.19	0.020	– 0.10	0.235
PTH	0.18	0.158	0.27	0.028
IGF-1	–0.01	0.921	–0.02	0.864

Bold: statistically significant at $p < 0.05$ after Tukey-Kramer adjustment for multiple comparison.