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## Fractures in Relation to Menstrual Status and Bone Parameters in Young Athletes

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### Abstract

**Introduction**—To compare fracture prevalence in oligo-amenorrheic athletes (AA), eumenorrheic athletes (EA), and non-athletes (NA) and determine relationships with bone density, structure and strength estimates.

**Methods**—175 females (100 AA, 35 EA, and 40 NA) 14–25 yo were studied. Lifetime fracture history was obtained through participant interviews. Areal BMD was assessed by DXA at the spine, hip and whole body (WB). Bone structure was assessed by HRpQCT at the radius and tibia, and strength by finite element analysis.

**Results**—AA, EA, and NA did not differ in age, sexual maturity, or height. AA had lower BMI, and older menarchal age than EA and NA ( $p < 0.001$ ). BMD Z-scores were lower in AA vs. EA at the total hip, femoral neck, spine, and whole body ( $p < 0.001$ ). Lifetime fracture risk was higher in AA than EA and NA (47%, 25.7%, 12.5%,  $p < 0.001$ ), largely driven by stress fractures in AA vs. EA and NA (32% vs. 5.9% vs. 0%). In AA, those who fractured had lower lumbar and WB BMD Z-scores, vBMD of outer trabecular region in radius and tibia, and trabecular thickness of the radius ( $p < 0.05$ ). In AA, those who had 2 stress fractures had lower lumbar and WB BMD Z-scores, total cross-sectional area, trabecular vBMD, stiffness and failure load at radius; and lower stiffness and failure load at tibia versus those with  $< 2$  stress fracture ( $p < 0.05$ ).

**Conclusion**—Weight-bearing athletic activity increases BMD, but may increase stress fracture risk in those with menstrual dysfunction. Bone microarchitecture and strength differences are more pronounced in AA with multiple stress fractures. This is the first study to examine fractures in relation to bone structure in adolescent female athletes.

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## Keywords

Female Athlete Triad; Stress Fracture; Amenorrhea; Bone Microarchitecture; Bone Mineral Density

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## Introduction

Many female athletes are at risk of developing the female athlete triad (Triad), the interrelationship of decreased energy availability, menstrual dysfunction, and poor bone health.(42) Low energy availability has independent negative effects on reproductive function (19) and bone, and low levels of gonadal steroids are also detrimental to bone. (17) Furthermore, low energy availability has negative effects on other metabolic hormones known to influence bone, including IGF-1, leptin and peptide YY. (19) A recent, prospective, multisite study demonstrated a higher incidence of bone stress injuries in athletes with specific Triad risk factors.(4) Of importance, the Triad may be particularly detrimental during adolescence, a time characterized by maximal increases in bone accrual towards attainment of peak bone mass.(26, 50, 52) Few studies have examined determinants of stress and other fractures in adolescent athletes, and particularly in those who are oligoamenorrheic.

Athletes in general are more prone to injuries including fractures. Stress fractures are fatigue fractures of bone caused by repeated submaximal stress and can delay return to sport by weeks to months.(24) These fractures are common in endurance athletes and often involve the foot, tibia, and fibula in long distance runners, track and field athletes, and dancers.(10) Stress fractures are reported in up to 10% of female athletes and 22% of female track and field athletes.(7, 15) Weight-bearing activity stimulates bone modeling and remodeling during childhood and adolescence and increases bone mineral density (BMD), (34, 55) which is also determined by genetics, body habitus, nutritional status, hormonal milieu, medications, and lifestyle choices (28, 45, 47). Although BMD is an important determinant of the ability of bone to withstand loading, (39) it does not always correlate with fracture risk in athletes.(38, 46) Given the debility associated with fractures, it is important to have a better understanding of factors that contribute to the risk for stress and other fractures in athletes.

Dual energy x-ray absorptiometry (DXA) is the clinical gold standard used to measure BMD. However, DXA assesses areal and not volumetric BMD, and thus underestimates BMD in short individuals while overestimating BMD in tall individuals. In addition, it cannot distinguish between cortical and trabecular bone.(36) In contrast, high-resolution peripheral quantitative computed tomography (HR-pQCT) provides measures of volumetric BMD (vBMD) of cortical and trabecular bone, and of bone microarchitecture.(5) We have previously reported characteristic differences in vBMD, bone microarchitecture and strength estimates at the distal radius and tibia (sites of non-weight bearing and weight bearing bone respectively) using HRpQCT and microfinite element analysis ( $\mu$ FEA) in oligoamenorrheic and eumenorrheic weight-bearing endurance athletes and non-athletes.(1, 2) Our data overall suggest that while repetitive weight-bearing activity improves microarchitecture and

strength of the tibia in adolescent athletes with a normal hormonal milieu, this effect is lost in those with menstrual dysfunction. Of note, studies thus far have not examined associations of bone structure and strength estimates with fracture history in adolescent amenorrheic athletes.

The purpose of this study was to examine fracture prevalence in adolescent and young adult athletes and non-athletes in relation to menstrual status, and bone density, structure and strength estimates. We hypothesized that in addition to menstrual dysfunction and lower measures of areal BMD (aBMD), impaired microarchitectural parameters (using HRpQCT) and reduced strength estimates (using  $\mu$ FEA) would predict risk for fracture (particularly stress fracture) in adolescent and young adult athletes.

## Subjects and Methods

### Subjects

We cross-sectionally studied 175 females between the ages of 14 and 25 years: 100 oligoamenorrheic athletes (AA), 35 eumenorrheic athletes (EA), and 40 non-athletes. Enrolled athletes ran at least 20 miles every week or were engaged in weight-bearing aerobic activity for at least 4 hours/week for at least 6 months preceding the study. Cyclists, swimmers, rowers and gymnasts were excluded because of variable weight-bearing potentially confounding BMD and microarchitecture results. Non-athlete participants were not engaged in any organized sports and exercised for less than 2 hours/week. All athletes and non-athletes had a BMI between the 10th and 90th percentiles. We defined oligoamenorrhea (for AAs) as the absence of menses for at least 3 months within a period of oligomenorrhea (cycle length >6 weeks) for at least 6 months preceding enrollment, or absence of menarche at 15 years or older. We defined eumenorrhea (for EA and non-athletes) as at least 9 menses (cycle length 21–35 days) in the preceding year with no oral contraceptive (OCP) use in the preceding 3 months. Subjects were recruited through advertisements in the Partners HealthCare system, medical clinics, local newspapers, and colleges. Exclusion criteria included conditions other than exercise-induced amenorrhea and use of medications other than calcium and vitamin D supplements that may affect bone metabolism, and other causes of amenorrhea such as premature ovarian failure, hyperprolactinemia, thyroid dysfunction, and hyperandrogenism, which were ruled out with a history, physical examination, and screening laboratory tests.

The Institutional Review Board of Partners HealthCare approved the study. Informed consent was obtained from subjects  $\geq$  18 years and parents of subjects <18 years old. Informed assent was obtained from subjects <18 years. DXA, HRpQCT, and FEA results from a subset of this population were previously published without reference to fracture histories.(1, 2)

### Experimental Protocol

Subjects were studied at the Clinical Research Center of our institution. Anthropometric measurements were obtained on the same electronic scale (to the nearest 0.1 kg) and wall-mounted stadiometer (to the nearest 0.1 cm). A study physician recorded lifetime fracture

and menstrual history, as well as details regarding exercise/athletic activity for the preceding 12 months during participant interviews. Tanner staging was determined by a study endocrinologist. Hand radiographs were obtained to determine bone age by the standards of Greulich and Pyle.(27) We used a chemiluminescent immunoassay to measure fasting 25-hydroxyvitamin D [25(OH)D] (sensitivity, 4 ng/ml; intraassay coefficient of variation, 2.9%–5.5%; DiaSorin, Stillwater, Minnesota). Calcium levels were assessed by Labcorp using standard methods. Resting energy expenditure (REE) values were obtained from measures of carbon dioxide production and oxygen consumption during rest using indirect calorimetry.

### **Bone Density Assessment**

DXA (Hologic QDR-Discovery A, Apex software version 13.3; Hologic Inc, Waltham, Massachusetts) was used to assess total hip, femoral neck, spine and whole body BMD and body composition. The coefficients of variation for BMD, fat mass, and lean mass for our institution are 0.8% to 1.1%, 2.1%, and 1.0%, respectively. The same scanner and software version were used for all participants.

### **Bone Microarchitecture Measurement and Finite Element Analysis**

HRpQCT was used to measure volumetric density, morphology, and microarchitecture at the ultradistal radius and tibia (XtremeCT; Scanco Medical AG, Bassersdorf, Switzerland) with an isotropic voxel size of 82  $\mu\text{m}^3$ .(8) Measurements were performed at the non-dominant wrist and leg unless there was a history of fracture at those sites, in which case the non-fracture side was measured. Outcome variables computed by automated analysis included area ( $\text{mm}^2$ ) and volumetric bone mineral density (vBMD) ( $\text{mgHA}/\text{cm}^3$ ) for total, trabecular, and cortical regions; cortical thickness (21) and perimeter; and trabecular number (1/mm), thickness (21), and spacing (21). The precision is 0.7–1.5% for densities and 2.5–4.4% for trabecular and cortical microarchitecture.

In addition to the standard evaluation protocol provided by the HRpQCT manufacturer, we also performed detailed cortical bone analysis by a semi-automated segmentation technique as previously described.(2, 11–13, 43). We used the 3D HRpQCT images to perform linear  $\mu\text{FEA}$  and calculate apparent biomechanical properties under uniaxial compression, as previously described, specifically stiffness and failure load.(2, 9, 13, 32, 37, 53) Micro FEA-derived estimates of failure load using these methods are strongly correlated ( $r^2 = 0.75$ ) with experimentally measured failure loads that produce Colles' fractures in human cadaveric radii.(44) We also calculated the proportion of load carried by the cortical and trabecular compartments (%) at the distal and proximal ends of the region of interest. All HR-pQCT data were acquired on a single instrument by one operator, who performed standard evaluations (periosteal contouring). All finite element analyses (endosteal contouring) were also performed by one study investigator blinded to study groups.

### **Statistical Analysis**

We used JMP (version 10; SAS Institute, Inc., Cary, NC) for all analyses and report data as means  $\pm$ SD. For three-group comparisons, we performed an overall ANOVA for normally distributed data, followed by the Dunnett's analysis to assess differences between AA vs.

EA and AA vs. non-athletes. For two-group comparisons, we used the Student t-test for normally distributed data. For non-normally distributed variables we used the Kruskal-Wallis or Wilcoxon tests. The Fisher's exact test was used to analyze differences among groups for categorical variables, and the Bonferroni correction was used to adjust for multiple comparisons as and when necessary. Fracture incidence rates were calculated by dividing the number of AA, EA, or non-athlete controls with at least one fracture after age 12.5 years by person-years of observation time; 12.5 years was chosen because it is the average age of menarche in U.S. girls.(3). For stress fractures analysis, as having more than one stress fracture often becomes concerning clinically, raising questions about Triad risk factors(19), we divided the AA group into those who had <2 stress fractures versus those who had had 2 or more stress fractures and compared these subgroups. Multivariate analysis was used to determine whether differences in bone density and structural parameters persisted after controlling for menarchal age.

## Results

### Clinical Characteristics

Most subjects classified themselves as Caucasian (n= 134), followed by Asian American (n= 18), more than one race (n= 15), African American (n= 6), and Native American (n= 1). The race distribution did not differ across groups. Sixty-three percent of the athletes were runners, 21% participated in weight-bearing team sports (such as basketball, soccer, lacrosse, hockey and tennis), 6% were dancers, and 10% were involved in a variety of weight-bearing activities, including cardio machine training. The distribution of the different varieties of weight-bearing activities did not differ across groups. Age, bone age, Tanner stage, and height did not differ among AA, EA and non-athlete groups. Age of menarche was greater, and BMI, percent ideal body weight, and fat mass lower in AA than the other two groups. Lean mass was lower in AA versus EA, and body fat percentage was lower in AA versus non-athletes. Resting energy expenditure was lower in AA versus EA. Vitamin D levels were higher in AA compared to the other two groups. Twenty-six percent of AA, 5.7% of EA and none of the non-athletes had a history of disordered eating behavior. Average hours of exercise per week, and the percentage of athletes whose main exercise activity was running did not differ between AA and EA (Table 1).

### Bone Density and HRpQCT findings

Results for DXA and HRpQCT are shown in Table 2. While EA had significantly greater femoral neck, total hip, lumbar spine, and total body BMD Z-scores than AA, AA did not demonstrate a similar benefit from exercise, as they did not significantly differ from non-athletes for BMD at any measured site. Differences in BMD among groups persisted after controlling for menarchal age, a factor known to impact pubertal bone accrual.

HRpQCT measurements at the **radius** showed lower % cortical area and cortical thickness, greater cortical porosity, and lower total vBMD in AA than non-athletes. Percent cortical porosity trended higher in AA versus EA. Micro-FEA analysis demonstrated lower stiffness and failure load at the radius in AA versus EA. At the **tibia**, total and trabecular cross-sectional area were greater in the AA versus non-athletes, suggesting greater moment of

inertia at weight bearing bone. However, cortical porosity was higher and cortical vBMD lower in the AA compared with non-athletes. Stiffness and failure load trended lower in AA than EA, but were higher in AA than non-athletes. Percent load carried by trabecular bone at the most proximal and the most distal tibial slices was greater in AA versus non-athletes. Unlike areal bone density, some differences in bone structure and strength parameters were no longer evident after controlling for menarchal age using multivariate analysis.

### Fracture Comparisons across Groups

A larger proportion of AA than EA and non-athletes reported a history of fracture (stress and non-stress) (47 % vs. 25.7 % vs. 12.5 %) (Table 3). This was driven mostly by stress fractures, as 32% of AA, 5.9% of EA, and none of the controls had ever had stress fractures in their lifetime. The majority of stress fractures occurred after the average age of menarche in U.S. girls, i.e. 12.5 years, when amenorrhea would be expected to exert a significantly negative impact on bone metabolism (Table 3). The incidence rate (cases per 10,000 person-years) for all types of fractures after age 12.5 years was calculated in AA (558.2, 95% CI: 398.8 – 760) and EA (312.4, 95% CI: 125.6 – 643.6), but there was no significant difference in the rates between the two groups ( $p=0.15$ ). The incidence rate of stress fractures after age 12.5 years was also calculated, yielding a significant different incidence in AA (432.6, 95% CI: 293.9 – 614) versus EA (89.3, 95% CI 10.8 – 322.4), ( $p=0.017$ ). No non-athlete sustained fractures after 12.5 years of age. Because many subjects experienced more than one lifetime fracture, Figure 1 shows the percentage of AA, EA, and non-athletes who experienced only stress fractures, only non-stress fractures, or both at any time of their lives. Of note, differences among groups for fractures persisted after excluding patients with a history of eating disorders. The AA group had the largest number of subjects with a history of disordered eating behavior. After excluding subjects with eating disorders, the proportion of AAs with any fracture, stress fractures, non-stress fractures, stress fractures after 12.5 years and non-stress fractures after 12.5 years was 50.0%, 35.1%, 18.9%, 33.8% and 10.8% respectively compared with 47.0%, 32.0%, 20.0%, 31.0% and 10.0% when subjects with eating disorders were included. Only two eumenorrheic athletes and no non-athlete had a history of disordered eating.

Figure 2a illustrates the proportion of subjects in each the three groups who sustained one or more fractures at any particular age. Whereas the non-athletes only experienced fractures between the ages of 7 and 12 in this cohort, the two athlete groups continued to experience fractures during adolescence, when they were presumably more active than the non-athletes. In addition, fractures continued to occur in AA (but not EA) with further increases in age. A similar, but even more striking pattern was observed when examining the proportion of subjects with stress fractures in the three groups according to age (Figure 2b). None of the non-athletes experienced stress fractures, and a greater proportion of AA than EA had stress fractures at nearly every age. Table 3 shows the location and type of fracture incurred by subjects. Stress fractures of the lower extremity were more common in AA versus the other two groups.

We next examined the individual groups (AA, EA, and non-athletes) to determine whether there were differences in the clinical characteristics of those who had a history of fracture

versus those who did not (data not shown). There were no significant differences in fat mass, percent body fat, lean mass, average hours of exercise, or types of exercise between those who had fractured in the AA group versus those who had not. When examining the EA subgroup, we found that those who had fractured were older ( $20.3 \pm 2.6$  years versus  $18.4 \pm 2.3$  years,  $p=0.045$ ), and had higher fat mass ( $18.5 \pm 3.9$  versus  $14.4 \pm 3.5$  kg,  $p=0.006$ ) and percent body fat ( $26.7 \pm 3.4$  versus  $23.5 \pm 3.9$ ,  $p=0.03$ ) compared to those who had never fractured. Similarly, among non-athletes, those with a history of fracture were older ( $21.9 \pm 2.2$  versus  $19.5 \pm 2.0$  years,  $p=0.01$ ), had higher BMIs ( $24.4 \pm 2.6$  versus  $21.8 \pm 2.0$  kg/m<sup>2</sup>,  $p=0.01$ ), fat mass ( $22.9 \pm 6.4$  versus  $16.2 \pm 4.2$  kg,  $p=0.003$ ) and percent body fat ( $36.4 \pm 5.4$  versus  $27.4 \pm 5.0\%$ ,  $p<0.001$ ).

### Bone Parameters in Fracture versus Non-fracture Subjects

Table 4 shows pertinent DXA and HRpQCT results for AA based on fracture history. Whole body and spine BMD Z-scores were lower in those AA who had fractured versus those who had not. Volumetric BMD of the outer portion of the trabecular region was lower at both the radius and tibia in AA with a history of fracture versus those without a history of fracture. At the radius, trabecular thickness was lower and trabecular von Mises stress (the amount of stress the trabecular compartment can withstand before permanently deforming) trended lower in the fracture versus non-fracture groups. No differences were noted in tibial microarchitecture in these AA subgroups.

When comparing BMD and HRpQCT data of EA who had fractured versus those who had not, EA with fractures had lower trabecular number ( $1.8 \pm 0.29$  versus  $2.03 \pm 0.25$ /mm,  $p=0.04$ ), greater trabecular spacing ( $0.49 \pm 0.11$  versus  $0.43 \pm 0.06$  mm,  $p=0.03$ ), with lesser percent load carried by trabecular bone at the most distal slice of the radius ( $0.51 \pm 0.10$  versus  $0.58 \pm 0.08$  %,  $p=0.04$ ). There were no differences found at the tibia in EA who had fractured versus those who had not (data not shown). In non-athletes who had fractured versus those who had not, no differences in BMD or HRpQCT data at the radius or tibia were found except that those with a history of fracture had lower percent load carried by trabecular bone at the most distal slice of the tibia ( $0.48 \pm 0.05$  versus  $0.55 \pm 0.07$  %,  $p=0.02$ ) as well as the most proximal slice of the tibia ( $0.29 \pm 0.05$  versus  $0.34 \pm 0.06$  %,  $p=0.049$ ) (data not shown).

Finally, we divided the AA group into those who had <2 stress fractures versus those who had had  $\geq 2$  stress fractures, as having more than one stress fracture often becomes concerning clinically and raises questions about Triad risk factors.<sup>(19)</sup> Clinical characteristics were similar in both groups, except that those with  $\geq 2$  stress fractures had less fat mass ( $10.6 \pm 3.1$  versus  $13.8 \pm 4.8$  kg,  $p=0.01$ ) and lower percent body fat ( $19.5 \pm 4.9$  versus  $23.5 \pm 5.7$  %,  $p=0.009$ ).

Table 4 shows DXA and microarchitecture comparisons in AA with <2 stress fracture versus AA with  $\geq 2$  stress fractures. The group with  $\geq 2$  stress fractures had significantly lower lumbar spine BMD Z-scores and their whole body BMD Z-scores trended lower than those with fewer stress fractures. At the **radius**, total cross-sectional area, trabecular vBMD and vBMD of the outer portion of the trabecular region were lower in the group with  $\geq 2$  stress fractures, and inner trabecular vBMD trended lower. In addition, stiffness and failure load

were lower in AA with  $\geq 2$  stress fractures. Similarly, at the **tibia**, stiffness and failure load were lower in those with  $\geq 2$  stress fractures versus those with fewer fractures.

## Discussion

This is the first study to examine bone microarchitecture and bone strength estimates in female adolescent and young adult athletes according to menstrual and fracture history.

### Age and Fractures

The incidence of fractures, especially at the radius, peaks during early adolescence (18, 33, 35) from a dissociation between peak statural bone growth and peak mineralization, as well as increased cortical porosity.(32, 54) In our study, a larger proportion of adolescent and young adult AA had fractures compared to EA and non-athletes, and this difference was mostly driven by a higher prevalence of stress fractures in AA. We also found that AA experienced fractures later in adolescence compared to EA and non-athletes, with a later peak than reported in healthy children (early adolescence).(18)

### Menstrual Status and Fractures

Few studies have evaluated associations between menstrual dysfunction and stress and non-stress fractures in athletes, and findings are not consistent. In a study of 18–26 year old female distance runners, Kelsey *et al.* reported a non-significant increased risk for stress fractures in those with irregular periods, (31) while Barrack *et al.* showed that an accumulation of Triad risk factors, but not oligoamenorrhea alone, increased the odds of developing a stress injury in young athletes.(4) In contrast, Nattiv, *et al.* did report greater severity of stress fracture (by MRI staging) in collegiate athletes with oligo-amenorrhea versus eumenorrhea.(41) Menstrual irregularity was noted in 75% of female athletes with stress injuries at predominantly trabecular bone sites, compared to only 12.5% of those with stress injuries at cortical sites. However, the study did not report comparisons of menstrual status in those who did or did not sustain stress injuries.(41) Our results of increased prevalence and incidence of stress fracture, particularly of the lower extremity, in AA versus EA and non-athletes are consistent with findings in other retrospective studies of female athletes, although these did not assess fracture risk in non-athletes.(6, 14, 21, 40) These studies also reported menstrual status in athletes with and without a history of fracture, rather than the other way around.(6, 14, 21, 40)

### Area Bone Mineral Density and Fractures

Similar to menstrual status, data for associations of areal BMD with fractures are not consistent. Duckham *et al.*, and others found no differences in areal BMD in those with or without stress fractures, (6, 14, 21) although another study did report a greater likelihood of oligoameneorrhea and lower areal BMD at the spine and femoral neck in athletes with fracture versus those without fracture.(40) In our study, within EA and non-athlete groups, there were no differences in BMD Z-scores in those with or without fractures. However, among AA, lumbar and whole body (but not total hip or femoral neck) BMD Z-scores were lower in those with a history of fracture, and in those with  $\geq 2$  stress fractures versus those with  $<2$  stress fractures. The lack of association of hip BMD Z-scores with fracture may



relate to weight bearing-activity partially counteracting the negative effects of a hormonally depleted state at the weight-bearing and predominantly cortical bone at the hip.

### **Bone Microarchitecture and Strength Estimates and Fractures**

Our findings of altered bone structure and reduced strength estimates in AA are similar to our previous reports in a subpopulation of these subjects (22), as well as in anorexia nervosa and postmenopausal women.(1, 2, 22, 23). Overall, at the non-weight bearing radius, AA had greatest cortical porosity, and lowest cortical area and thickness, total volumetric BMD, stiffness and failure load. The decreased proportion of cortical bone in AA may be from enhanced endosteal resorption in the hypo-estrogenic state, as in menopause, when trabecularization of cortical bone at the endosteal border results in increased porosity.(23, 56) Our findings of negative effects of the amenorrheic state on mostly cortical but not trabecular bone (for the radius), are consistent with studies in the Kronos Early Estrogen Prevention Study in post-menopausal women, in which estrogen replacement had beneficial effects on cortical, but not trabecular microarchitecture at the radius. (23) Of interest, menarchal age was greater in AA than in EA, and after controlling for menarchal age, many differences across groups were no longer evident, particularly at the non-weight-bearing radius. This emphasizes the importance of normal menarchal timing in optimizing bone accrual. It is possible that other hormonal abnormalities associated with low energy availability and amenorrhea in athletes, such as low IGF-1 or higher cortisol levels (20), and reduced bone turnover as previously reported in AA (16), also contribute to differences in bone structural parameters (and bone density) across groups.

At the weight-bearing tibia, AA had greater total and trabecular area and cortical porosity, and lower cortical density than non-athletes. Stiffness and failure load trended lower than in EA, but were higher than in non-athletes. Greater cross-sectional area in athletes is likely from increased weight bearing activity, consistent with other studies in athletes involved in high and moderate impact sports.(48) This would lead to greater moment of inertia and resistance to bending, and lower strain for a given force; (25) and would explain the higher strength estimates in AA versus non-athletes. Increased cortical porosity in AA is likely from delayed mineralization of the expanding tibia, compounded by estrogen deficiency.

We examined microarchitecture and estimated strength differences in those with or without a history of fracture within each group. There were no microarchitecture differences between fracture and non-fracture subgroups of EA and non-athletes, suggesting that factors other than bone quality were at play. These may have included the degree of mechanical trauma, training volume, and biomechanics of gait. However, AA who fractured had lower vBMD in the outer trabecular region (meta VBMD) at both the radius and tibia. This may be from lower estrogen levels in AA leading to increased endosteal bone resorption and therefore lower density of the outer trabecular region. Trabecular thickness was lower at the radius (but not tibia) in AA who fractured, and it is possible that weight-bearing effects on the tibia are protective. One study examined quadrant specific tibial bone microarchitecture using HRpQCT in 19 athletes ages 18–45 with lower limb stress fractures and 19 controls not differentiated by menstrual status, (49) and found lower distal tibial trabecular vBMD and lower tibial cortical area in those with stress fractures, particularly in the posterior and

lateral cortical regions. (49) We may have found more tibial differences had we separated the analyses according to region.

Finally, when we specifically compared those AA who had sustained <2 versus ≥ 2 stress fractures, we found more pronounced differences in bone quality and strength across groups. At the radius, total cross-sectional area, total trabecular vBMD, and vBMD at both the inner and outer portions of the trabecular region were lower in the group with more fractures. This is similar to findings in postmenopausal women with a history of fragility fractures, who also had decreased vBMD in the inner and outer trabecular regions at the radius and tibia, with more pronounced changes at the radius.(51) In our study, AA with ≥ 2 stress fractures had lower stiffness and failure load at both the radius and the weight-bearing tibia, suggesting that those who do fracture do not demonstrate the beneficial effects of weight-bearing at the tibia.

### Strengths and Limitations

Strengths of this study include its large sample of oligoamenorrheic athletes, thorough menstrual and training history, and BMD as well as microarchitectural assessments in groups. Limitations include its cross-sectional design, and retrospective self-report of fractures, training, and menstrual status. However, previously published work has demonstrated that self-report of fracture history (occurrence and timing) is sensitive and specific, particularly for distal forearm fractures.(29, 30)

### Conclusions

Oligo-amenorrheic adolescent and young adult athletes lack much of the bone health benefits of weight-bearing exercise, such as enhancement of overall BMD and improved stiffness and failure load at weight-bearing sites. This makes them more susceptible to stress fractures than eumenorrheic athletes and non-athletes despite higher vitamin D and calcium levels. Bone microarchitectural and strength differences are more pronounced in those amenorrheic athletes who experienced multiple stress fractures, suggesting either a dose-response of amenorrhea on bone microarchitecture and strength, or individual differences in bone susceptibility to amenorrhea, leading to more bone injuries. Further work is needed to better characterize the differences in bone microarchitecture in a variety of oligoamenorrheic athletes. For sports clinicians, this study also suggests a high level of suspicion of low energy availability and menstrual dysfunction in female athletes who present with stress injuries.

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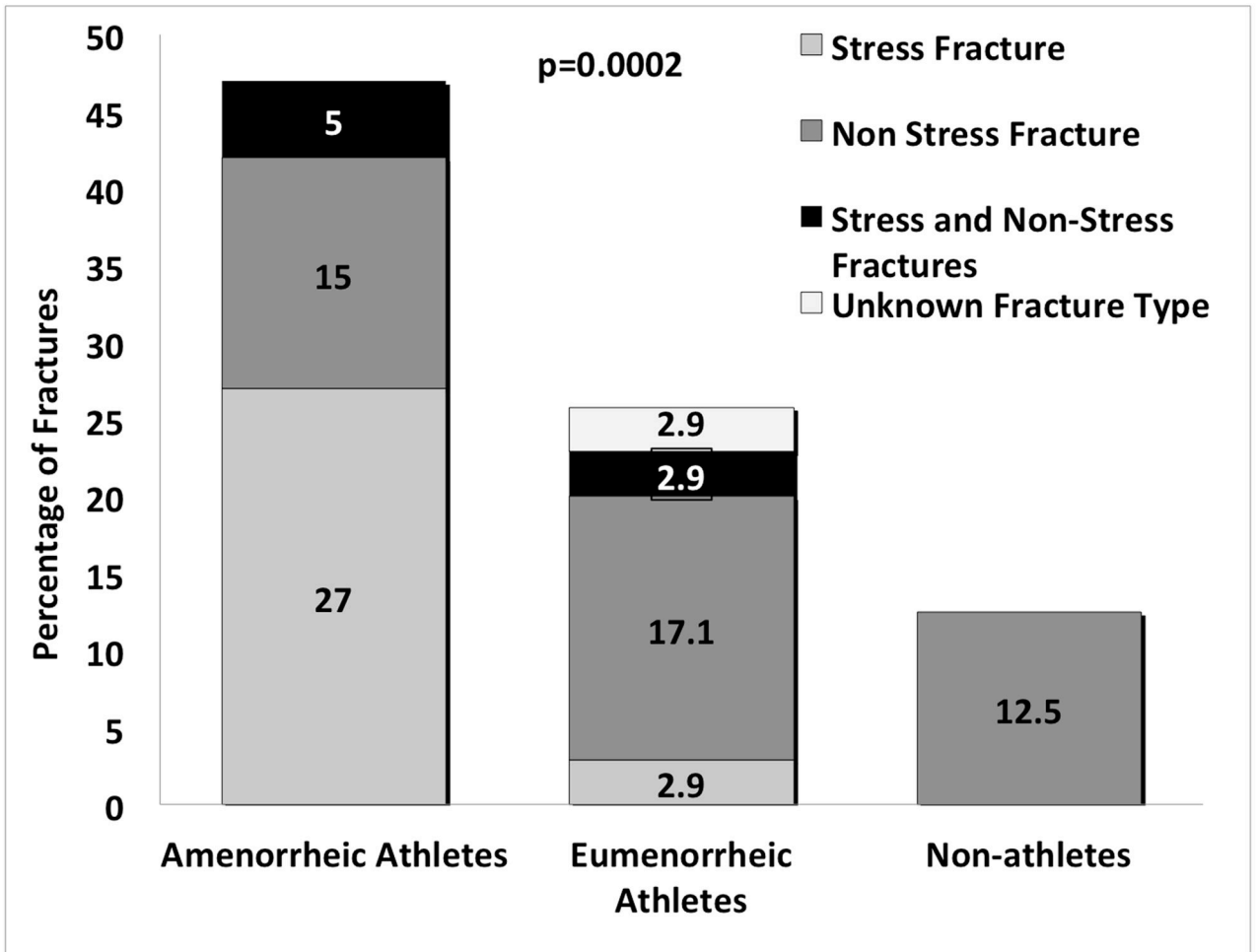
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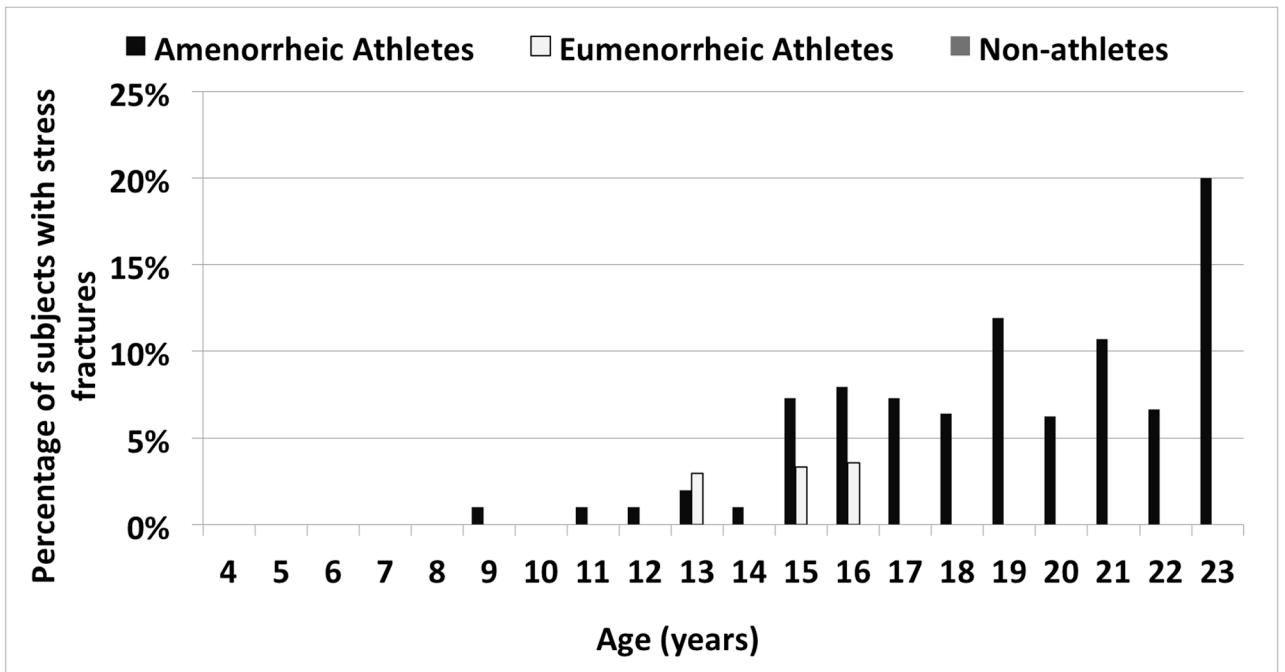
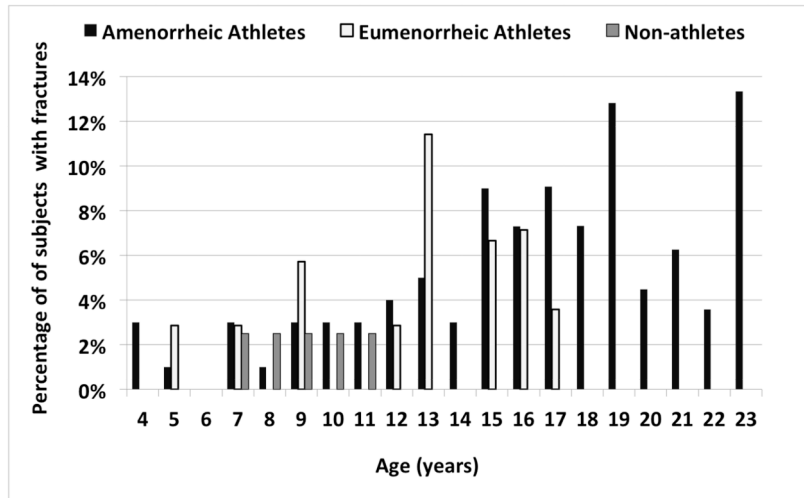
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**Figure 1.** Percentage of Fractures in Amenorrheic Athletes (AA), Eumenorrheic Athletes (EA) and Non-athletes (NA)



**Figure 2.** Proportion of Amenorrheic Athletes (AA), Eumenorrheic Athletes (EA) and Non-athletes (NA) who Fractured Each Year Between 0 and 25 Years  
 Figure 2a. All Types of Fractures  
 Figure 2b. Stress Fractures



Clinical Characteristics of Amenorrhoeic Athletes (AA), Eumenorrhoeic Athletes (EA) and Non-athletes (NA)

Table 1

	AA (n=100)	EA (n=35)	NA (n=40)	ANOVA		AA vs EA		AA vs NA: P	
				P	P	P	P		
Age (years)	19.7±2.5	18.9±2.5	19.8±2.1	0.22	-	-	-	-	
Bone age (years)	17.5±1.1	17.4±1.1	17.6±1.0	0.52	-	-	-	-	
Age of menarche (years)	13.8±1.9	12.5±1.5	12.4±1.2	<0.0001	0.0004	<0.0001	<0.0001	<0.0001	
Duration since last menses (months)	8.9±12.7	-	-	-	-	-	-	-	
Tanner Stage	4.7±0.6	4.8±0.5	4.9±0.4	0.17	-	-	-	-	
Height (cm)	165.0±6.2	164.7±7.2	162.3±6.6	0.07	-	-	-	-	
BMI (kg/m <sup>2</sup> )	20.4±2.3	22.6±2.3	22.1±2.3	<0.0001	<0.0001	<0.0001	0.0003	0.0003	
% Ideal BMI	95.9±10.3	107.5±12.6	103.4±11.6	<0.0001	<0.0001	<0.0001	0.001	0.001	
Fat mass (kg)	13.3±4.7	15.4±4.0	17.0±5.0	<0.0001	0.04	0.04	<0.0001	<0.0001	
Percent body fat	22.9±5.7	24.3±4.0	28.6±5.8	<0.0001	0.33	0.33	<0.0001	<0.0001	
Lean mass (kg)	41.8±5.2	45.3±6.4	40.0±4.3	<0.0001	0.002	0.002	0.13	0.13	
REE (calories)	1216±173	1363±216	1223±189	0.0006	0.0003	0.0003	0.98	0.98	
Vitamin D (ng/mL)	38.4±13.5	30.3±13.1	25.1±13.2	<0.0001	0.006	0.006	<0.0001	<0.0001	
Calcium (mg/dL)	9.3±0.4	9.1±0.7	9.1±0.5	0.04	0.15	0.15	0.04	0.04	
Hours/week of exercise	10.5±5.8	10.0±4.2	1.7±2.5	<0.0001	0.84	0.84	<0.0001	<0.0001	
Type of exercise (%)									
Running	66.0	57.1	0		0.27	0.27	-	-	
Other	34.0	42.9	0				-	-	
History of eating disorders (%)	26.0	5.7	0	0.0001	0.13	0.13	0.01	0.01	

AA: oligoamenorrhoeic athletes, EA: eumenorrhoeic athletes, NA: non-athletes Data presented as means ± standard deviations or as percentage where noted.

ANOVA used for 3 group comparisons followed by Dunnett's testing when ANOVA was significant with AA as the comparison group. Significant p values are in bold.

REE: resting energy expenditure

**Table 2**  
 Areal Bone Density (DXA), HRpQCT and Finite Element Analyses for the Radius and Tibia in Amenorrhoeic Athletes (AA), Eumenorrhoeic Athletes (EA) and Non-athletes (NA)

	AA	EA	NA	ANOVA p-value	AA vs EA p-value	AA vs NA p-value
<b>DXA (Areal BMD Z-scores)</b>	N = 100	N = 35	N = 40			
Femoral Neck	-0.17±1.06	0.38±0.93	-0.41±0.83	<b>0.003<sup>a</sup></b>	<b>0.01<sup>a</sup></b>	0.35
Total Hip	0.05±1.01	0.80±0.87	-0.06±0.78	< <b>0.0001<sup>a</sup></b>	<b>0.0001<sup>a</sup></b>	0.79
Lumbar Spine	-0.77±1.21	0.00±0.88	-0.40±0.93	<b>0.002<sup>a</sup></b>	<b>0.001<sup>a</sup></b>	0.15
Whole Body	-0.64±1.02	0.19±1.05	-0.71±0.96	<b>0.0001<sup>a</sup></b>	<b>0.0001<sup>a</sup></b>	0.92
<b>HRpQCT and FEA: Radius</b>	N=87	N=34	N=38			
Total area (mm <sup>2</sup> )	263.7±45.0	272.2±42.1	256.7±40.7	0.32	-	-
% Ct. area	18.4±5.9	19.3±5.1	21.7±6.6	<b>0.02</b>	0.69	<b>0.008</b>
Ct. thickness (mm)	0.70±0.20	0.75±0.16	0.83±0.25	<b>0.008</b>	0.41	<b>0.004</b>
Ct. porosity (%)	0.012±0.008	0.008±0.004	0.008±0.005	<b>0.006<sup>a</sup></b>	<b>0.05</b>	<b>0.007<sup>a</sup></b>
Ct. vBMD (mg HA/cm <sup>3</sup> )	816.3±67.5	824.6±54.6	845.2±72.8	0.09	-	-
Tb. vBMD (mg HA/cm <sup>3</sup> )	165.4±31.6	177.0±37.0	174.4±35.8	0.16	-	-
Outer Tb. vBMD (mg HA/cm <sup>3</sup> )	223.3±30.7	234.1±35.4	231.7±35.2	0.19	-	-
Inner Tb. vBMD (mg HA/cm <sup>3</sup> )	125.3±32.9	137.4±38.9	134.8±37.2	0.16	-	-
Total vBMD (mg HA/cm <sup>3</sup> )	299.7±56.5	314.2±51.5	333.4±63.6	<b>0.01</b>	0.37	<b>0.006</b>
Stiffness (kN/m)	72.5±14.0	79.5±12.3	77.9±14.3	<b>0.02</b>	<b>0.03</b>	0.09
Failure load (kN)	3.7±0.7	4.0±0.6	4.0±0.7	<b>0.02</b>	<b>0.03</b>	0.09
(Tb.F/TF) distal (%) <sup>*</sup>	53.4±8.1	55.7±9.0	50.2±9.4	<b>0.03</b>	0.33	0.12
(Tb.F/TF) proximal (%) <sup>*</sup>	20.8±7.3	22.5±7.4	20.3±7.3	0.43	-	-
<b>HRpQCT and FEA: Tibia</b>	N=87	N=34	N=38			
Total area (mm <sup>2</sup> )	669.8±102.8	698.7±91.5	615.8±99.0	<b>0.002<sup>a</sup></b>	0.33 <sup>a</sup>	<b>0.01</b>
Tb. area (mm <sup>2</sup> )	547.6±106.2	568.6±94.4	494.3±101.0	<b>0.006</b>	0.57	<b>0.02</b>
% Ct. area	18.69±4.78	18.94±4.10	20.05±4.97	0.33	-	-
Ct. thickness (mm)	1.22±0.25	1.27±0.23	1.25±0.24	0.52	-	-

	AA	EA	NA	ANOVA p-value	AA vs EA p-value	AA vs NA p-value
Ct. porosity (%)	0.019±0.011	0.017±0.009	0.014±0.010	<b>0.03<sup>a</sup></b>	0.61	<b>0.01<sup>a</sup></b>
Ct. vBMD (mg HA/cm <sup>3</sup> )	867.4±37.0	874.4±36.2	893.0±40.51	<b>0.003</b>	0.63	<b>0.001</b>
Tb. vBMD (mg HA/cm <sup>3</sup> )	203.1±28.4	208.4±34.6	192.5±33.2	0.08	-	-
Outer Tb. vBMD (mg HA/cm <sup>3</sup> )	266.7±31.1	273.0±36.1	255.1±36.2	0.07	-	-
Inner Tb. vBMD (mg HA/cm <sup>3</sup> )	159.9±28.3	164.5±34.7	149.9±32.9	0.11	-	-
Total vBMD (mg HA/cm <sup>3</sup> )	328.1±46.9	334.8±52.3	335.1±58.2	0.69	-	-
Stiffness (kN/m)	227.9±30.5	242.1±36.8	211.1±33.7	<b>0.0005<sup>a</sup></b>	0.07	<b>0.02<sup>a</sup></b>
(Tb.F/TF) distal (%) <sup>*</sup>	59.6±7.0	59.6±5.8	53.7±6.7	<b>&lt;0.0001<sup>a</sup></b>	<b>1.00</b>	<b>&lt;0.0001<sup>a</sup></b>
(Tb.F/TF) proximal (%) <sup>*</sup>	38.7±6.9	39.0±6.1	33.5±6.2	<b>0.0002<sup>a</sup></b>	0.96	<b>0.0002<sup>a</sup></b>
Tb VM (N/mm <sup>2</sup> ) <sup>**</sup>	63.7±4.6	63.1±5.3	60.9±6.3	<b>0.03<sup>a</sup></b>	0.84	<b>0.02<sup>a</sup></b>

AA: oligomenorrheic athletes, EA: eumenorrheic athletes, NA: non-athletes Data presented as means ± standard deviations or as percentage where noted.

ANOVA used for 3 group comparisons followed by Dunnett's testing when ANOVA was significant with AA as the comparison group.

<sup>a</sup>P value < 0.05 after controlling for age of menarche

Ct: Cortical; Tb: Trabecular

\* (Tb.F/TF) distal or proximal; percent load carried by trabecular bone at most distal (or proximal) slice.

\*\* Tb VM: Trabecular von Mises stress (amount of stress the trabecular compartment can withstand before permanently deforming)

**Table 3**  
Percentage of Amenorrhoeic Athletes (AA), Eumenorrhoeic Athletes (EA) and Non-athletes (NA) who Experienced Stress and Non-stress Fractures and Fracture Location

	AA		EA		NA		Fisher's Exact Test		AA vs EA		AA vs NA	
	N=100	N=35	N=40	N=40	P	P	P	P	P	P		
<b>Fracture (%)</b>	47.0	25.7	12.5	<0.0001 <sup>a</sup>	0.04 <sup>a</sup>	0.0004 <sup>a</sup>						
<b>Stress fracture (%)</b>	32.0	5.9	0	0.01 <sup>a</sup>	0.004 <sup>a</sup>	<0.0001 <sup>a</sup>						
Lower Extremity (%)	27.0	5.9	0	<0.0001 <sup>a</sup>	0.02 <sup>a</sup>	<0.0001 <sup>a</sup>						
Upper Extremity (%)	4.0	0	0	0.50	-	-						
Non-extremity or spine (%)	3.0	0	0	0.58	-	-						
<b>Non-stress fracture (%)</b>	20.0	20.6	12.5	0.56	-	-						
Lower Extremity (%)	8.0	8.8	2.5	0.51	-	-						
Upper Extremity (%)	12.0	14.3	7.5	0.61	-	-						
Non-extremity or spine (%)	3.0	0	2.5	0.82	-	-						
<b>Stress fracture after 12.5 yrs (%)<sup>*</sup></b>	31.0	5.9	0	<0.0001 <sup>a</sup>	0.005 <sup>a</sup>	<0.0001 <sup>a</sup>						
<b>Non-stress fracture after 12.5 yrs (%)<sup>*</sup></b>	10.0	14.7	0	0.04 <sup>a</sup>	0.54	0.12						

AA: oligomenorrhoeic athletes, EA: eumenorrhoeic athletes, NA: non-athletes

Data presented as percentage of each group. Fisher's exact test was used for both 2 and 3 group comparisons.

<sup>\*</sup> Stress or non-stress fractures that occurred after the average age of menarche, 12.5 years.

<sup>a</sup> P value <0.05 after excluding subjects with eating disorders

**Table 4**

DXA Z-Scores and HRpQCT Data from the Radius and Tibia in Amenorrhoeic Athletes according to Fracture History [no fracture versus 1 fracture (stress and non-stress), and <2 stress fractures versus 2 stress fractures]

	No Fractures	1 Fractures	P	< 2 Stress Fractures	2 Stress Fractures	P
<b>DXA (BMD Z-scores)</b>	<b>n=53</b>	<b>n = 47</b>		<b>n=84</b>	<b>n=16</b>	
Femoral Neck	-0.01±1.06	-0.33±1.05	0.14	-0.09±1.07	-0.55±0.96	0.11
Total Hip	0.16±1.03	-0.09±0.97	0.21	0.12±1.02	-0.33±0.86	0.10
Lumbar Spine	-0.54±1.28	-1.02±1.08	<b>0.045</b>	-0.61±1.20	-1.58±0.87	<b>0.003</b>
Whole Body	-0.40±1.10	-0.90±0.87	<b>0.01</b>	-0.55±1.02	-1.09±0.94	<b>0.05</b>
<b>HRpQCT Radius</b>	<b>n =45</b>	<b>n =42</b>		<b>n=71</b>	<b>n=13</b>	
Total area (mm <sup>2</sup> )	263.3±45.7	264.1±44.8	0.94	267.9±45.8	240.7±32.9	<b>0.045</b>
% Ct. area	18.5±6.8	18.2±4.7	0.77	18.4±6.2	18.4±3.9	0.99
Ct. thickness (mm)	0.71±0.23	0.70±0.15	0.76	0.71±0.21	0.68±0.12	0.61
Ct. porosity (%)	1.3±0.9	1.1±0.7	0.29	1.2±0.9	0.8±0.5	0.07
Ct. vBMD (mg HA/cm <sup>3</sup> )	813.8±79.0	819.1±52.2	0.72	814.9±71.5	823.8±41.0	0.67
Tb. thickness (mm)	0.073±0.011	0.067±0.009	<b>0.03</b>	0.071±0.011	0.067±0.009	0.25
Tb. vBMD (mg HA/cm <sup>3</sup> )	170.8±4.7	159.1±27.9	0.09	168.5±32.2	148.1±21.2	<b>0.03</b>
Outer Tb. vBMD (mg HA/cm <sup>3</sup> )	230.0±31.9	215.7±27.8	<b>0.03</b>	226.8±31.1	204.5±21.0	<b>0.02</b>
Inner Tb. vBMD (mg HA/cm <sup>3</sup> )	129.8±35.8	120.0±28.8	0.18	128.3±33.8	109.0±21.9	<b>0.05</b>
Total vBMD (mg HA/cm <sup>3</sup> )	305.9±64.9	292.5±44.6	0.28	302.2±59.1	285.9±38.4	0.34
Stiffness (kN/m)	74.4±14.2	70.4±13.5	0.19	74.3±13.7	63.0±12.1	<b>0.007</b>
Failure load (kN)	3.79±0.70	3.58±0.69	0.17	3.78±0.68	3.18±0.60	<b>0.004*</b>
(Tb.F/TF) distal (%)*	54.3±7.2	52.3±8.9	0.26	54.0±8.1	49.6±7.2	0.07
(Tb.F/TF) proximal (%)*	21.4±7.1	20.1±7.6	0.44	21.3±7.6	18.0±5.0	0.13
TbVM (N/mm <sup>2</sup> )**	52.9±6.9	49.8±7.3	<b>0.049</b>	51.9±7.1	49.0±7.6	0.19
<b>HRpQCT Tibia</b>	<b>n =45</b>	<b>n = 42</b>		<b>n=73</b>	<b>n=14</b>	
Total area (mm <sup>2</sup> )	668.3±108.0	671.4±98.3	0.89	674.3±104.0	646.5±96.6	0.36
% Ct. area	19.2±5.2	18.2±4.3	0.32	18.9±4.9	17.8±3.9	0.46
Ct. thickness (mm)	1.25±0.28	1.19±0.22	0.27	1.24±0.26	1.15±0.19	0.23
Ct. porosity (%)	2.0±1.2	1.8±0.9	0.45	1.9±1.1	2.0±1.0	0.79

	No Fractures	1 Fractures	P	< 2 Stress Fractures	2 Stress Fractures	P
Ct. vBMD (mg HA/cm <sup>3</sup> )	865.8±43.8	869.1±28.6	0.68	867.4±4.4	867.3±28.1	1.00
Tb. vBMD (mg HA/cm <sup>3</sup> )	208.4±33.3	197.5±20.7	0.07	204.6±30.0	195.1±15.8	0.25
Outer Tb. vBMD (mg HA/cm <sup>3</sup> )	273.9±35.2	258.9±24.1	<b>0.02</b>	268.6±33.0	256.6±15.7	0.19
Inner Tb. vBMD (mg HA/cm <sup>3</sup> )	163.9±33.7	155.7±20.6	0.18	161.2±29.7	153.3±18.9	0.34
Total vBMD (mg HA/cm <sup>3</sup> )	335.1±53.6	320.5±37.7	0.15	330.5±49.0	315.4±32.4	0.27
Stiffness (kN/m)	230.9±31.3	224.7±29.6	0.35	230.7±30.3	213.8±28.0	<b>0.05*</b>
Failure load (kN)	11.5±1.5	11.2±1.5	0.35	11.5±1.5	10.7±1.4	<b>0.048*</b>
(Tb.F/TF) distal (%)**	59.6±7.3	59.5±6.7	0.96	59.5±7.1	60.1±6.2	0.75
(Tb.F/TF) proximal (%)**	38.6±7.3	38.8±6.7	0.89	38.6±7.1	39.9±6.5	0.88
TbVM (N/mm <sup>2</sup> )***	63.94±5.01	63.40±4.25	0.60	63.68±4.72	63.66±4.37	0.99

Data presented as means ± standard deviations.

The Student t-test was used for normally distributed 2 group comparisons. The Wilcoxon Rank Sum test was used for data not normally distributed (\*).

Ct.: cortical; Tb: trabecular

\*\* (Tb.F/TF) distal or proximal: percent load carried by trabecular bone at most distal (or proximal) slice.

\*\*\* Tb VM: Trabecular von Mises stress (amount of stress the trabecular compartment can withstand before permanently deforming)