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Age and sex differences in tibia morphology in healthy adult Caucasians

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Abstract

Variability in peripheral Quantitative Computed Tomography (pQCT) measurement sites limits direct comparisons of results between studies. Further, it is unclear what estimates of bone strength are most indicative of changes due to aging, disease, or interventions. The purpose of this study was to examine age group and sex differences in tibia morphology. Additional purposes of this study were to determine which tibia site or sites are most sensitive for detecting age and sex differences.

Methods—Self-identifying Caucasian men (n=55) and women (n=59) ages 20-59 years and separated by decades had their non-dominant tibias measured with pQCT (Stratec XCT 3000) at every 10% of the limb length from 5%-85% (distal to proximal). Volumetric BMD and BMC of the total, cortical and trabecular bone were determined, as well as periosteal (PeriC) and endosteal (EndoC) circumferences, and cortical thickness (CTh).

Results—There were significant ($p<0.01$) site effects for all BMC, vBMD, PeriC and EndoC measures. Large sex differences (men>women) in Tot.BMC (21-28%) were paralleled by differences in Cort.BMC (21-25%) ($p<0.01$). Site*sex interaction effects were significant ($p<0.05$) for BMC (peak sex difference: 5%, 15%, 25%, 85% sites) and circumference (peak sex difference: 65% site) variables. CTh and total vBMD were lowest ($p<0.05$) in 50-59 yr group, and EndoC was highest in the 50-59 yr group. Site*age interactions existed for Cort.vBMD, Tot.BMC (85% site), and EndoC (25%, 35%, 55%-85% sites). Correcting for bone free lean body mass (BFLBM) greatly reduced sex differences, eliminating sex*site interaction effects, but sex main effects remained significant. Correcting for BFLBM did not eliminate age effects.

Conclusion—The magnitude of age and sex differences in tibia variables varied by measurement site demonstrating the need for standardization of measurement sites.

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Keywords

pQCT; morphology; lean mass; vBMD

1.0 INTRODUCTION

Osteoporosis and low bone mass are prominent public health problems, that are causal risk factors for hip and vertebral fractures [1,2]. Prevention of osteoporosis to prevent fragility fractures is of interest, as hip fractures increase mortality risk [1]. The primary measure for the diagnosis of osteoporosis is areal BMD (aBMD) from Dual Energy X-ray Absorptiometry (DXA), a surrogate of bone strength, as fracture risk increases up to a factor of 3 for each standard deviation decrease in aBMD [2]. However, fracture risk also increases with suboptimal bone geometry and changing material properties of bone, and many fragility fractures occur before an individual meets the DXA-based criteria for osteoporosis [3,4]. Thus, utility of DXA is limited as it is unable to measure cross-sectional bone geometry or separate cortical and trabecular bone data to aid in the prediction of bone strength.

Peripheral Quantitative Computed Tomography (pQCT) complements DXA because it can assess total, cortical, and trabecular volumetric BMD (vBMD), mass, area, and tissue distribution in cross-sectional slices of long bones. In both men and women, cortical and trabecular bone losses markedly affect the structural integrity of the bone, and these losses can individually be detected with pQCT [5-9]. Currently, the International Society for Clinical Densitometry (ISCD) Position Stand reports that the distal radius can be used to predict hip fracture risk for postmenopausal women [10]. However, pQCT cannot be used to predict fracture risk or diagnose osteoporosis in other groups [10], since large, population-wide databases coupled with epidemiological outcomes are not established for pQCT measures such as they are for DXA. Also, pQCT is unable to measure the most clinically relevant sites (proximal femur and lumbar spine).

Although the tibia is commonly measured with pQCT, there are currently no recommendations for using the tibia for bone health assessment or hip fracture risk prediction. Given that the tibia is exposed to multiple modes, frequencies, durations, amplitudes and rates of mechanical loading from physical activities, its characteristics may be more closely related to the hip than the forearm. Detecting bone loss from pathologies, aging, or disuse, or predicting fracture risk with the tibia will be more valuable if it is known at which tibia site(s) bone loss can be detected most readily, and under what bone loss conditions. Variability in selected tibia sites between studies can make comparison of results or development of screening recommendations more difficult. The purpose of this study was to examine the relationship of age and sex with tibia morphology in adults. Additional purposes of this study were to determine which tibia site or sites are most sensitive for detecting age and sex differences in morphology.

2.0 METHODS

2.1 Research Design

This cross-sectional study employed a mixed factorial research design with two group variables (sex and age decade) and one repeated measures variable (limb site). This study tested healthy men and women who were grouped by decade between 20-59 years. Participants had their non-dominant leg, defined as the non-preferred kicking leg, scanned with pQCT at 9 cross-sectional sites, ranging from 5% to 85% of the limb length. All methods were approved by the University of Oklahoma Institutional Review Board.

This study was developed as a proof-of-concept study for larger, future trials to determine ideal standardized testing sites in a more heterogeneous sample. We estimated sample size needs for medium effect sizes ($f^2=0.15$) for site, sex, and age group main effects at an alpha level of $p=0.05$. At 10 subjects per group (8 groups: 2 sex, 4 age) and 9 sites, power was estimated to be 96.9% for the sex main effect, 91.2% for the age main effect, and 79.3% for the site main effect.

2.2 Participants

Participants for this study were healthy, self-identified Caucasian men and women ages 20-59 years. Both pre and postmenopausal women were recruited for this study, but premenopausal women were eumenorrheic, and none were taking sex steroids for at least the previous 12 months. Women completed a menstrual history questionnaire to provide information about menstrual cycle characteristics, menopausal status, and previously-used oral contraceptive or hormone replacement therapy. A Block 2005 food frequency questionnaire was used to estimate calcium intake. The bone-specific physical activity questionnaire (BPAQ) was used to estimate lifetime loading.(Beck)

Potential participants were excluded if they were taking sex steroids, were completely sedentary, defined as self-reporting fewer than 75 minutes of moderate or vigorous physical activity per week, also if they self-reported participating in more than 15 hours of moderate or vigorous exercise per week, if they weighed more than 300 lbs., had any conditions or were taking medications known to cause significant BMD loss or gain, or if they had any metal devices implanted in the tibia. Also, women were excluded if they were pregnant, thought they might be pregnant, or if they were breastfeeding.

2.3 Body Composition

Body weight and height were measured using a wall stadiometer and a Tanita BWB-800 digital scale (Tanita Corporation of America, Inc., Arlington Heights, IL). DXA (GE Lunar Prodigy, Prodigy enCORE software version 13.31.016, Madison, WI) was used to measure bone free lean body mass (BFLBM) and fat mass (FM) of the total body. Scan speeds were determined by the measured thickness of the subject at the naval (Thick = >25 cm and Standard = 13 – 25 cm). A refractometer (VEE GEE®, Model CLX-1) was used to measure urine specific gravity to determine hydration status prior to being scanned on the DXA. All participants were within our predetermined normal hydration range (1.004~1.029).

2.4 Peripheral Quantitative Computed Tomography (pQCT)

A pQCT scanner XCT 3000 with software version 6.00 (Stratec Medizintechnik GmbH, Pforzheim, Germany) was used to measure and analyze cortical vBMD (mg/cm^3) and BMC (mg/mm); trabecular vBMD (mg/cm^3) and BMC (mg/mm); and total bone vBMD (mg/cm^3) and BMC (mg/mm) at 9 sites of the non dominant tibia from 5-85% (distal to proximal) of the tibia length in 10% increments. The measured sites are shown in Figure 1. Other variables that are indicative of bone size and shape that were recorded were periosteal (PeriC) (mm) and endosteal circumferences (EndoC) (mm), and cortical thickness (CTh). For the 5% and 85% sites, the total and trabecular bone analyses were performed using the Stratec Contour Mode 3 and Peel Mode 4, with an outer threshold of $169 \text{ mg}/\text{cm}^3$ and an inner threshold of $650 \text{ mg}/\text{cm}^3$ because of the thinner cortical shell at these sites. Sites 15%-75% used Contour Mode 1 and Peel Mode 2 with $710 \text{ mg}/\text{cm}^3$ as the inner and outer thresholds. For sites 15%-85%, the cortical bone analyses were performed using Cortical Mode 2 with a threshold of $710 \text{ mg}/\text{cm}^3$.

In the Bone Density Research Laboratory, the *in vivo* (%CV) precision for measuring total bone vBMD, BMC and area at the tibia 4%, 38%, and 66% sites ranged from 0.3-1.9%, and least significant change (LSC) for following an individual over time was 3.19-6.86 mg/mm (BMC), 5.58-24.79 mg/cm^3 (vBMD), and 12.23-24.68 mm^2 (area). Precision ranges for measuring vBMD, BMC and area variables for the trabecular and cortical compartments were 0.5-1.5% (LSC: 3.32 mg/cm^3 (vBMD), 8.83 mg/mm (BMC), 29.38 mm^2 (area)) and 0.5-1.7% (LSC: 5.60-8.98 mg/mm (BMC), 24.61-30.82 mg/cm^3 (vBMD), 6.73-19.04 mm^2 (area)), respectively. All pQCT scans were performed by the same qualified technician.

2.5 Statistical Analyses

Data are reported as mean $\pm$ SE for all dependent variables. Statistical analyses were performed using SPSS for Windows version 18.0 (SPSS, Inc., Chicago, IL). Four (decade) by two (sex) by n (site) repeated measures ANOVAs were used to detect significant site, age, sex, site*age, site*sex, age*sex, and site*age*sex effects on all tibia variables at each site. Bonferroni post hoc tests were used for pairwise age-group and site comparison when testing for main effects. When a site*sex interaction was significant, independent t-tests were performed to determine sex differences at each site. When a significant site*age interaction occurred, one-way ANOVAs were used to determine age group differences at each site. When a significant age*sex interaction occurred, sex differences were determined for each age group, and age group differences were determined within each sex. Specifically, total BMC, vBMD, area, and periosteal circumference were compared at sites 5%-85%. Trabecular BMC, vBMD and area were compared between the 5%, 15%, and 85% sites. Cortical BMC, vBMD, area, endosteal circumference, and cortical thickness were compared between sites 15%-85%. The level of significance was set at $p = 0.05$. The assumption of sphericity was violated in every pQCT-related repeated measures ANOVA, so the Greenhouse-Geisser adjustment was used to determine significance.

3.0 RESULTS

3.1 Participant Characteristics

There were a total of 114 participants. Of the women participants, 32.2% ($n = 19$) were postmenopausal. Of the premenopausal women ($n = 38$), 71% ($n = 27$) were previous oral contraceptive users, and the time since last usage ranged from 1 year to 31 years. Two women were perimenopausal, according to self-report. The time since menopause ranged from 1 to 20 years, and 6 of the 19 postmenopausal women had had a hysterectomy. Of the postmenopausal women, 4 were previous hormone replacement therapy (HRT) users, and the time since HRT use ranged from 1 year to 8 years.

Table 1 shows the age, height, weight, body composition variables (bone-free lean body mass (BFLBM), fat mass (FM), body fat %), calcium intake, and total BPAQ scores of each group. Two-way ANOVAs were used for age and sex with body composition variables. There was not a significant age difference within sex groups. Men were significantly ($p < 0.01$) taller and heavier than women, and they had significantly ($p < 0.01$) greater total BFLBM. There were no significant age effects for height or BFLBM. The age effect for weight was not significant ($p = 0.060$), but there was a significant ($p < 0.01$) age effect for total body FM, where the 40s age group had higher fat mass values than the 20s age group. The overall weight range for this study was 46.8 kg (women) -131.8 kg (men), and body fat percentage ranged from 15.3-51.5% in women and 6.2-44.6% in men. A age*sex interaction effect was significant ($p < 0.05$) for calcium intake, as intake increased between age groups for women, but decreased between age groups for men.

3.2 Total and Cortical BMC and vBMD

Figures 2 and 3 present the total vBMD and BMC values for each age and sex group at each site, respectively. There were significant ($p < 0.001$) site ($\eta^2_{\text{partial}} = 0.893$), site*sex ($\eta^2_{\text{partial}} = 0.098$), site*age ($\eta^2_{\text{partial}} = 0.123$), and sex ($\eta^2_{\text{partial}} = 0.577$) effects. The 55%, 65%, and 75% sites were not significantly different from each other. Total BMC was 21-27.9% lower in women than men, with the largest difference occurring at the 5% site and the smallest difference at the 75% site. All sites were significant for sex. Age was only significant at the 85% site, but pairwise comparisons did not find significant differences between decades. When correcting for total BFLBM, the site*sex interaction was no longer significant. When correcting for total FM, age became significant ($p < 0.05$), with 20s having higher values than 50s, and the 5% and 85% sites had the most marked declines.

There were significant ($p < 0.001$) site ($\eta^2_{\text{partial}} = 0.974$), sex*site ($\eta^2_{\text{partial}} = 0.086$), age*site ($p < 0.05$, $\eta^2 = 0.051$), and age ($\eta^2_{\text{partial}} = 0.184$) effects for total vBMD. The 25% and 55% sites were the only sites that were not significantly different from each other. Sex differences in total vBMD peaked at the 5% site, where values were 9.9% lower in women, and sex differences were significant at only the 5%, 15%, and 85% sites ($M > W$). Total vBMD values were 5.4-17.2% lower in the 50s than the 20s, and differences were highest at the 5%, 15%, 75%, and 85% sites. There was a trend for 40s to have greater total vBMD than 50s (3.3-7.2% lower).

Figures 4 and 5 present the cortical BMC and vBMD values for each age and sex group at each site, respectively. There were significant ($p < 0.001$) site ($\eta^2_{\text{partial}}=0.894$), site*sex interaction ($\eta^2_{\text{partial}}=0.063$), and sex effects ($\eta^2_{\text{partial}}=0.566$) for cortical BMC. The age effect neared significance ($p=0.063$, $\eta^2_{\text{partial}}=0.066$). The only sites that were not significantly different from each other were 35% versus 75% and 45% versus 65%. Sex differences were significant at all sites, and cortical BMC was 21.3-24.6% lower in women. Sex differences were larger at the tibia ends (15%, 85%). The site*sex interaction was no longer significant when BFLBM was used as a covariate. Interestingly, the age effect became significant when FM was used as a covariate, but pairwise comparisons were not significant.

Significant ($p < 0.01$) site ($\eta^2_{\text{partial}}=0.919$), site*age ($\eta^2_{\text{partial}}=0.073$), and sex effects ($\eta^2_{\text{partial}}=0.128$), and a trend ($p = 0.083$, $\eta^2_{\text{partial}}=0.021$) for site*sex existed for cortical vBMD. The 15% site was not significantly different from the 65% site, and the 25% was not significantly different from the 35% site. There was a trend for age at the 25% site, and age groups were within 2% of each other at all sites. Cortical vBMD values were 0.9-2.0% higher in women, and this was significant at sites 25%-75%. The sex difference at the 85% site neared significance ($p = 0.052$). When correcting for BFLBM, the site*sex interaction trend disappeared, but when correcting for FM, site*sex became significant, as sex effects were significant ($p < 0.05$) at all sites except for the 15% site.

3.3 Circumferences and Cortical Thickness

Figure 6 presents the periosteal circumferences (PeriC) from 5% to 85%, and Figures 7 and 8 show the endosteal circumferences (EndoC) and cortical thickness values from 15% to 85% of the tibia length, respectively. There were significant ($p < 0.001$) site ($\eta^2_{\text{partial}}=0.951$), site*sex ($\eta^2_{\text{partial}}=0.043$), sex ($\eta^2_{\text{partial}}=0.542$) effects for PeriC, and there was near significance ($p = 0.055$, $\eta^2_{\text{partial}}=0.046$) for a site*age interaction. Only sites 25% vs. 55% were not significantly different from each other. Men had 9.7-13.0% higher PeriC values than women, and sex effects were significant at all sites. Correcting for BFLBM made the site*sex interaction nonsignificant, and made the site*age and age*sex interactions significant ($p < 0.05$). In this analysis, PeriC appeared to peak in 30s, with a trend for 30s > 20s. The sex effect was <1% in 20s, but was approximately 6%, 7%, and 4.5% in 30s, 40s, and 50s. The age effect failed to reach significance ($p = 0.057$) after correcting for BFLBM. Correcting for FM also made the site*age interaction significant ($p < 0.05$).

Site ($\eta^2_{\text{partial}}=0.964$), site*age ($\eta^2_{\text{partial}}=0.057$), site*sex ($\eta^2_{\text{partial}}=0.059$), age ($\eta^2_{\text{partial}}=0.110$), and sex ($\eta^2_{\text{partial}}=0.242$) effects were all significant ($p < 0.02$) for EndoC. All sites were significantly different from each other ($p < 0.01$, except 25% vs. 45%, $p < 0.05$). Twenties had 4.1-8.7% and 1.8-13.1% lower EndoC values than 30s and 50s, respectively ($p < 0.05$). The difference between 20s and 30s peaked at the 55% site, and the peak difference between 20s and 50s was at 35% site. Differences between 20s and 30s were smaller at more distal sites, whereas the smallest difference between 20s and 50s was at the 85% site. Age differences were significant ($p < 0.05$) at sites 25%, 35%, and 55%-85%, and a trend ($p < 0.10$) existed at sites 15% and 45%. Women had 6.5-12.0% lower EndoC values

than men, and the largest differences were closest to the midshaft, but differences were significant at all sites. Site*sex interaction and sex effects were not significant for EndoC after correcting for BFLBM, however, site*age*sex became significant ($p < 0.05$).

Cortical thickness had significant ($p < 0.001$) site ($\eta^2_{\text{partial}}=0.946$), site*sex ($\eta^2_{\text{partial}}=0.053$), age ($\eta^2_{\text{partial}}=0.139$), and sex ($\eta^2_{\text{partial}}=0.412$) effects. The difference between the 35% and 55% sites was not significant ($p = 0.067$), but all other sites were significantly different from each other. The 50s group had significantly ($p < 0.05$) lower values than all other age groups. The age effect was significant ($p < 0.05$) at sites 35%-55%, and there was a trend ($p < 0.10$) for the age effect at sites 25%, 65%, and 75%. Sex differences were significant ($p < 0.01$) at all sites. Site*sex effects were no longer significant after using BFLBM as a covariate.

3.4 Trabecular BMC and vBMD

Figures 9 and 10 present the trabecular BMC and vBMD findings. There were significant ($p < 0.01$) site ($\eta^2_{\text{partial}}=0.182$), age ($\eta^2_{\text{partial}}=0.102$), sex ($\eta^2_{\text{partial}}=0.246$), and site*sex*age interaction ($p < 0.03$, $\eta^2_{\text{partial}}=0.083$) effects for trabecular BMC. Male trabecular BMC values were 23.3-24.7% higher than women values overall. The 50s group had significantly ($p < 0.01$) lower values than the 20s group. At the 5% site, sex differences were largest in the 20s (41% lower in women), and smallest in the 50s (10.8% lower in women.) Finally, at the 85% site, 20s women had 5.1% higher values than men, but 30s, 40s, and 50s women had 11.3%, 42.5%, and 40.1% lower values than men. Correcting for BFLBM eliminated the site effect.

There were significant ($p < 0.01$) site ($\eta^2_{\text{partial}}=0.260$) and age*sex interaction ($\eta^2_{\text{partial}}=0.105$) effects for trabecular vBMD. Trabecular vBMD was 40% and 73% higher in 20s women than 20s men at the 5% and 85% sites, respectively. It was 34% and 52% higher in 30s women than 30s men, but it was 26% and 56% lower in 40s women than 40s men, and 32% and 53% lower in 50s women than 50s men. Also, while trabecular vBMD in women was highest in the 20s age group, it was highest in the 40s age group in men. At the 5% site, 20s women had significantly ($p < 0.05$) greater trabecular vBMD values than 50s women, and at the 85% site, 20s women were significantly ($p < 0.05$) greater than 40s and 50s women. The age*sex interaction effect remained significant when correcting for BFLBM, but the site effect was no longer significant. Correcting for FM did not affect the trabecular vBMD results.

Table 2 presents the sites of peak sex or age group differences when the sex, sex*site, age, age*site, or age*sex interactions were significant. The multiple sites reported for cortical BMC were because the sex differences at these sites were within 0.2% of each other. The multiple sites for peak age differences in total vBMD, CTh, and EndoC are peak differences for different age group comparisons.

4.0 DISCUSSION

The primary rationale behind the current study was that previous investigations measuring bone characteristics using pQCT have used a variety of tibia sites and have focused on a

variety of different bone variables from the pQCT output [5,7,8,11-17]. This variety limits direct comparisons among studies and has inhibited the development of standardized testing procedures and reference databases, which is a major strength of DXA. This study focused on measuring the tibia at 9 sites along the length of the tibia in generally healthy, non-elderly adult Caucasian men and women. The main findings were that most tibia sites have significantly different amounts of volumetric bone mineral density, bone mineral content, circumferences, and cortical thicknesses. Sex differences were primarily in size and content. In addition, a major proportion of sex differences were explained by differences in muscle mass.

Women have been shown to have greater fragility fracture risk than men after middle age [1,2]. Areal BMD has been used as a surrogate of bone strength and an indicator of fracture risk, but larger bones give higher aBMD values, even if the actual density is similar. We found large sex differences in bone size variables, instead of vBMD, which is in agreement with previous studies that reported bone size differences without large differences in vBMD between men and women [12,18]. In fact, data from this study suggests that women had greater cortical vBMD, and at some sites, total vBMD, than men. Although the cross-sectional nature of this study in healthy adults limits the ability to form conclusions about the reason for greater fragility fracture risk in women, it is plausible that bone density loss is a secondary factor for sex differences in fracture rates: because smaller bones have lower resistance to fracture than larger bones with the same density [4], any decrements to the material properties may be more devastating to whole bone strength in smaller bones than in larger bones.

Muscle mass accounted for a much larger proportion of sex differences in BMC than of sex differences in vBMD. In fact, correcting for BFLBM eliminated sex*site interaction effects, although many main effects for sex remained significant. Total fat mass did not account for sex differences in bone characteristics in our study, and since women had greater fat mass values than men, some sex differences in bone characteristics were slightly amplified when controlling for fat mass values. Previous pQCT-based sex comparisons of bone characteristics have reported greater bone content, size, and strength between men and women after correcting for body size [12, 18]. Our data suggests that muscle mass has a much stronger relationship with bone characteristics than fat mass, but sex differences in other factors besides body composition (i.e. hormonal) are likely to explain sex differences in bone characteristics. Previous studies have reported fat mass as a predictor of aBMD in untrained postmenopausal women [20,21], since fat mass is a source of gravitational loading and also a potential source of estrogen production [22]. It appears that we may have had smaller sex differences for some variables than those found by Capozza et al., who had also performed serial scans of the tibia [11]. This is likely due to their much smaller study sample size and age range and the sedentary status of their participants. They did not report any body composition measures, which may have also contributed to the level of group divergence. We included a wide range of physical activity levels, and we did not include or exclude for specific activities to make our findings more generalizable, as different physical activities have shown to have varying positive [13-17, 23-25] and negative [26-28] associations with bone health.

The general lack of age*sex interaction effects in this study was surprising, as bone loss has repeatedly been shown to accelerate during menopause [29-33]. This may simply be a statistical power issue related to sample size, as correcting for BFLBM caused age*sex interactions to become significant for PeriC and age*sex*site interactions to become a trend for total BMC. The main finding was that sex effects were greatest in 40-49 year olds for these variables. This enhanced sex difference is not likely due to menopausal status, as there were 14 (out of 15) postmenopausal woman in the 50s group, and only 4 postmenopausal women in the 40s group. Two women in this latter group were perimenopausal.

Significant age effects were not detected as consistently as sex effects. The trend for age effects for total and cortical BMC likely did not meet significance because of the number of sites that were not different between age groups, and we were likely underpowered for detecting differences at sites where age-related declines may be slower in the tested age range. Other potential reasons for fewer age group differences are the lack of age group differences in lean tissue values and the significant age group differences in fat mass values. Since muscular contractions are thought to be a major source of bone loading and muscle function is predictive of bone strength, maintaining lean tissue and perhaps function may have prevented some age-related bone loss [19,34-37]. Although fat mass was not related to bone characteristics, correcting for fat mass made some age differences in bone characteristics (total and cortical BMC, and the age*site for PeriC) significant when they were previously a trend. Essentially, some age effects were reduced because of increased fat mass in older age groups, but not nearly to the extent that lean mass affected sex effects. Further, decreases in lean mass equivalent to the increases seen in fat mass would have likely caused a greater detrimental effect on bone characteristics between age groups.

The 5% and 85% sites were sites with greater amounts of group divergence in total bone variables. Also, the 35% and 65% sites, near already commonly used sites (38% and 66%) were sites at which several group differences peaked. More group differences peaked at the 65% than the 35% site, and the 65% site has the added benefit of being a site for assessing calf MCSA when assessing region-specific soft tissue changes. The 15% site does not appear to add anything to the above four sites. The 'ideal' site(s) also partly depends on the chosen outcome variable. It should be noted that results do not suggest 4%, 14%, 38%, and 66% sites most frequently used in the literature are inappropriate, but does suggest a need for caution when directly comparing studies that used different sites for measurement. Results of this study suggest that the 85% site may be a useful and underutilized site for pQCT testing.

The cross-sectional design of this study precludes the ability to address rates of bone change in different age groups or between sexes. While certain tibia sites had larger sex and age group differences, long-term longitudinal studies are needed to confirm which sites are most sensitive for detecting change due to loading changes, aging, menopause, or other pathological conditions. Further, prospective studies are needed to determine fracture risk probabilities with pQCT. Drastic departures from the most commonly used sites are likely not necessary.

In conclusion, content, density, and geometry values in the tibia significantly varied by measurement site. Further, the measurement site affected the magnitude of observed age group and sex differences in morphology variables. Correcting for BFLBM greatly reduced sex differences, eliminating sex*site interaction effects, but sex main effects remained significant. Correcting for BFLBM did not eliminate age effects. Taken together, these findings demonstrate the need for standardization of measurement sites.

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Highlights

- Large sex differences in bone content; much smaller differences in vBMD.
- Sex differences largely accounted for by differences in lean mass.
- Age group and sex differences dependent on measurement site.

Figure 1.
Example cross-sectional images at each measured site.

Figure 2. Total BMC at Each Tibia Site from 5% to 85% of the Limb Length. Mean $\pm$ SE. * $p < 0.01$; Sex effect significant at all sites. † $p < 0.05$ Significant age effect at 85%.

Figure 3. Total Volumetric Bone Mineral Density (vBMD) at Each Tibia Site from 5% to 85% of the Limb Length. Mean $\pm$ SE. † $p < 0.01$ ($p < 0.05$ at 25%); Age effect significant at all sites. * $p < 0.01$; ** $p < 0.05$; Significant sex effect at 5%, 15%, 85%.

Figure 4. Cortical BMC at Each Tibia Site from 15% to 85% of the Limb Length. Mean $\pm$ SE. * $p < 0.01$; Sex effect significant at all sites.

Figure 5. Cortical vBMD at Each Tibia Site from 15% to 85% of the Limb Length. Mean $\pm$ SE. * $p < 0.01$; Sex effects were significant at sites 25%-75%. Trend for sex at 85% ($p = 0.052$).

Figure 6. Periosteal Circumference at Each Tibia Site from 5% to 85% of the Limb Length. Mean $\pm$ SE. Significant ($p < 0.01$) site, site*sex, sex effects. * $p < 0.01$ Sex effect significant at all sites.

Figure 7. Endosteal Circumference at Each Tibia Site from 15% to 85% of the Limb Length. Mean $\pm$ SE. $p < 0.05$ Significant site, site*age, site*sex, age, and sex effects. $p < 0.01$ (except 25% vs. 45%, $p < 0.05$) * $p < 0.01$ Sex effect significant at all sites. † $p < 0.05$ Significant age effect.

Figure 8. Cortical Thickness at Each Tibia Site from 15% to 85% of the Limb Length. Mean $\pm$ SE. Significant ($p < 0.01$) site, site*sex, age, and sex effects. † $p < 0.05$: Age effect. * $p < 0.01$: Sex effect significant at all sites.

Figure 9. Trabecular BMC of the 5% and 85% sites. Mean $\pm$ SE. Significant site, sex, and site*sex*age effect ($p < 0.01$). All sites are significantly different. * $p < 0.01$ Sex effect. † $p < 0.05$; 20-29 group different from 50-59 group in men. # $p < 0.05$; 20-29 group different from 40-49 and 50-59 groups in women.

Figure 10. Trabecular Volumetric Bone Mineral Density of the 5%, 15%, and 85% Sites. Mean $\pm$ SE. Significant site and age*sex interaction effects, $p < 0.05$. # $p < 0.05$ Significant difference between 20-29 group and 50-59 group in women. ‡ $p < 0.05$ Significant difference between 20-29 group and 40-49 group in women.

Table 1Physical and Behavioral Characteristics for Each Group. (Mean $\pm$ SE)

Variable	Age & Sex Group	20-29 M = 15 W = 15	30-39 M = 15 W = 14	40-49 M = 10 W = 15	50-59 M = 15 W = 15
Age ^c	Men	24.3 $\pm$ 0.8	34.6 $\pm$ 0.8	46.1 $\pm$ 0.9	54.8 $\pm$ 0.8
(yrs)	Women	21.2 $\pm$ 0.3	35.3 $\pm$ 0.9	47.0 $\pm$ 0.5	55.2 $\pm$ 0.7
Ht ^a	Men	176.9 $\pm$ 1.6	180.4 $\pm$ 1.3	177.3 $\pm$ 1.9	178.9 $\pm$ 2.2
(cm)	Women	165.2 $\pm$ 1.3	163.6 $\pm$ 2.4	164.4 $\pm$ 1.4	165.3 $\pm$ 1.6
Wt ^a	Men	82.9 $\pm$ 4.1	91.7 $\pm$ 3.5	90.0 $\pm$ 5.9	88.1 $\pm$ 4.6
(kg)	Women	65.0 $\pm$ 3.1	69.2 $\pm$ 3.7	78.7 $\pm$ 5.7	73.1 $\pm$ 4.2
BFLBM ^a	Men	63.5 $\pm$ 2.4	61.0 $\pm$ 1.2	60.2 $\pm$ 2.6	59.9 $\pm$ 2.1
(kg)	Women	39.8 $\pm$ 1.1	40.9 $\pm$ 1.9	44.7 $\pm$ 2.0	37.5 $\pm$ 2.9
FM ^c	Men	16.2 $\pm$ 2.1	27.1 $\pm$ 3.0	26.3 $\pm$ 3.9	24.8 $\pm$ 2.9
(kg)	Women	21.9 $\pm$ 2.3	24.8 $\pm$ 2.5	31.7 $\pm$ 4.0	37.5 $\pm$ 2.9
Total body fat % ^{ac}	Men	19.0 $\pm$ 2.2	28.5 $\pm$ 2.4	28.1 $\pm$ 2.6	27.3 $\pm$ 1.7
	Women	33.1 $\pm$ 1.8	35.4 $\pm$ 2.3	38.1 $\pm$ 2.9	39.7 $\pm$ 2.2
Ca Intake (mg/day) ^f	Men	1432 $\pm$ 196	1021 $\pm$ 104	1137 $\pm$ 159	976 $\pm$ 122
	Women	870 $\pm$ 122	919 $\pm$ 103	1265 $\pm$ 150	1265 $\pm$ 150
Total BPAQ ^{bdf}	Men	34.4 $\pm$ 5.2	36.8 $\pm$ 8.2	40.0 $\pm$ 10.1	30.3 $\pm$ 5.6
	Women	95.7 $\pm$ 15.7	65.2 $\pm$ 24.1	58.1 $\pm$ 17.5	19.0 $\pm$ 3.5

Significant sex difference

^a p < 0.01,^b p < 0.05.

Significant age effect

^c p < 0.01^d p < 0.05.

Significant age*sex interaction

^e p < 0.01,^f p < 0.05.

Ht: Height; Wt: Weight; BFLBM: Bone-Free Lean Body Mass; FM: Fat Mass; BPAQ: Bone-specific Physical Activity Questionnaire.

Table 2

Site(s) of Peak Sex or Age Group Difference for Each Bone Variable Where Sex, Sex*Site Interaction, Age, Age*Site, or Age*Sex Interaction Effects are Significant.

Variable	Site(s) of Peak Sex Difference	Site(s) of Peak Age Difference
Total BMC	5%	85%
Total vBMD	5%	5%, 85%
Trabecular BMC	5%	85%
Trabecular vBMD	85%	85%
Cortical BMC	15, 25, 85%	NS
Cortical vBMD	65%	25%
Cortical Thickness	15%	35%, 55%
Periosteal Circ.	65%	NS
Endosteal Circ	65%	35%, 55%

BMC: Bone Mineral Content; vBMD: Volumetric Bone Mineral Density; Circ: Circumference.