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Changes in Bone Structure and Mass With Advancing Age in the Male C57BL/6J Mouse

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ABSTRACT

To determine whether the mouse loses bone with aging and whether the changes mimic those observed in human aging, we examined the changes in the tibial metaphysis and diaphysis in the male C57BL/6J mouse over its life span using microcomputed tomography (μCT). Cancellous bone volume fraction (BV/TV) decreased 60% between 6 weeks and 24 months of age. Loss was characterized by decreased trabecular number (Tb.N), increased trabecular spacing (Tb.Sp), and decreased connectivity. Anisotropy decreased while the structure model index increased with age. Cortical bone thickness increased between 6 weeks and 6 months of age and then decreased continuously to 24 months (−12%). Cortical bone area (Ct.Ar) remained constant between 6 and 24 months. Fat-free weight reached a peak at 12 months and gradually declined to 24 months. Total mass lost between 12 and 24 months reached 10%. Overall, the age-related changes in skeletal mass and architecture in the mouse were remarkably similar to those seen in human aging. Furthermore, the rapid early loss of cancellous bone suggests that bone loss is not just associated with old age in the mouse but rather occurs as a continuum from early growth. We conclude that the C57BL/6J male mouse maybe a useful model to study at least some aspects of age-related bone loss in humans. (J Bone Miner Res 2002;17:1044–1050)

Key words: aging, bone, mouse, osteoporosis, bone structure

INTRODUCTION

AGE-RELATED BONE loss occurs in both men and women and is a major underlying cause of osteoporotic fractures in the elderly.[1–3] Because of the increasing technical ability to manipulate and study gene expression in the mouse, there is a growing interest in and use of the aged mouse as an animal model to study age-related bone loss in humans. Indeed, because of the remarkable differences in peak bone mass across mouse strains, much work is now concentrated on seeking genetic loci associated with high and low bone mass.[4–6] However, the question arises as to whether mice lose bone with age and if so whether the pattern of loss and change in bone architecture is similar to that in human aging.

In humans, peak bone mineral density (BMD) as assessed by quantitative computed tomography (QCT), and dual-energy X-ray absorptiometry (DXA) is reached between the ages of 10 and 19 years.[7,8] However, bone mineral content (BMC) continues to increase to the age of 30–35 years as a consequence of continued radial growth of the bone and increased mineralization.[9] With further aging, BMD and BMC decrease.[8,10–12] Aging is associated also with structural changes in bone.[13–22] Cancellous bone volume de-

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creases, trabecular number (Tb.N) decreases, trabecular spacing (Tb.Sp) increases, and cortical bone thins. Trabecular thickness (Tb.Th) remains unchanged or decreases with age.[16,20–22]

Studies in the female mouse, although limited, generally show a decrease in bone mass with age.[23–25] Vertebral mass is reported to peak around midlife (13 months) and gradually decrease thereafter.[24] Middiaphyseal cortical thickness and percent cortical area (Ct.Ar) in the femur also decrease with age. Bone density in the femoral neck peaks at 12 months and falls by 18% by 32 months.[25] However, little is known about the structural or architectural changes that occur in mouse bone during aging other than that cancellous bone volume decreases. Furthermore, age-related bone loss in the female mouse is complicated by estrogen deficiency.[26,27] It is not clear to what extent the reported changes in bone thickness and mass are a consequence of aging per se or hormone deficiency.

In the male mouse, conventional bone histomorphometry indicates that cancellous bone volume peaks around 12 months of age and then decreases by nearly 50% by extreme old age.[24] Bergman et al.[28] report similar findings in the femur of the Balb/c mouse between 4 and 24 months. Perkins et al.[29] report that between 6 and 24 months cortical thickness decreases by ~14% in both C57BL/6J and Balb/c mice. However, data are not available on cancellous structure or architecture. Data are available on bone changes with age in several senescence-accelerated mouse models but these models do not necessarily represent the normal course of bone aging.[30,31]

To determine whether changes in bone structure and mass occur in the aging male mouse and whether these changes are similar to those in humans, we studied C57BL/6J male mice from 1.5 to 24 months of age using microcomputed tomography (μCT). The data show that bone in the male mouse undergoes substantial change with advancing age and that the changes are remarkably similar to those in human aging.

MATERIALS AND METHODS

Animals

Forty-eight, 4-week-old male C57BL/6J mice (mean life span, ~27 months) were obtained from The Jackson Laboratory (Bar Harbor, ME, USA) and maintained in our animal colony until they reached 1.5, 3, 5.5, 12, 18, and 24 months of age (n = 8 mice/group).[32,33] Animals were housed 4–5 mice/cage (≤5.5 months of age) and 2–3 mice/cage (>5.5 months of age) and were maintained on a 12:12 h light-dark cycle with access to water ad libitum and a standard rodent diet (8640 Harlan Teklad 22/5 [W]; Harlan Teklad, Madison, WI, USA) containing 1.13% calcium and 0.94% phosphorus. Mice were weighed periodically throughout the study and killed by cervical dislocation. The Animal Care and Use Committee at the University of Colorado at Boulder approved the protocol for this study.

Preparation of bones

At the time of euthanasia the right tibia was removed and cleaned of adherent tissue. Bones were defatted by sequential extraction in ethanol and diethyl ether using a Soxhlet apparatus and dried overnight at 95°C, and their length and fat-free weight were determined before being submitted to μCT.

μCT

The tibias were imaged using μCT (μCT-20; Scanco Medical AG, Bassersdorf, Switzerland) with a resolution of 18 μm in all three spatial dimensions.[34] The scans were performed starting at the growth plate and extending into the shaft distally in 18-μm sections for a total of ~120 slices per scan. From this volume, 50 slices below the growth plate, constituting 0.9 mm in length, were selected for evaluation. This region of interest was chosen based on preliminary observations indicating that in the older animals all of the cancellous bone was found within this volume. Distal regions were devoid of cancellous bone. The trabecular part of the proximal tibia was identified semiautomatically.[35] The gray-scale images were processed using a low-pass filter (width of 0.5, support = 1 pixel or kernel size of 3 pixels) to remove noise, and a fixed threshold was used to separate the bone and marrow phase. A second image was obtained along the diaphysis to assess changes in cortical bone structure. This scan was performed at a resolution of 18 μm in all three spatial dimensions and consisted of 10 18-μm sections, starting 5 mm proximally from the tibial-fibular junction. From this volume, six slices were selected for evaluation.

The bone from 6-week-old animals was less mineralized (as assessed by the gray-level intensities) than the bone in older animals, which stayed relatively constant. Thus, a different threshold was selected for the 6-week group compared with the other age groups, all of which were analyzed at an identical threshold level. To express the threshold units in units of hydroxyapatite (HA) rather than arbitrary units, to enable comparisons for later scans, and between scanners we scanned a 97% pure HA phantom (BABI-HAP-D; Berkeley Advanced Biomaterials, Inc., Berkeley, CA, USA) using the same resolution and imaging set up. The gray level of the HA was 17973.5, assuming 97% purity and the density of pure HA to be 3 g/cm^3, the threshold selected for cortical bone was 0.955 gHA/cm^3 for the 6-week-old animals and 1.01 gHA/cm^3 for the other age groups, and the threshold for trabecular bone was 0.584 gHA/cm^3 for the 6-week-old animals and 0.743 gHA/cm^3 for the others. From the resulting binarized images of the trabecular region, structural parameters were computed. Bone volume fraction (BV/TV), Tb.Th, Tb.N, and Tb.Sp were calculated with the direct distance transformation method.[36] Using a triangle meshing technique previously described,[36] degree of anisotropy (DA)[36] and structure model index (SMI)[37] were calculated. Connectivity density (Conn.Dn) was determined using the Euler method.[38]

The cortex was segmented using a low-pass filter and a fixed threshold as described previously, and from the binarized image cortical area Ct.Ar, medullary area (Me.Ar) and

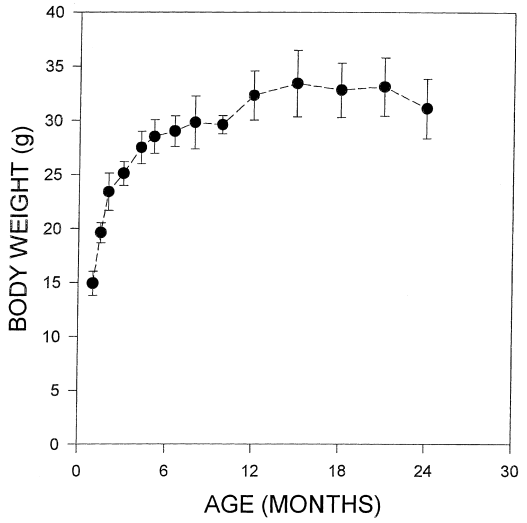

FIG. 1. Age-related changes in body weight (mean ± SD).

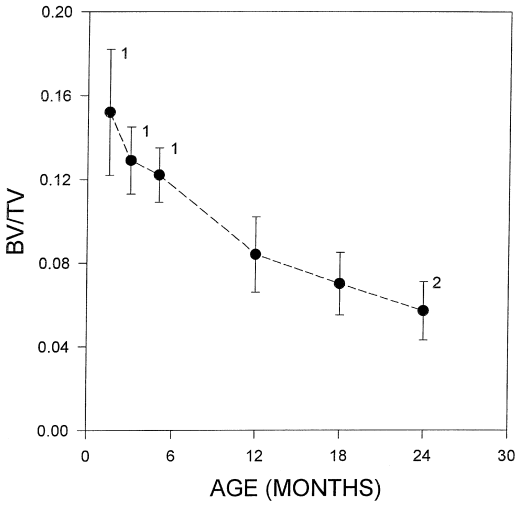

FIG. 2. Age-related changes in cancellous BV/TV (mean ± SD) in the proximal tibia. $^1p < 0.001$ and $^2p < 0.05$ compared with 12 months, one-way ANOVA, and Dunnett's test.

cross-sectional area (X.Ar) were calculated and averaged over all slices.

Data analysis

Data are presented as mean ± SD and analyzed using one-way analysis of variance (ANOVA) and Dunnett's post hoc test using the 12-month-old animals as the control group.

RESULTS

The change in body weight with age is shown in Fig. 1. Body weight reached a stable level by 12 months of age and remained unchanged to 24 months of age.

Quantitative analysis of the proximal tibial metaphysis is shown in Figs. 2–4. Cancellous bone volume decreased continuously from 1.5 to 24 months of age (Fig. 2). Using 12 months as a basis from which to measure bone changes (Dunnett's test), aging from 12 to 24 months resulted in a 32% decrease in cancellous BV/TV ($p < 0.05$). Between 5.5 and 24 months, bone volume decreased by 52% ($p < 0.001$). Tb.N also decreased continuously, but the change between 12 and 24 months was not significant (Fig. 3). Between 5.5 and 24 months Tb.N decreased by 37% ($p < 0.001$). Tb.Sp increased continuously from 1.5 to 24 months (Fig. 3). Between 12 and 24 months and between 5.5 and 24 months Tb.Sp increased by 41% and 64%, respectively. Tb.Th increased roughly 18% between 1.5 and 5.5 months but then remained relatively constant to 24 months (Fig. 3). Conn.Dn, a measure of trabecular connectedness, decreased continuously from 1.5 to 12 months, whereas beyond 12 months of age, Conn.Dn remained constant (Fig. 3). Anisotropy, or the degree of asymmetry in trabecular bone orientation, decreased from ~2.2 (highly oriented) at 5.5 months to 1.65 (less oriented) at 18 months (Fig. 4). The SMI gradually increased ($p < 0.02$) from 2.76 ± 0.23 at 1.5 months to 3.06 ± 0.40 at 24 months of age (Fig. 4). Mineral density of the cancellous bone as estimated from the gray scale appeared to increase from 0.84 gHA/cm^3 at 1.5 months to 1.2 gHA/cm^3 at 12 months but then remained relatively constant into old age.

Analysis of the midtibial diaphysis is shown in Fig. 5. Total bone area increased continuously from 1.5 to 12 months of age. Between 12 and 24 months, no change in bone area was observed. Cortical thickness increased from ~160 μm at 1.5 months to 230 μm at 5.5 months and then decreased continuously to 185 μm at 24 months. Between 12 and 24 months, thickness decreased by 12%. Cortical bone area increased from 0.43 mm^2 at 1.5 months to ~0.64 mm^2 at 5.5 months and then remained remarkably constant to 24 months. Me.Ar increased with age (data not shown). Cortical density, as estimated from the gray scale, appeared to increase from 1.84 gHA/cm^3 at 1.5 months to 1.96 gHA/cm^3 at 5.5 months and then continued to increase gradually to 2.00 gHA/cm^3 at 24 months ($p < 0.05$).

Three-dimensional images of the cancellous bone and cross-sectional images of the cortical bone from animals aged 1.5, 3, 5.5, 12, 18, and 24 months are shown in Fig. 6. The loss of bone volume and decrease in anisotropy with age in the cancellous compartment and the increase in Me.Ar and cortical thinning in the diaphysis with age are visually apparent.

The total fat-free weight and length of the tibia are reported in Table 1. Fat-free weight reached a peak at 12 months and gradually declined to 24 months. Total mass lost between 12 and 24 months reached 10% ($p < 0.01$). Length peaked between 3 and 5.5 months and did not change thereafter.

DISCUSSION

Our results show that both cancellous and cortical bone undergo dramatic change during growth and aging in the male

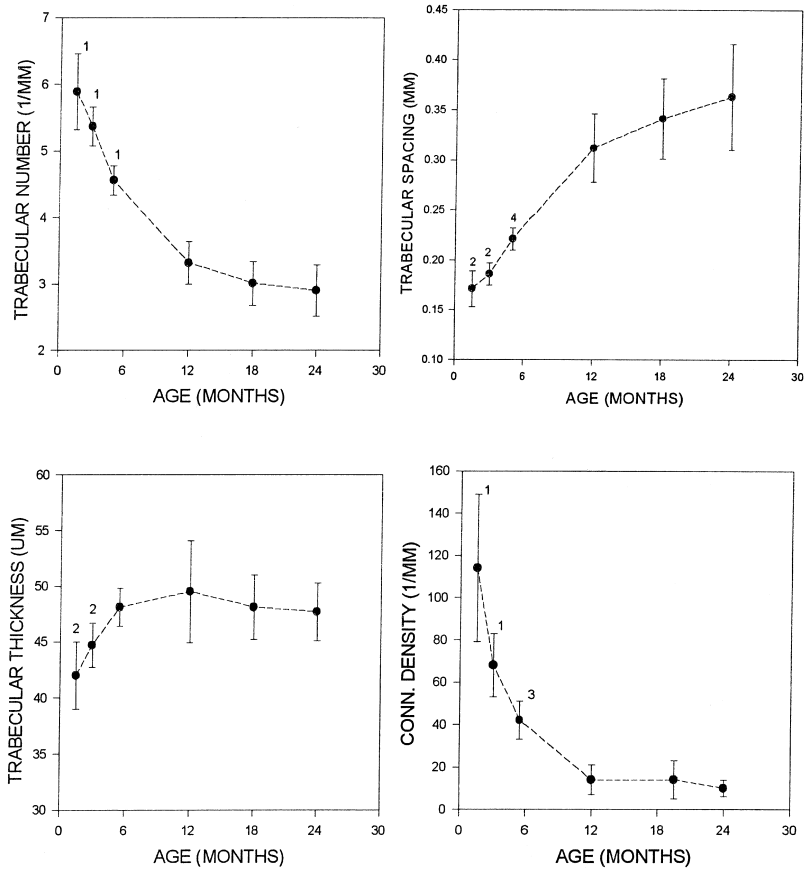

FIG. 3. Age-related changes in Tb.N, Tb.Sp, Tb.Th, and Conn.Dn (mean ± SD) in the proximal tibia. $^{1}p < 0.001$, $^{2}p < 0.005$, $^{3}p < 0.01$, and $^{4}p < 0.05$ compared with 12 months, one-way ANOVA, and Dunnett's test.

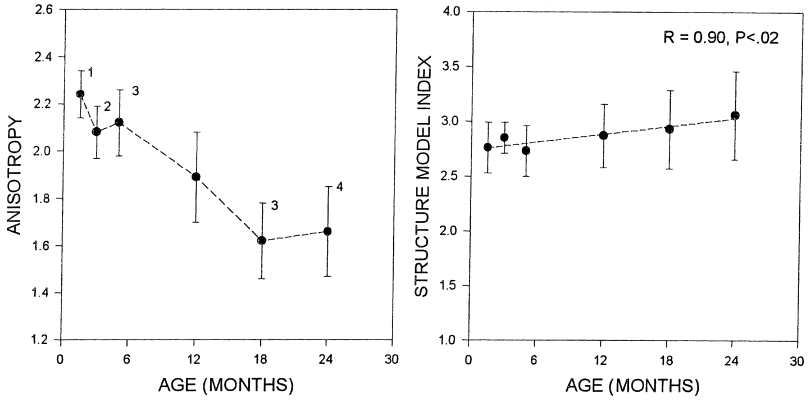

FIG. 4. Age-related changes in trabecular anisotropy and SMI (mean ± SD) in the proximal tibia. $^{1}p < 0.001$, $^{2}p < 0.005$, $^{3}p < 0.01$, and $^{4}p < 0.05$ compared with 12 months, one-way ANOVA, and Dunnett's test.

C57BL/6J mouse. They also reveal that the pattern of change is remarkably similar to that observed in human aging.

In human aging, cancellous bone volume, Tb.N, and Tb.Th decrease and Tb.Sp increases.[13–22] Furthermore, during the early stages of aging in the human there is a preferred loss of horizontal trabeculae leading to an increase in anisotropy.[39] This is followed by a period of trabecular perforation and an eventual decrease in anisotropy. In the human tibia, Tb.Th decreases with age but Tb.N shows only a small insignificant trend downward.[22] The SMI in the tibia gradually increases but the increase does not reach significance until late in life when trabeculae undergo a sharp change from platelike to less platelike.[22]

We observe similar but not identical cancellous bone changes in the aging male mouse. Like in humans, cancellous volume and Tb.N decrease and Tb.Sp increases with advancing age. However, unlike reports in the human Tb.Th does not appear to decrease with age in the mouse, suggesting that bone loss in this model is not a consequence of trabecular thinning at least in the proximal tibia. Also, unlike humans, there does not appear to be a preferential loss of horizontal trabeculae. Anisotropy decreases in a

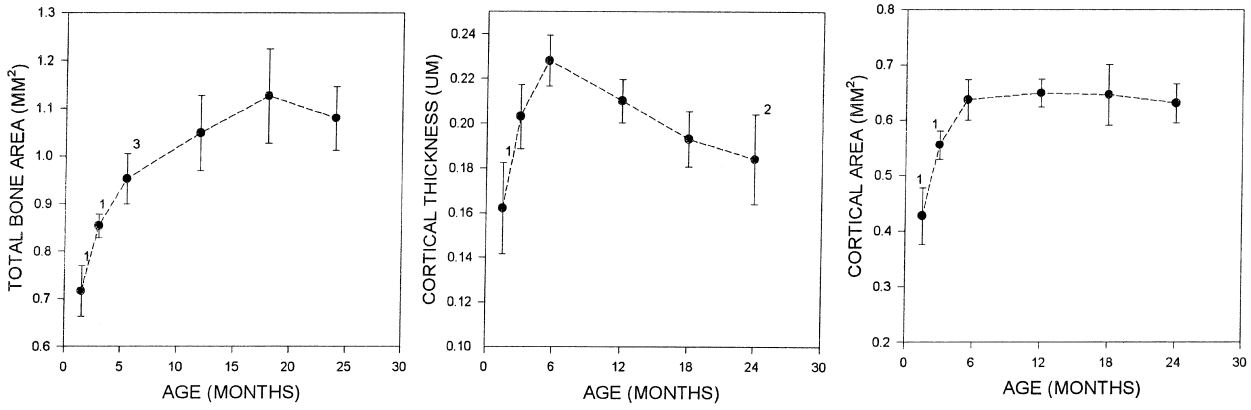

FIG. 5. Age-related changes in cross-sectional diaphyseal area (total bone area), cortical thickness, and Ct.Ar (mean ± SD) 5 mm proximal to the tibial-fibular junction. $^1p < 0.001$, $^2p < 0.005$, and $^3p < 0.05$ compared with 12 months, one-way ANOVA, and Dunnett's test.

nearly continuous fashion in the mouse. Comparing the SMI in humans and mice tibias is revealing. SMI values in the human proximal tibia gradually increase from 0.5 to 0.8 (relatively platelike) between the ages of 10 and 70 years. Between 70 and 90 years, they increase more sharply, reaching peak values of ~1.5. SMI values in the male mouse are much higher (2.76, rodlike) to begin with and like in humans, gradually increase with age (become more rodlike).

The differences between the human and mouse with respect to trabecular thinning and loss patterns may represent differences in the mechanisms of bone loss, reflect the influences of dissimilarities in mechanical loading, be consequent to differences in skeletal architecture between humans and C57BL/6J male mice, or be related to differences in the skeletal sites analyzed (our data were collected from the tibia whereas most human data have been collected from the vertebrae). Interestingly, the serum concentration of testosterone does not decrease with postmaturational aging in the C57BL/6J male mouse.[40] Thus, androgen deficiency does not appear to play a role in age-related bone loss in this model. Regardless of the mechanisms, the changes in cancellous bone with age are remarkably similar in humans and mice.

In contrast, the age-related change in cancellous bone in the rat is radically different.[41] Vertebral bone volume and Tb.Th increase with age and Tb.N remains unchanged.

With respect to cortical bone, human aging is associated with an increase in X.Ar because of continued periosteal growth, a decrease in cortical thickness, an increase in cortical porosity, and a decrease in cortical density.[17–19] The same changes occur in the tibia from the male mouse with the exception of the decrease in cortical density as estimated by the gray scale in our animals. X.Ar increases and cortical thickness decreases. The male mice appear to differ from humans only in that cortical density appears to increase. This may be caused by the absence of cortical remodeling in the mouse and the gradual increase in bone matrix mineralization that naturally occurs with time.[42,43] However, decreased density has been reported in the femoral neck of female mice.[25] The reason for this discrepancy is not clear but may be related to gender and skeletal site.

The timing of the changes in bone associated with aging also is remarkably similar in mice and men. In humans, total bone mass peaks between 30 and 40 years of age or assuming a life span of 75–80 years, roughly halfway through life. We and others find that bone mass in the mouse also peaks around midlife (i.e., at 12–13 months).[24] Cancellous bone volume in the tibia reaches a peak at or before 1.5 months (5% of mean life span) in the mouse and then decreases rapidly. Unfortunately, cancellous measurements are limited in children and adolescent humans, but cancellous volume also may peak relatively early in human life (10–19 years or 20% of mean life span).[7,8] The other structural parameters of cancellous bone have not been studied adequately in young humans to make comparisons with mice.

The structural changes in cancellous bone that occur with postmaturational aging in the male mouse are similar to those induced by ovariectomy in the female mouse.[44,45] Using a similar μCT approach to measure cancellous structure, ovariectomy results in a decrease in cancellous BV/TV, Tb.Th, Tb.N, and Conn.Dn and an increase in Tb.Sp. However, cortical thickness is reported to remain unchanged in the ovariectomized animal.[45]

The use of μCT imaging in this model permits us to examine the different compartments, that is, trabecular and cortical bone, in our aging mouse model. However, it must be noted that the resolution of the μCT images was 18 μm, and Tb.Th ranged from 42 to 50 μm in this model. Although this spatial resolution is ~⅓–½ the trabecular dimensions, clearly, higher-resolution images would permit a more accurate estimate of the Tb.Th. Thus, the measures of thickness determined in this study may differ from those derived from histology. In fact, comparative studies between histology and μCT have established that although there may be some resolution-dependent effects and μCT provides only static structural information, compared with the cellular function that histology provides, μCT is a valid modality for assessing trabecular structure in animal models and human specimens.[45–47] In images in which the spatial resolution was lower (i.e., on the order of trabecular bone dimensions), we have shown that despite the fact that measured param-

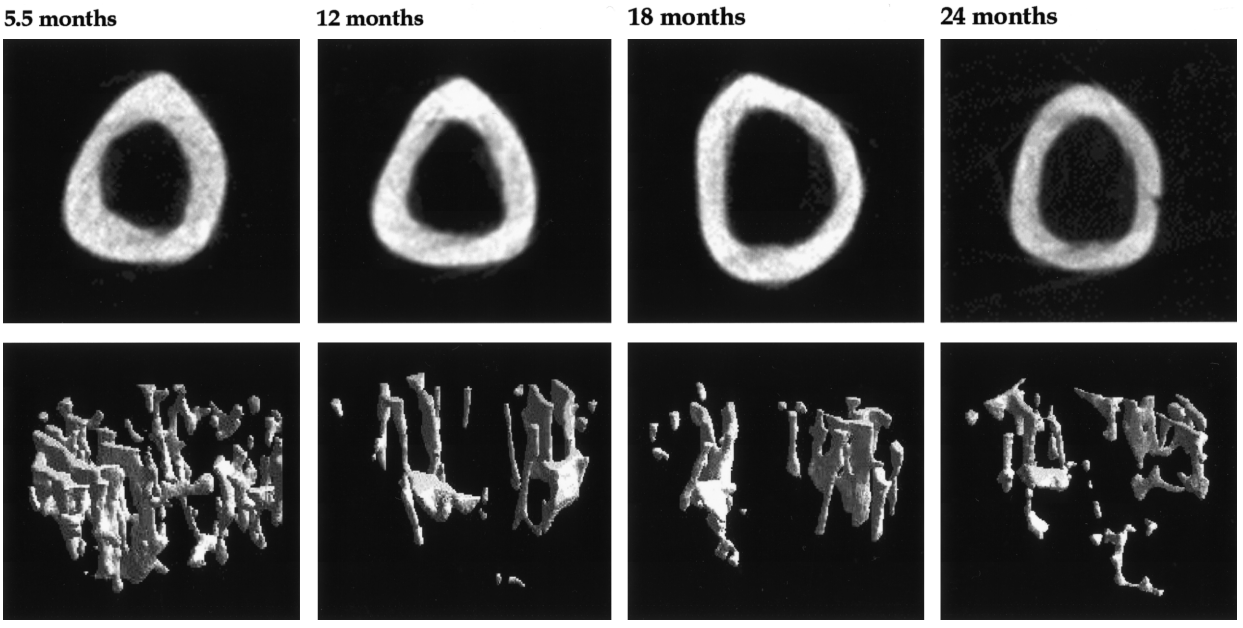

FIG. 6. Representative (median) μCT images of the tibial diaphysis 5 mm proximal to the tibial-fibular junction (top) and cancellous bone in the proximal tibia (bottom) from mice aged 5.5, 12, 18, and 24 months.

TABLE 1. EFFECT OF AGE ON FAT-FREE WEIGHT AND LENGTH OF THE TIBIA (MEAN ± SD)

Age (months)	*Fat-free weight (mg)*	*Length (mm)*
1.5	24.3 + 1.8 ($p < 0.001$)*	15.7 + 0.3
3.0	30.1 + 1.3 ($p < 0.001$)*	17.3 + 0.1
5.5	34.8 + 1.9	17.7 + 0.3
12	35.5 + 2.5	17.9 + 0.3
18	34.9 + 2.3	17.9 + 0.3
24	32.0 + 2.2 ($p < 0.01$)*	17.7 + 0.2

* One-way ANOVA and the Dunnett's test, compared with the 12-month group.

eters in the limited resolution range differ from those measured from very high resolution images, the correlation between the measures is very good.[48,49] Therefore, the trends of change in Tb.Th that we observe are clearly valid, although the absolute magnitude may be prone to a resolution-based bias. The measures of spacing and other parameters do not suffer from this resolution-based effect, however.

Collectively, our data suggest that the changes in skeletal structure that occur during aging in the mouse are similar to those that occur during human aging. Therefore, the mouse may be a useful model to study at least some aspects of age-related bone loss in humans. However, despite the similarities in the age-related loss of bone in humans and mice, the mechanisms responsible for loss may not be identical. Differences in body mass, skeletal structure, and life span may influence the dynamics of bone metabolism and how bone is lost with aging.

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