Postpubertal Architectural Developmental Patterns Differ Between the L_3 Vertebra and Proximal Tibia in Three Inbred Strains of Mice*

Helen R Buie, Christopher P Moore, and Steven K Boyd

ABSTRACT: An understanding of normal microarchitectural bone development patterns of common murine models is needed. Longitudinal, structural, and mineralization trends were evaluated by in vivo \(\mu\)CT over 12 time points from 6–48 wk of age at the vertebra and tibia of C3H/HeN, C57BL/6, and BALB/C mice. Longitudinal growth occurred rapidly until 8–10 wk, slowed as the growth plate bridged, and fused at 8–10 mo. Structural augmentation occurred through formation of trabeculae at the growth plate and thickening of existing ones. In the vertebrae, BV/TV increased rapidly until 12 wk in all strains. Between 12 and 32 wk, the architecture was stable with BV/TV deviating <1.1%, 1.6%, and 3.4% for the C57BL/6, BALB/C, and C3H/HeN mice. In contrast, the tibial architecture changed continuously but more moderately for BV/TV and TbTh compared with the vertebra and with comparable or larger changes for TbN and TbSp. Age-related trabecular deterioration (decreased BV/TV and TbN; increased TbSp and structure model index) was evident at both sites at 32 wk. In all strains, the cortex continued to develop after trabecular values peaked. The temporal plateau of BMD was variable across mouse strains and site, whereas tissue mineral density was attained at \(\sim\)6 mo for all sites and strains. Geometric changes at the tibial diaphysis occurred rapidly until 8–10 wk, providing the C57BL/6 mice and C3H/HeN mice with the highest torsional and compressive rigidity, respectively. In summary, key skeletal development milestones were identified, and architectural topology at the vertebra was found to be more stable than at the tibia.

J Bone Miner Res 2008;23:2048–2059. Published online on August 4, 2008; doi: 10.1359/JBMR.080808

Key words: murine, bone architecture, growth, vertebra, tibia

INTRODUCTION

MURINE MODELS have become an important tool for the study of genetics and disease because of the ability to control factors that are not possible in clinical studies. Animals can be inbred to acquire a homogeneous genetic background (i.e., identical twins) with specific phenotypes and a variety of bone characteristics.(1–6) In addition, animal studies allow a high degree of environmental control and offer a unique opportunity to systematically study disease(7,8) and injury.(7,8) The use of these models for bone research, however, requires a detailed understanding and characterization of normal patterns of development, particularly nearing skeletal maturity. Some important aspects of skeletal development to consider include longitudinal growth, mineral accumulation, and structural changes.

Bone length in mice is in part genetically determined(1–9); however, it is unclear if variation results exclusively from differences in longitudinal growth rates or also temporal differences in growth patterns. It has been established that growth is fastest during the postnatal period and begins to slow at puberty (\(\tau\) : ~26–31 days; \(\phi\) : ~28–35 days).(9–4) which males have longer bones than females.(9–11)

Reported growth patterns after puberty are inconsistent(1,10,12,13) even within a given mouse strain, and studies have primarily focused on C57BL/6J mice. Whereas some of these patterns have been established through cross-sectional study designs, intergroup variability can pose problems when characterizing temporal development trends. Longitudinal study designs, in which a group of animals is examined repeatedly over time, would complement current knowledge of bone development by establishing temporal trends.

Mineral accumulation has been primarily assessed by measuring BMD, but this is more difficult to interpret compared with tissue mineral density (TMD). For example, BMD increases more rapidly during puberty compared with the postnatal period(9) but may reflect slowing growth rates rather than increased mineral deposition because BMD depends on both mineral content and total bone volume (i.e., volume of bone and marrow). TMD on the other hand, which depends on mineral content and tissue bone volume (i.e., volume of bone only), was found to increase more rapidly during the postnatal period.(13) Large differences in BMD between two mice strains that have similar mineral size, shape, and organization within the collagen matrix was found to result from variations in tissue bone volume,(14) further highlighting the difficulties in relating...
MURINE SKELETAL GROWTH AND ARCHITECTURAL STABILITY

BMD to mineral accumulation. TMD provides a more direct understanding of mineralization but has rarely been reported. Recently, bone mineralization was shown to proceed rapidly in postnatal BALB/cByJ mice, with TMD reaching 51% of mature values at 1 day of age and 80% by 40 days. Variation in TMD across anatomic sites has not been examined, but BMD is clearly site specific. Despite these limitations, timing of peak BMD measured by Beamer et al. is commonly adopted as an indicator of skeletal maturity.

Developmental patterns of bone structure have primarily focused on the cortex or the trabecular compartment of C57BL/6J mice. Architectural parameters have been found to peak early and at different times for the cortical and trabecular compartments. Similar developmental patterns were found for the tibia, femur, and vertebra, with more pronounced changes in the long bones. No stable period of architecture was identified in the vertebra and tibia in three common inbred strains. Mice were sedated with isoflurane anesthetic and maintained on low doses for the duration of the measurements (~40 min per mouse). An ophthalmic ointment (BNP Sterile Ophthalmic Ointment; Vetcom) was applied to each eye to prevent dryness and damage. Using a novel whole body scanning device (Scanco Medical AG), scans were acquired (55 kV, 109 μA, 200-ms integration time, 38.9-mm field of view, 2000 projections on 360°, 2048 CCD detector array, cone-beam reconstruction) of the third lumbar (L₃) vertebra and both proximal tibias at a nominal isotropic resolution of 19 μm. The L₃ vertebra was selected for evaluation over other larger lumbar vertebrae because it fit in one stack of μCT images. The delivered radiation dose was ~188 mGy based on previous measurements in the laboratory using an ionizing chamber probe. This dose is approximately four times lower compared with other in vivo studies. The scanner was calibrated weekly using hydroxyapatite (HA) phantoms. After each scan, the mice were weighed (Ohaus Scout SC2020), and body mass was recorded. After the final measurement, the mice were killed by CO₂ asphyxiation. The protocol for this study was approved by the Animal Care and Use Committee at the University of Calgary.

**Image processing**

Volumes extracted for analysis included the metaphysis of the vertebral body, the left and right proximal tibial metaphyses (a 1.5-mm slab extending away from the growth plate), and the left tibial diaphysis (a 0.5-mm slab beginning 5.0 mm distal to and extending away from the proximal growth plate). Semiautomated hand-drawn contours were used to isolate regions for analysis, and an automated approach was used to separate the cortical and trabecular compartments. For the tibia, the long axis of the bone was first aligned with the superior–inferior axis before defining the different regions of interest. These regions of interest were then transformed to allow the analysis to be carried out in the bone’s original scan orientation. Finally, all grayscale images were processed using a Gaussian filter (sigma = 0.7, support = 1) to reduce noise and a global threshold (22% of the maximal grayscale value) to extract the bone from surrounding soft tissue.

**Analysis**

Bone architecture was assessed by direct 3D methods (Image Processing Language v. 5.01a; Scanco), densitometry from the X-ray attenuation, strength indicators from cross-sectional geometry, and longitudinal growth from vertebral body length. Temporal longitudinal growth patterns should not depend on the site examined because relative growth rates are comparable across sites. Architectural measurements for the vertebral body and the tibial metaphysis included bone volume ratio (BV/TV, %), trabecular thickness (TbTh, μm), trabecular separation (TbSp, μm), trabecular number (TbN, mm⁻²), structure model index (SMI), degree anisotropy (DA), connectivity density (ConnD, mm⁻³), and cortical thickness (CtTh, μm). Mineral accumulation was assessed by densitometric measures of BMD (mg HA/cm³) and TMD (mg
HA/cm³) for the whole L3 vertebra and for the proximal tibial metaphysis. BMD and TMD are determined by normalizing mineral content from the X-ray attenuation by bone volume (bone and marrow) and tissue volume (bone only), respectively. Relative TMD was also calculated as a percentage of plateau values (TMD, %). Cortical area (CtA, mm²) and polar moment of inertia (J, mm⁴) of the tibial diaphysis were measured to provide indications of the bone’s ability to withstand axial and torsional loading, respectively. In addition, total cross-sectional area (TA, mm²) and medullary area (MA, mm²) provided indications of change to the periosteal and endocortical surfaces.

Standard descriptive statistics were evaluated for all variables. To check if the high- and low-frequency scanning groups could be pooled, architectural outcomes were tested by a two-way ANOVA for mouse strain and treatment (high versus low dose). All continuous dependent variables were averaged across time, and one- and two-way repeated-measures ANOVAs were used to determine effects of strain, site, and limb. Simple effects testing was performed where appropriate. Temporal trends were evaluated by a one-way repeated-measures ANOVA excluding incomplete datasets. Statistical analyses were performed with SPSS 16.0 for Windows (SPSS, Chicago, IL, USA). Differences were considered statistically significant for p < 0.05.

RESULTS

There were no significant differences in the variables studied between the high- and low-frequency groups (Fig. 1); therefore, the groups were pooled to increase statistical power.

The total number of datasets available for analysis was reduced because of accidental death, disease, operator error, and motion artifacts. Three C3H mice were found dead at 44 wk after a water bottle flooded the cage. Five B6 mice (three high-frequency group; two low-frequency group) developed ulcerative dermatitis, which did not improve with topical antibiotic treatment (Topagen Spray; Schering Canada) and were killed at 25, 29, 29, 32, and 37 wk. Female B6 mice are prone to this disease and may be more susceptible during the summer months, with similar timing to the tibia. Complete fusion of the growth plate occurred between 8 and 10 mo. Growth curves were not significantly different between the B6 and BAL mice. C3H mice, on the other hand, had a longer rapid growth period and grew 40% faster between 6 and 8 wk, leading to significantly longer bones (p < 0.001).

Body mass

Body mass increased rapidly until 6 wk of age and then continued linearly at a slow and steady rate (Fig. 2A). On average, mass was not significantly different between the C3H and B6 mice, and both were heavier than the BAL mice (p < 0.001) because of more rapid increases after week 6. Body mass continued to increase for the B6 mice through 48 wk, whereas it stabilized in the C3H and BAL mice at weeks 32 and 42, respectively.

Longitudinal growth

Longitudinal growth patterns were strain dependent and proceeded rapidly until 8–10 wk, followed by a slow continuous growth until fusion of the growth plate (Fig. 2A). Longitudinal growth has previously been shown to be most rapid during the postnatal period and slows at puberty. We found that growth slows further at 8–10 wk because of bridging of the growth plate, which we observed at the tibia. There was insufficient resolution to detect bridging elements at the vertebra in this study but is apparent in previous studies, with similar timing to the tibia. Complete fusion of the growth plate occurred between 8 and 10 mo. Growth curves were not significantly different between the B6 and BAL mice. C3H mice, on the other hand, had a longer rapid growth period and grew 40% faster between 6 and 8 wk, leading to significantly longer bones (p < 0.001).

Mineralization

Mineral accumulation was similar for all three strains but was only evident from TMD measurements (Fig. 2B). BMD measurements were highly variable between anatomic site and strain with peak or plateau values attained between 14 and 34 wk. Plateau values for TMD on the other hand...
occurred at ∼26 wk for all sites and strains. A complete TMD (%) curve was obtained by including data from Miller et al. (13) and can be described by the general growth curve \( r^2 = 0.954 \) given in Eq. 1.

\[
\text{TMD} = \frac{21.06 + 82.81}{1 + 2.31 \cdot \exp[-0.77(Age_{\text{in weeks}} - 0.08)^{0.43}]}
\]  

[Eq. 1]

**Structure**

Temporal changes in architectural parameters are plotted in Figs. 3 and 4, and representative 3D structures are shown in Figs. 5 and 6. Strain-related differences in architecture were significant at both the vertebra and tibia and site-to-site differences for the B6 and BAL mice (Table 1).

On maturity, the vertebral body was architecturally stable, with some trabeculae at 6 wk persisting through week 48 (Fig. 5). Vertebral BV/TV increased by 113–159% between 6 and 12 wk, primarily because of increases in TbTh, which ranged from 60% to 84% during this period. Between 12 and 32 wk, the mean BV/TV increased <1.1%, 1.6%, and 3.4% in the B6, BAL, and C3H mice, respectively. During this period, changes in TbTh, TbN, TbSp, SMI, and DA were minimal, but significant reductions in ConnD occurred for the B6 and the C3H mice. Cortical maturity was delayed compared with the trabecular compartment, with CtTh attaining maximum values at 17 wk in the B6 and C3H mice and 25 wk in the BAL strain. Typical changes with advanced age (decreased BV/TV and TbN; increased TbSp and SMI) onset at 32–34 wk, but decreasing BV/TV trends only reached significance for the B6 and C3H strains (B6, \( p = 0.003 \); C3H, \( p = 0.008 \); BAL, \( p = 0.211 \)).

In contrast to the stability of the vertebra, there were continuous changes in architecture at the tibia with age (Figs. 3 and 4). Initial increases in BV/TV and TbTh were more gradual (less pronounced) than in the vertebra at 33–60% and 9–25%, respectively. In contrast, trends in TbN and TbSp were similar to the vertebra but with comparable or larger changes. There was an early decline in ConnD at both sites. The temporal plateau of BV/TV at the tibia differed among mouse strain and did not coincide with those of the vertebra. However, timing of peak CtTh in the B6 and BAL strains was similar for the vertebra and tibia. As with the vertebra, age-related bone loss was seen at 32–34 wk (decreasing BV/TV trends: B6, \( p = 0.005 \); C3H, \( p = 0.009 \); BAL, \( p = 0.023 \)).

Developmental trends for the left and right tibias were comparable, but there were significant differences in trabecular architecture. For all strains, architectural parameters were on average higher \( (p < 0.001) \) in the right limb compared with the left, with larger differences between 12 and 26 wk. For all the mice, cortical thickness was not significantly different between limbs at any time point.

Temporal changes in cross-sectional geometry of the tibial diaphysis are provided with representative sections in Fig. 7. Data from Richman et al. (19) are included for data before week 6, and strain-related differences in structure are provided in Table 2. Initial increases in cortical area from week 6 arose primarily from apposition to the endocortical surface, which occurred rapidly in the C3H and B6 mice until 8–10 wk and more moderately until week 20 in
the BAL mice. The B6 mice developed bones with larger total cross-sectional areas compared with the other strains, leading to enhanced torsional resistance, whereas the C3H mice developed high cortical area providing superior compressive resistance. Structural increases in compressive and torsional rigidity occurred early in the B6 and C3H mice and more gradually in the BAL mice.

**DISCUSSION**

Longitudinal, structural, and mineral developmental patterns were determined from an in vivo study providing a detailed understanding of normal growth patterns at skeletal maturity in B6, BAL, and C3H mice at the vertebra and tibia. Despite common use of these mice, skeletal development of the BAL and C3H strains has not been well characterized. Whereas the B6 mice have been examined previously,\(^{10,13}\) this study yields a comprehensive description of key developmental stages at maturity. Establishment of normal development patterns is not only necessary to effectively plan studies but can also aid in interpretation of existing ones. For example, our laboratory recently performed an ovariectomy or sham operation on these three strains of mice at 12 wk of age and examined bone loss at the tibia in vivo over a 5-wk period. Whereas continuous decreases in BV/TV occurred for the B6 and C3H mice, for unknown reasons, the BAL showed some recovery by the end of the study. This recovery, however, can be explained by knowing that BV/TV is increasing during this period for the BAL mice but not for the B6 and C3H. Knowledge of
normal bone changes can also be important for studies providing only endpoint data, which is common in exercise studies of rodents. In these studies, it is difficult to know if exercise induced positive bone changes or simply prevented deterioration.

The longitudinal analysis reinforces our current understanding of bone development by clarifying the temporal changes in architecture. Structural augmentation occurred in all three strains of mice through formation of new trabeculae at the growth plate and thickening of existing ones. All three strains of mice exhibited a period of high architectural stability in the vertebra, whereas continuous changes occurred in the tibia. This developmental difference most likely resulted from the slow growth period while the growth plate was bridging, which resulted in replacement of a large proportion of the metaphyseal region for the tibia compared with the vertebra. Despite these structural differences, tissue mineralization patterns (TMD) were similar across both sites and all three mouse strains. Longitudinal growth patterns differed in both rate and key developmental milestones with strain-related differences in bone length primarily established during the rapid growth period.

A limitation of this study is that earlier time points may be underestimated because of partial volume effects and undermineralization (75–100% of mature values) of the bone. However, higher-resolution in vivo scans were not possible, and multiple thresholds to account for variations in mineralization could not be selected objectively or consistently through manual or iterative methods. Nonetheless, this limitation is likely not too severe because, for the B6 mice, the patterns we found were generally consistent with...
those of cross-sectional studies of C57BL/6J mice, which have a similar origin but are from a different supplier.\textsuperscript{(11,20)} Also, the source, seasonality, and litter size may be factors that could result in small differences in the development patterns reported here; therefore, these data should not be considered a replacement for the inclusion of normal control experimental groups in future research.

Radiation effects are a consideration with in vivo scanning. In this study, the high and low radiation groups produced the same architectural trends from 6 to 48 wk of age despite being subjected to different radiation doses. Previous reported effects of radiation are variable and range from none in mature rats\textsuperscript{(21,30)} to detectable in growing mice.\textsuperscript{(21)} In the latter study, radiating one limb four times was found to result in decreases of BV/TV and TbN and increases of TbSp compared with the contralateral limb, but doses were almost 4-fold higher than in this study (712 versus 188 mGy), and the scanning interval was much shorter (1–2 versus 2–12 wk).\textsuperscript{(21)} In this study, radiation effects are unlikely because differences between the high and low radiation groups were not significant, and the high radiation group did not consistently have lower BV/TV and TbN or higher TbSp than the low radiation group.

Longitudinal growth patterns in the mouse are consistent with other reports and clarify some previous inconsistencies.\textsuperscript{(1,10,13)} Longitudinal growth of the vertebra proceeded rapidly until \(\sim 8–10\) wk, followed by a slow continuous growth as the epiphyseal plate became bridged and fully fused at \(\sim 8–10\) mo. Previous reports also describe rapid and slow growth periods with either no further growth after 6 mo\textsuperscript{(10,13)} or continuous growth through 12 mo.\textsuperscript{(13)} These discrepancies can be attributed to less frequent sampling and intergroup variability. Reports of growth patterns in the rat are similar to those found here, with comparable timing for the rapid to slow transition\textsuperscript{(31)} and fusion of the growth plate.\textsuperscript{(32)} Longitudinal growth rates and timing were similar between the BAL and B6 strains, whereas they were higher and extended in the C3H mice, leading to notably larger mice at the end of the rapid phase. Both rate and temporal differences in growth patterns during the rapid growth phase were important in establishing large differences in bone length between strains.

To our knowledge, this is the first study to examine temporal changes to the vertebral structure in vivo with sufficient resolution to examine individual trabeculae. Growth clearly followed the model of endochondral ossification and growth of long bones,\textsuperscript{(33,34)} with new trabeculae forming only at the growth plates. High architectural stability was
found after maturation, which is consistent with studies of rats that have found that minimal modeling occurs in the vertebra from 8 wk onward.\(^{(35,36)}\) In a previous cross-sectional study, rapid structural declines were reported in female B6 mice immediately after peak values were reached at 2 mo.\(^{(11)}\) Some possibilities for the discrepancy may be differences in site (L3 versus L5), region of analysis (distance to the growth plate), supplier (Charles River versus Jackson), body composition, and processing methods (constant global versus more sophisticated thresholding). Interestingly, the structural trends for the male B6 mice\(^{(11)}\) were very similar to those found here.

In contrast to the high stability of the vertebral structure, the structure was continuously changing in the proximal tibial metaphysis. Structural development patterns for the tibia were consistent with previous findings for long bones.\(^{(11,20)}\) The higher variability at this site may arise from greater degrees of modeling because of continued longitudinal growth.\(^{(36)}\) Significant differences in trabecular but not cortical properties were found between left and right limbs at maturity (data not shown). Asymmetry in bone properties has been reported in humans\(^{(37–44)}\) and rodents.\(^{(30,45)}\) The use of the contralateral limb as an internal control, which has become common in bone studies\(^{(38,46–51)}\) should therefore be used with discretion (e.g., randomize left and right limbs).

The onset of age-related bone declines we observe is similar in timing to the progressive lengthening of estrus cycles observed in virgin C57BL/6J mice by 9 mo of age.\(^{(52)}\) In a clinical study, BMD was shown to decline in perimenopausal woman with increased cycle length and irregularity (oligomenorrhea) but not those with regular cycles (eume-
Cycle irregularity was associated with decreased estradiol and increased follicle-stimulating hormone, changes that favor bone resorption. The age-related declines we observed may therefore result from reduced ovarian function preceding ovarian failure.

Patterns of bone loss in the mouse differed somewhat from human patterns of bone loss, consistent with long-term studies of C57BL/6J mice. Characteristic age-related changes in humans include reduced BV/TV, TbN, and TbTh and increased TbSp. Preferential loss of horizontal trabeculae leads to increased DA, whereas thinning leads to more rod-like trabeculae reflected by increased SMI. For both the tibia and vertebra, we found that bone loss in mice resulted primarily from decreased trabecular number as opposed to thinning. The lack of change in DA over time suggests there is no preferential loss of horizontal trabeculae in mice. However, similar to humans, we observed that bone loss in the vertebra of the mice was more prominent in the vascular region (i.e., center of the vertebra). The period of bone loss was relatively

### Table 1. Two-Way ANOVA of Architectural Outcomes, as Measured From µCT and Averaged From 6 to 48 wk by Site (L3, Lumbar Spine, Proximal Tibia [PT])

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Site</th>
<th>C3H/HeN (mean ± SE)</th>
<th>BALB/C (mean ± SE)</th>
<th>C57BL/6 (mean ± SE)</th>
<th>Site F (df) [p]</th>
<th>Strain F (df) [p]</th>
<th>Site × strain F (df) [p]</th>
</tr>
</thead>
<tbody>
<tr>
<td>BV/TV (%)</td>
<td>L3</td>
<td>26.4 ± 0.75*</td>
<td>5.1 ± 0.86†</td>
<td>33.9 ± 0.83‡§</td>
<td>671.3 (1,30) [&lt;0.001]</td>
<td>15.5 (2,30) [&lt;0.001]</td>
<td>230.4 (2,30) [&lt;0.001]</td>
</tr>
<tr>
<td></td>
<td>PT</td>
<td>26.0 ± 1.0*‡</td>
<td>21.6 ± 0.7†</td>
<td>8.8 ± 0.3*‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TbTh (μm)</td>
<td>L3</td>
<td>98 ± 2†</td>
<td>98 ± 2†</td>
<td>89 ± 2*‡</td>
<td>152.9 (1,30) [&lt;0.001]</td>
<td>26.9 (2,30) [&lt;0.001]</td>
<td>45.3 (2,30) [&lt;0.001]</td>
</tr>
<tr>
<td></td>
<td>PT</td>
<td>97 ± 2*‡</td>
<td>86 ± 1†</td>
<td>73 ± 1*‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TbSp (μm)</td>
<td>L3</td>
<td>347 ± 5*†</td>
<td>247 ± 2*†</td>
<td>209 ± 3*†</td>
<td>234.6 (1,30) [&lt;0.001]</td>
<td>21.4 (2,30) [&lt;0.001]</td>
<td>126.5 (2,30) [&lt;0.001]</td>
</tr>
<tr>
<td></td>
<td>PT</td>
<td>359 ± 14*‡</td>
<td>347 ± 1*†</td>
<td>602 ± 18*§</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TbN (mm⁻³)</td>
<td>L3</td>
<td>3.33 ± 0.05*†</td>
<td>4.51 ± 1.0*†</td>
<td>5.10 ± 0.06*‡</td>
<td>608.2 (1,30) [&lt;0.001]</td>
<td>11.8 (2,30) [&lt;0.001]</td>
<td>272.1 (2,30) [&lt;0.001]</td>
</tr>
<tr>
<td></td>
<td>PT</td>
<td>3.32 ± 0.11*†</td>
<td>3.22 ± 0.08*†</td>
<td>1.83 ± 0.05*‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ConnD (mm⁻³)</td>
<td>L3</td>
<td>67.5 ± 2.4*†</td>
<td>76.7 ± 2.3*†</td>
<td>106.1 ± 4.5*†</td>
<td>488.0 (1,30) [&lt;0.001]</td>
<td>1.38 (2,30) [0.267]</td>
<td>216.4 (2,30) [&lt;0.001]</td>
</tr>
<tr>
<td></td>
<td>PT</td>
<td>67.0 ± 4.1*†</td>
<td>46.3 ± 2.5*†</td>
<td>19.5 ± 2.1*‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SMI</td>
<td>L3</td>
<td>1.00 ± 0.09*†</td>
<td>0.31 ± 0.10*†</td>
<td>0.80 ± 0.10*‡</td>
<td>790.4 (1,30) [&lt;0.001]</td>
<td>917.7 (2,30) [&lt;0.001]</td>
<td>110.4 (2,30) [&lt;0.001]</td>
</tr>
<tr>
<td></td>
<td>PT</td>
<td>1.37 ± 0.07*†</td>
<td>1.63 ± 0.06*†</td>
<td>2.67 ± 0.04*‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DA</td>
<td>L3</td>
<td>1.31 ± 0.01*†</td>
<td>1.46 ± 0.01*†</td>
<td>1.43 ± 0.02*‡</td>
<td>115.3 (1,30) [&lt;0.001]</td>
<td>39.4 (2,30) [&lt;0.001]</td>
<td>23.3 (2,30) [&lt;0.001]</td>
</tr>
<tr>
<td></td>
<td>PT</td>
<td>1.44 ± 0.01*†</td>
<td>1.49 ± 0.01*†</td>
<td>1.64 ± 0.02*‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CtTh (μm)</td>
<td>L3</td>
<td>216 ± 4*†</td>
<td>175 ± 3*†</td>
<td>162 ± 3*†</td>
<td>879.1 (1,30) [&lt;0.001]</td>
<td>105.6 (2,30) [&lt;0.001]</td>
<td>30.1 (2,30) [&lt;0.001]</td>
</tr>
<tr>
<td></td>
<td>PT</td>
<td>249 ± 2*†</td>
<td>234 ± 2*†</td>
<td>194 ± 2*†</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Significantly different (p < 0.05) than *BALB/C, †C57BL/6, and §C3H/HeN within the site.

Significantly different (p < 0.05) between L3 and PT sites within the strain.

BV/TV, bone volume ratio; TbTh, trabecular thickness; TbSp, trabecular separation; TbN, trabecular number; ConnD, connectivity density; SMI, structure model index; DA, degree anisotropy; CtTh, cortical thickness.

![Figure 7](image-url)
short in this study; for long-term changes, the reader may consult Halloran et al.\textsuperscript{(20)} or Glatt et al.\textsuperscript{(11)}

The vertebra and tibia shared some similarities in development despite differences in structural stability. Trends for TbN and TbSp were similar across sites but with comparable or larger changes in the tibia. For the B6 and C3H mice, structural inhomogeneity increased with age at both sites because trabeculae were not formed and resorbed consistently (TbN was time dependent). In addition, age-related changes onset with similar timing at both sites.

In all mice, the mechanisms of architectural augmentation were (1) formation of new trabeculae at the growth plates and (2) thickening of existing trabeculae. The ability to form new trabecular connections away from the growth plate has been a contentious issue\textsuperscript{(62)} because of the reduced strength and increased risk of fracture caused by decreased trabecular number and connections associated with aging and diseases.\textsuperscript{(63)} such as osteoporosis. In this study, new trabeculae did not form away from the growth plate, even at a young age, providing support for the hypothesis that trabeculae are not reformed once lost. The loss of connectivity is often thought to arise from resorption of trabeculae. Interestingly there was an early rapid decrease in ConnD for all strains and sites, including the BAL mice where TbN was maintained. In this case, decreased connectivity was associated with the transformation of rods into plates and not resorption of trabeculae, highlighting the need for careful interpretation of changes in ConnD.

Mineralization patterns were similar for the three strains of mice based on relative TMD but not BMD. BMD varied temporally and in magnitude by site and strain, whereas TMD values provided a much simpler pattern with similar mineral accumulation for all sites and strains. This finding is comparable to those of Rubin et al.,\textsuperscript{(14)} who found mineral shape and distribution was similar for B6 and C3H mice despite large differences in BMD. Roschger et al.\textsuperscript{(64)} also found constant mineral density distributions for normal individuals despite ethnic groups, skeletal site, age, and sex.

In our study, the plateau of TMD values after peak structural values is reasonable and within the normal 3- to 6-mo lag for new bone to achieve complete mineralization.\textsuperscript{(65)} In addition, our results overlap with those of a previous report,\textsuperscript{(13)} which together provide a complete mineralization curve (Eq. 1) and a means of adjusting threshold values when analyzing growing bones by \(\mu\)CT.

Structural adaptations corresponding to strength changes at the tibial diaphysis occurred more quickly in the B6 and C3H mice than BAL mice. In the B6 and C3H mice, compressive and torsional rigidity (CtA and J) increased significantly during the period of rapid longitudinal growth and became stable or changed gradually. For B6 mice, changes in torsional rigidity (J) are consistent with those reported by Somerville et al.,\textsuperscript{(10)} and changes in compressive rigidity (CtA) similar to trends for cortical thickness by Glatt et al.\textsuperscript{(11)} In BAL mice, geometric changes were gradual, similar to the architectural trends. Overall, the B6 and C3H mice had the highest torsional and compressive rigidities, respectively, whereas BAL mice had the lowest properties in both cases.

In summary, this study provides a detailed characterization of normal skeletal development patterns for three inbred strains of mice commonly used in bone research. The findings were consistent with previous data from the literature, and repeated-measures analysis provided novel insight into the patterns of development. Future studies should concentrate on the period up to 12 wk for growth-related changes, 12–32 wk for mature values, and from 32 wk onward for age-related deterioration. For future studies with mice in the mature phase, specific timing should be selected based on the mouse strain, site, and region of interest (cortical versus trabecular). Furthermore, because of the high stability of the vertebra compared with the tibia (and possibly other long bones), this site is well suited for studying the effects of different interventions, particularly if in vivo analysis is not feasible. Finally, of the strains considered, the BAL mice had the most homogeneous and least variable bone structures, with reasonable proportions of trabecular bone at both the tibia and vertebra. Altogether, this study provides critical knowledge of normal bone changes and skeletal milestones necessary to optimally plan and interpret bone studies examining the effect of age, injury, and disease.

**ACKNOWLEDGMENTS**

This work was supported by grants from the Alberta Heritage Foundation for Medical Research, Natural Sciences and Engineering Research Council of Canada (NSERC), the Canadian Institute of Health Research (CIHR), and the Alberta Ingenuity Fund (AIF). The authors acknowledge the support of Dr Tak Fung for assistance with the statistical analysis and Dr Heather MacDonald for insight in bone development.
REFERENCES


44. Watson KM, Stitson DJ, Davies HM 2003 Third metacarpal

Address reprint requests to:
Steven K Boyd, PhD
Department of Mechanical and Manufacturing Engineering
University of Calgary
2500 University Drive NW
Calgary, AB T2N 1N4, Canada
E-mail: skbboyd@ucalgary.ca

Received in original form February 25, 2008; revised form May 15, 2008; accepted July 31, 2008.