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Original Article

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Differences in vertebral structure and strength of inbred female mouse strains

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Abstract

This study assessed mouse strain-related differences in vertebral biomechanics and histomorphometry in inbred mice strains shown to differ in bone mineral content (BMC) and areal density (BMD) (as measured by pDEXA). Lumbar vertebrae L3 to L5 were collected from three mice strains (C3H/HeJ[C3], C57BL/6J[B6], and DBA/2J[D2], n=12/strain, 4-month-old female, 22.2±0.3g). BMC and BMD were measured in L3 and L4 using peripheral dual energy x-ray absorptiometry. The L4 vertebral body was then mechanically tested in compression to determine structural properties (ultimate/yield load, stiffness) from load-displacement curves and derive apparent material properties (ultimate/yield stress, and modulus of elasticity). L5 was processed for histomorphometric evaluation. Vertebral BMC and BMD were greater in C3 than in B6 and D2 mice. Vertebral trabecular/cancellous bone volume was smaller in C3 than in D2 and B6 mice. Trabecular bone formation rates were greater in D2 than in B6 and C3 mice. Osteoid surface was smaller in C3 mice than in B6 and D2 mice. Differences in osteoclast and mineralizing surfaces were not detected among the three mouse strains. In addition, there were no significant differences in biomechanical properties between the three strains. Despite the greatest BMC and areal BMD in C3 mice, the lack of strain-related differences in vertebral body strength data suggests that the biomechanical properties may be affected by the bone distribution and/or complex combination of cortical and cancellous bone at this site.

Keywords: *B6, D2, C3,* Genetic, Vertebral Body, Strain, Density, Histomorphometry, Bone Strength, Mice

Introduction

Physical properties of bone (size, shape, density, etc.) are regulated by a complex interaction of genetic (heritability) and environmental factors[1-4]. Studies in humans have shown that 50 to 80 percent of variability in bone mass is genetically determined (heritable)[1-3]. The linkage of specific chromosomal loci with high bone mass and osteogenesis imperfecta provides evidence that bone mass and strength are genetically regulated[5-8].

The majority of studies[9-23] pertaining to genetic regulation of bone phenotypes have been carried out with females of inbred and recombinant mice strains that included C3H/HeJ (C3), C57BL/6J (B6), DBA/2J (D2), and B6xC3. Among the various bone characteristics (size, strength, adaptation), bone mass and density (areal and volumetric BMD) were widely used to study genetically-regulated skeletal differences among all the three inbred mice (C3, B6, D2)[10,11], between C3 & B6[12,19,20,22], and between B6 & D2[23]. These mice strains have been shown to differ in their adult (4-month-old, female) femoral and vertebral peak bone mass, volumetric density (vBMD, pQCT)[11], bone architecture, size (cortical thickness, femoral cross sectional area, trabecular thickness)[20], and femoral structural strength[10,20]. The adult female C3 mouse strain has greater femoral (4-month-old) and vertebral (12-month-old) cortical bone density (pQCT)[11] than the B6 and D2 strains. In addition, as shown in various studies, C3 mouse strain has greater mid-shaft femoral structural strength as compared to B6[10,20] and D2[10]. However, the tibial bone adaptation response, in 4-month-old adult female mice, to a known and equal mechanical stimulus[9] or disuse[14] is lowest in C3.

Inbred mice are identical where genetic makeup is similar within each inbred strain, and therefore offer a useful model for studying the strain (genetic) specific control of bone physical properties (size, shape, mass, density, strength etc.).

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In addition, the influence of environmental factors (e.g., nutrient intake) on bone mass and strength can be investigated within genetically identical animals[13,16,18]. Genetic analysis[12] suggests that there are at least three quantitative trait loci (QTLs) that are responsible for the BMD at both femoral and vertebral body sites, and therefore presents a good opportunity for studies verifying that the same QTLs are also linked to bone strength properties.

Genetic differences in bone mass, structure, strength, and adaptation have been characterized in cortical bone of several inbred[9-12,14,15,17,18-20] and recombinant[21,23] strains of mice. Although micro-CT analyses of vertebral bodies in 4-month-old C3 mice showed fewer trabeculae, the compressive structural strength did not differ from B6[20]. The pQCT measurement in L5 vertebral body confirmed C3's greater total BMC and vBMD than B6, however, no histomorphometric and strength data were measured in these 4-month-old female mice[12]. In addition, C3's (12-month-old) vertebral cortical density (vBMD, pQCT) was the highest when compared to B6 and D2[11]. In the vertebral bodies of growing mice (1.5- and 3-month-old), percent trabecular bone volume and mineral apposition rate were greater in C3 as compared to B6[22]. However, there are no published investigations in which both dynamic histomorphometry and strength (both structural and apparent material) differences among the three strains (C3, B6, D2) have been assessed using vertebral bodies from the same animals. Thus there is no simultaneous explanation of differences in vertebral body bone formation and quality (strength).

A detailed vertebral body histomorphometric analysis and biomechanical testing may help to understand the mechanism of cancellous bone adaptation and structure in these inbred mice strains. The vertebral body (cancellous) bone mass and strength, along with histomorphometric data, are critical for understanding the heritability of the bone physical properties in future studies. In addition, the extent of inter strain-related variation in bone turnover and cortical and cancellous tissue volume in vertebral bone in these animals are not well defined. Therefore, the focus of this paper is to characterize strain-related differences in vertebral body bone mass, strength, size, and histomorphometry in 4-month-old female C3, B6, and D2 mice.

Material and methods

C3, B6, and D2 female inbred mice (n=12/strain) were obtained from Jackson Laboratories (Bar Harbor, ME). Mice from each strain were housed (6/cage) together until 4-month of age in shoebox cages under standard environmental conditions. The animals were maintained on a normal laboratory diet (Harlan Teklad Rodent Diet, 1.36%Ca, 1.0%P, 23%protien, WI, USA) with food and water provided *ad libitum*. The Creighton University Animal Research Committee approved the experimental protocols.

Each mouse was injected with calcein (Sigma Chemical Co., St. Louis, MO; 7 mg/kg, sq.) at 10 and 3 days prior to death to quantify active mineralization sites. At the time of necroscopy (for sample collection), each mouse was first anesthetized by intraperitoneal injection with ketamine (50mg/kg) and xylazine (10mg/kg) (Fort Dodge Lab. Inc., IA) and then blood was drawn via cardiac puncture causing death by exsanguination. Lumbar vertebrae (L3-4 and L5) were excised and cleaned of soft tissue. L5 vertebral bodies were placed in 70% ethanol for histomorphometric assessment. L3-4 vertebral bodies were placed in saline solution and stored frozen (-20°C) for densitometric, morphometric, and biomechanical analyses.

Bone Densitometry: Lumbar (L3-4) vertebrae BMC and BMD were measured using peripheral dual energy x-ray absorptiometry (pDEXA) (Norland Corp.; Ft. Atkinson, WI) with a resolution of 0.2 μm x 0.2 μm and scan speed of 2 μm/sec. Each vertebral body was scanned *ex vivo* along the cranial-caudal direction for density measurements. During each scan and analysis, the whole vertebral body was considered the region of interest.

Cancellous Bone Histomorphometry: Lumbar (L5) vertebral bodies were processed using standard histomorphometric techniques to analyze for static and dynamic endpoints. The lumbar vertebrae (L5) were placed in Villanueva stain for 3 days, dehydrated in graded ethanols and acetone, and embedded undecalcified in modified methyl methacrylate[24]. Frontal sections (5 μm thick) were cut from each vertebra with a vertical bed microtome (Jung Supercut 2050) and affixed to slides. Two slides were stained by the Goldner method[25] and used for determining structural and static endpoints. Two slides were coverslipped without further staining and used for determining dynamic endpoints of bone formation. Histomorphometric data were collected with the Bioquant TCW software (R&M Biometrics, Nashville, TN). The data are reported in accordance with standard bone nomenclature[26].

For data collection, the measurement area consisted of secondary spongiosa at distances greater than 0.5 μm away from the cranial and caudal growth plates. The following measurements were taken: at 20X, total tissue area (Tt.Ar); at 40X, total trabecular area (Tb.Ar) and perimeter (Tb.Pm); at 200X, single and double labeled perimeter (sL.Pm and dL.Pm); and at 400X, the interlabel width of double labels (Ir.L.Wi), osteoid perimeter (O.Pm), and osteoclast perimeter (Oc.Pm). The following structural and static calculations were made: cancellous bone surface (BS=Tb.Pm), cancellous bone volume (BV=Tb.Ar/Tt.Ar), trabecular thickness (Tb.Th=2 x B.Ar/BS), trabecular number [Tb.N=(BV/TV)/Tb.Th], trabecular spacing [Tb.Sp=(1/Tb.N)-Tb.Th], osteoid surface [OS=(O.Pm/Tb.Pm) x 100], and osteoclast surface [Oc.S=(Oc.Pm/Tb.Pm) x 100]. The following dynamic calculations were made: single labeled surface [sLS=(sL.Pm/Tb.Pm) x 100], double labeled surface [dLS=(dL.Pm/Tb.Pm) x 100], mineralizing surface [(MS=dLS+0.5sLS) x 100], mineral apposition rate [MAR=Ir.L.Wi/Ir.L.t (interlabel time period)], and bone formation rate (BFR/BS=MS x MAR).

In addition to the above, an average of 42 measurements

per slide were done to quantify cortical thickness along the cranial-caudal direction (cortical shell, at the mid-section of the vertebral body) with 4X magnification and averaged.

Biomechanical Testing: Vertebral bodies (L4) were isolated from posterior elements using the Dremel tool (Dremel, Racine, WI) and prepared with flat and parallel cranial and caudal ends by removing only the soft cartilage (inter-vertebral body disc) material and exposing the bone surface. These vertebral bodies were subjected to mechanical testing in compression using a servo-controlled mechanical testing machine (Instron 5543, Canton, MA). All biomechanical tests were conducted at room temperature and at a rate of 3mm/minute. Structural parameters that include ultimate load, yield load, and stiffness were measured from the load-displacement curve for each bone specimen. Ultimate load is the maximum load that a specimen (maximum height of the curve) takes before fracturing. Yield load is the load at which the permanent damage/deformation is incurred in the specimen. The yield load was estimated to be the intersection point of the load displacement curve and a line parallel to the linear portion of the load-displacement curve but offset 0.2% of the initial specimen length[27-29]. The cross sectional area of each vertebra was measured on a medial and lateral (transverse) plane directly adjacent to the fracture site (Figure 1). The fracture site was identified under the microscope as the site that showed a rough surface as one plane of the bone start to slide onto the adjacent plane along cranial axis. The cross sections were traced at 50X on a profile projector (Nikon, V-10). Cross sectional areas were calculated using the program ARM (Biomechanics Lab, Creighton University) on a VAXstation 2000 (Digital Equipment Corporation; Maynard, MA). The apparent material properties (ultimate/yield stress and modulus of elasticity)[30] were derived from the structural properties and cross sectional size (at mid section anterior posterior plane) of each vertebral body. The apparent material strength does not represent the true intrinsic material strength properties. In addition, the apparent material strength properties include contribution from both the cortical shell and the trabecular bone. The ultimate stress, yield stress, and compressive modulus of elasticity (E) were calculated from the following equations:

$$\text{Ultimate or Yield stress} = F/A \quad (1)$$

$$E = \text{stiffness}*L/(A) \quad (2)$$

Where F is either ultimate or yield force (load) obtained from the load-displacement curve of each mechanical test. The ultimate and yield stresses are calculated (Equation 1) by using ultimate and yield forces respectively. L is the vertebral body length along the cranial-caudal axis, and A is the cross sectional area[27].

Statistical Analysis: Data were analyzed using one-way ANOVA followed by a Tukey HSD post-hoc test[31]. Data were expressed as mean±SEM. In addition, multivariate analysis using the analysis of covariance (ANCOVA) approach (SAS Inst, Inc., Cary, NC, USA) was also performed to determine all the variables that have significant effects on the structural strength (ultimate load) of the vertebral body. All p-values obtained from the statistical tests were considered significant if $p<0.05$.

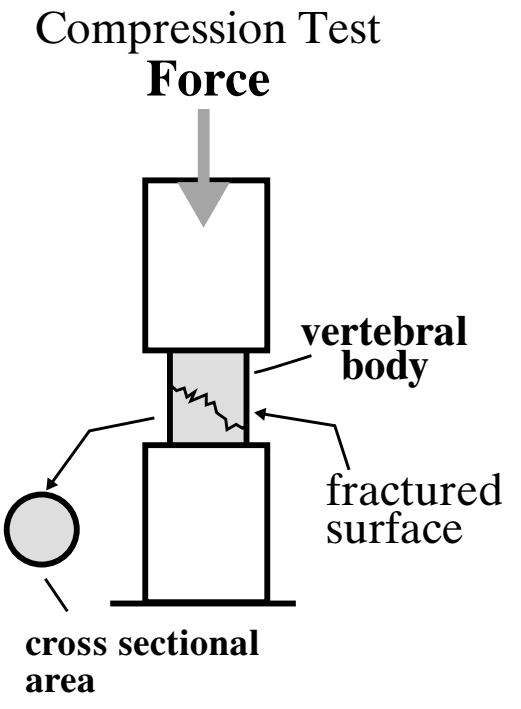

Figure 1. Compression test of vertebral body. Each vertebral body was tested along cranial-caudal axis. Prepared vertebral bodies with parallel and flat ends were subjected to compressive force/load. Bone fractures occur at mid-length of the vertebral body where cross section area is also measured to calculate ultimate stress, yield stress, and modulus.

Results

The body weights were similar (mean±SEM: 23.0± 0.3 [C3]; 22.0± 0.3 [B6]; 21.6± 0.4 [D2]) among the three mouse strains. Vertebral (L3-4) bone mineral content (BMC) and areal density (BMD) were respectively ~29% and 13% greater in C3 mice than in B6 and D2 mice (Table 1). The BMC and BMD were not different between B6 and D2 mice. The mean cross sectional area of the L4 vertebral body (biomechanical test specimen) was ~17% greater in C3 and D2 than in B6 mice (Table 2). The mean cross sectional area between C3 and D2 was not different.

Vertebral (L5) cancellous bone volume was ~47% greater in B6 and D2 mice than in C3 mice (Table 2). Bone volume was not different between B6 and D2 mice. Although trabecular number was ~66% greater and trabecular separation was ~74% lower in B6 and D2 mice compared to C3 mice, trabecular thickness was greatest (~13%) in the C3 mouse strain (Table 2). Trabecular number, spacing, and thickness were not different between B6 and D2 mice.

Dynamic histomorphometry of vertebral cancellous bone revealed that osteoclast surface, an index of bone resorption,

Variables	C3H/HeJ (C3)	C57BL/6J (B6)	DBA/2J (D2)
Weight (g)	23.0±0.3	22.0±0.3	21.6±0.4
BMC (mg)	14.2±0.6	10.5±0.3 [a]	11.3±0.4 [a]
BMD (mg/cm^2)	60.4± 1.0	53.0±0.6 [a]	52.5±0.6 [a]
Different than C3 [a] $p<0.05$.			

Table 1. Mice body weight, vertebral (L3-4) Bone mineral content and density.
(Mean ± SEM)

	C3H/HeJ (C3)	C57BL/6J (B6)	DBA/2J (D2)
Length (cranio-caudal mm)	2.6± 0.1 [b]	2.4±0.1	2.6±0.1 [b]
Cross sectional area (mm^2)	2.6±0.1 [b]	2.2±0.1	2.4±0.1 [b]
Cancellous bone volume (%)	18.4±1.6	26.3±1.2 [a]	27.8±25.3 [a]
Trabecular thickness (μm)	58.9±2.3	51.5±1.6 [a]	52.4±1.9
Trabecular number (#/μm)	3.1±0.2	5.1±0.1 [a]	5.3±0.1 [a]
Trabecular separation (μm)	327±21	192±4 [a]	184±5 [a]
Osteoclast surface (%)	0.35±0.14	0.10±0.04	0.19±0.04
Osteoid surface (%)	3.8±0.6	7.4±1.7	6.7±0.8
Mineralizing surface (%)	27.8±2.3	29.7±1.8	29.4±1.3
Mineral apposition rate (μm/d)	1.22±0.05	1.15±0.02	1.50±0.03 [a b]
Bone formation rate (μm^3/μm^2/d)	0.12±0.01	0.12±0.01	0.16±0.01 [a b]
Cortical shell thickness (mm)	0.10 ±0.01	0.11±0.01	0.13±0.01 [a]
Different from C3 [a] $p<0.05$ Different from B6 [b] $p<0.05$			

Table 2. Vertebral (L5) Histomorphometry
(Mean ± SEM)

did not differ among the three strains of mice. Osteoid surface was marginally (P=0.08) greater (~70%) in B6 and D2 mice than in C3 mice (Table 2). Osteoid surface was not different between B6 and D2 mice. Differences in mineralizing surface were not detected among the three strains of mice. However, mineral apposition rates and bone formation rates were respectively ~26% and ~33% greater in D2 than in either C3 or B6 mice (Table 2). The mineral apposition rates and bone formation rates were not different between C3 and B6 mice.

In addition to the cancellous bone endpoints, thickness of the vertebral body (L5) cortical shell was measured in each of the three mouse strains. The vertebral cortex was ~13% thicker in D2 than in either C3 or B6 mice (Table 2). The

Variables	C3H/HeJ (C3)	C57BL/6J (B6)	DBA/2J (D2)
Ultimate load (N)	13.4±1.7	12.2±1.0	11.6±1.3
Yield load (N)	7.9±1.6	8.0±1.0	6.6±0.8
Stiffness (N/mm)	134±13	108±14	106±11
Ultimate stress (N/mm^2)	5.3±0.8	5.7±0.5	4.7±0.4
Yield stress (N/mm^2)	2.9±0.6	3.7±0.4	2.7±0.2
Modulus (N/mm^2)	140±15	121±17	118±13

Table 3. Vertebral (L4) biomechanical properties
(Mean ± SEM)

vertebral cortex (cortical bone) was not different between B6 and C3 mice. In L4 test specimens vertebral lengths (cranio-caudal height) in C3 and D2 were significantly greater (~11%) than in B6 (Table 2). The cranio-caudal length was not different between C3 and D2 mice.

Significant differences in the structural (ultimate load, yield load, stiffness) and apparent material (ultimate stress, yield stress, modulus) strength properties of L4 were not detected among the three mouse strains. However, C3 mice had a marginally (P=0.09) higher modulus of elasticity than B6 and D2 mice (Table 3).

The structural strength (ultimate load) was significantly affected by cross sectional area (P=0.018), bone mineral density (P=0.045), and mice strain/breed (P=0.045). However, bone mineral content (P=0.066) and cortical thickness (P=0.067) only marginally affected the vertebral bone strength.

Discussion

This study further characterizes the vertebral body phenotypes in 4-month-old adult females from three-mouse strains (C3, B6, D2) and provides valuable information for future genotyping work. The use of adult (4-month-old, female) mice offered a steady state condition in skeletal growth. The vertebral bodies (L3-4) were analyzed for bone mineral content (BMC), areal bone mineral density (BMD) and biomechanical strength (L4). Histomorphometric (L5) measurements were made in three inbred mouse strains that are commonly used for research in bone genetics. Inbred strain-related differences in vertebral body BMC, areal BMD, cross sectional area, modulus, and both static and dynamic histomorphometric measurements were noted in these mouse strains.

Data regarding the phenotypic characterization of cortical and trabecular bone in C3 and B6 mice have been reported previously[12,20,22]. Turner et al.[20] presented whole vertebral body and mid-shaft femoral bone structural and strength properties using micro-CT and strength data in 4-month-old female B6 and C3 mice without histomorphometric analysis of the cancellous bone. In addition, Sheng et al.[22] also suggested site-specific differences in vertebral body and distal femur trabecular bone histomorphometric measurements in growing female B6 and C3 mice (1.5- and 3-month-old). Although pQCT measurement in vertebral body show C3's greater total BMC and vBMD than B6 in 4-month-old[12], and greater than B6 and D2 in 12-month-old mice[12], no histomorphometric and strength data were available in all three mice strains. The current study presents histomorphometric and strength data in vertebral bodies of 4-month-old female inbred (C3, B6, D2) mouse strains.

Despite differences in bone volume (with least in C3) and cross sectional area (with least in B6) (Table 2), there were no differences in either structural or apparent material properties among the three inbred mouse strains. The combination of bone volume (mass), and its distribution (cross sectional area) determines the structural strength of vertebral bodies. For example, in C3 mice, the larger vertebral body cross sectional area was expected to contribute towards greater structural strength (ultimate load). Based on our previous work in cortical (femur and tibiae) and cancellous bone in distal femur[10], the C3 mice were also expected to have the greatest mineralizing surface, mineral apposition and bone formation rates leading to the strongest vertebral bodies. The static histomorphometric measurements are helpful in explaining the discrepancy between bone mass (BMC, BMD) and vertebral body strength in C3 mice. For example, as compared to both B6 and D2, the lower bone volume resulting from relatively lower trabecular number may have wiped out any structural strength advantage resulting from greater cross sectional area in C3 mice.

In addition, as compared to both B6 and D2, the discrep-

ancy between low trabecular volume and greater BMC and BMD in C3 mice can be explained on the basis of compact or cortical bone in the vertebral body shell. The cortical bone may provide the bulk of the BMC and BMD and compensate for the low trabecular bone volume. Although the cortical shell thickness in C3 is similar to B6 and smaller than D2, it does contribute to the total area. Therefore, as compared to trabecular bone volume, there has to be more cortical bone[20] in C3 vertebral bodies in order to maintain relatively greater BMC and BMD.

The multivariate analysis confirms the significant contribution of bone mineral density, cross sectional area and mice strain on bone structural strength. In addition, the analysis also suggests that the bone mineral content and cortical thickness have marginal influences on vertebral bone structural strength. Although C3's fewer but thicker trabeculae and relatively greater (>B6) cross sectional area (Table 2) did contribute to structural strength, the contribution was not enough to have vertebral body strength different than D2 and B6 mice. In addition, the strain-related differences in the vertebral body cortical shell thickness were also responsible for a lack of variation in bone strength. For instance, as compared to C3 mice, the D2, with greater cortical thickness, may have adjusted for its lower BMC such that bone strength (both structural and apparent material) was similar.

It is clear that the morphology of vertebral bodies varied among the three inbred mouse strains with at least one of the variables different within each strain as compared to the other two. The vertebral structural and apparent material properties were similar, suggesting that the genetic regulation and complex combination of trabecular volume, cortical shell thickness, and areal distribution of mass produce a structure that has similar strength characteristics designed to carry similar body weight in these mice.

As compared to both the B6 and D2, the lack of trabecular bone in C3 vertebral bodies has been attributed to bone resorption due to stress shielding[20]. For example, Turner et al.[20] suggested stress shielding resulting from a thicker cortical shell in C3 mice was responsible for lack of trabecular bone in vertebral bodies. However, in this study, vertebral body cortical shell thickness was relatively greater in D2 and not different between C3 and B6 mice. Despite greater cortical shell thickness in D2 mice, the trabecular bone volume was greater than C3, suggesting that genetic regulation of trabecular bone may be independent of stress shielding.

Although we reported lower osteoclast and greater osteoid surface trends in the distal femur (cancellous bone) of C3 mice[10], no significant differences were found in vertebral body osteoclast surface among the three mouse strains. In addition, the vertebral body osteoid surfaces show lower trends in C3 as compared to the B6 and D2 mice, suggesting that genetic effects on cancellous bone formation in distal femur[10] and vertebral body are site-specific and may be complicated. For instance, as compared to B6 and D2, the C3 mice showed greater trabecular number (Tb.N) and bone volume (BV/TV) in the distal femur[10] but smaller Tb.N and BV/TV in the vertebral body. The Tb.N and BV/TV in B6 mice were greater at the distal femur site[10], but they were not different than D2 mice at the vertebral site, suggesting site-specific genetic regulation of cancellous bone formation in distal femur and vertebral bodies.

Both mineral apposition and bone formation rates in vertebral bodies in this study, and mineralizing surface in distal femoral sites in another study[10] were also different among these mouse strains at adult age (4-month-old). While both mineral apposition rates and bone formation rates in cancellous bone are greatest in the D2 vertebral body, no differences were found at the distal femur in these mouse strains[10]. In addition, in growing mice, both endocortical and periosteal bone formation in the C3 were greater than in B6 at both the tibial and femoral sites[19]. This suggests that in these mice, strain-related differences in bone mineral apposition and formation rates also vary with anatomical site such that the cortical bone (mid-shaft tibia & femur) different than the cancellous/trabecular bone (vertebral body and distal femur)[10].

Although, there were no differences between B6 and D2 mice in their bone volume, trabecular number and thickness (Table 2), the mineral apposition rate (MAR) and bone formation rate were greatest in D2 mice. The greater bone formation rate suggests that as compared to both C3 and B6, the D2 mice are still forming bone at a faster rate at 4 months of age and that the genetic regulation of bone formation is different.

It is important to note that as compared to B6 the C3 mice had greater vertebral body BMC partly due to greater length and cross sectional area (resulting in larger total volume). However, the lower cancellous bone volume (% BV/TV) was the result of fewer but thicker trabeculae, which was accompanied by lower cortical thickness of the vertebral body. And yet, when calculated using the projected area (pDEXA), the BMD was greater in the vertebral bodies of the C3 mice and responsible for higher (but not statistically significant) modulus (Table 3). In addition, despite similar vertebral size, C3's higher BMC may also explain their denser bones (more mineral packed/volume) as compared to D2 mice. Although modulus tended to be greater in C3 mice, the lack of correlation between BMC and structural strength parameters (ultimate, yield load and stiffness) may be due to the differences in the distribution of bone mass within the vertebral bodies of each mouse strain. The three inbred mice strains (C3, B6, D2) are good models to investigate the genetically regulated bone phenotypes (BMC, BMD, structure, etc.) and locating the responsible QTLs as demonstrated in several other studies[12,21,23].

A previous study[12] used a highly heritable volumetric BMD (pQCT) phenotype to define quantitative trait loci (QTL) in both femurs and vertebral bodies, suggesting a set of common QTLs involved in the regulation of BMD. The areal bone density (pDEXA), tissue distribution, and biomechanical strength data in the vertebral body (current study) and femur[10] can be used either to verify the QTLs for BMD and/or find additional QTLs for bone structure and

strength. Like volumetric BMD[12] it is logical to expect to find several QTLs associated with bone tissue distribution, structure and resulting strength. Genetic regulation of bone structure and strength in cortical and trabecular bone is key to understanding the skeletal bone adaptation response to an environment that induces mechanical stimuli or exercise[9,17]. Identification of QTLs related to bone structure and strength will lead to a better understanding of the gene-by-environment interaction of bone adaptation.

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