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Bone Brittleness Varies with Genetic Background in A/J and C57BL/6J Inbred Mice

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ABSTRACT

The contribution of genetic and environmental factors to variations in bone quality are understood poorly. We tested whether bone brittleness varies with genetic background using the A/J and C57BL/6J inbred mouse strains. Whole bone four-point bending tests revealed a 70% decrease in postyield deflection of A/J femurs compared with C57BL/6J, indicating that A/J femurs failed in a significantly more brittle manner. Cyclic loading studies indicated that A/J femurs accumulated damage differently than C57BL/6J femurs, consistent with their increased brittleness. Differences in matrix composition also were observed between the two mouse strains. A/J femurs had a 4.5% increase in ash content and an 11.8% decrease in collagen content. Interestingly, a reciprocal relationship was observed between femoral geometry and material stiffness; this relationship may have contributed to the brittle phenotype of A/J femurs. A/J femurs are more slender than those of C57BL/6J femurs; however, their 47% smaller moment of inertia appeared to be compensated by an increased tissue stiffness at the expense of altered tissue damageability. Importantly, these differences in whole bone mechanical properties between A/J and C57BL/6J femurs could not have been predicted from bone mass or density measures alone. The results indicated that bone brittleness is a genetically influenced trait and that it is associated with genetically determined differences in whole bone architecture, bone matrix composition, and mechanisms of cyclical damage accumulation. (J Bone Miner Res 2001;16:1854–1862)

Key words: genetics, bone biomechanics, skeletal fragility, inbred mice, bone density, bone quality

INTRODUCTION

THE NUMBER of osteoporosis-related hip fractures is expected to increase from 1.7 million in 1990 to 6.3 million in 2050.[1] Given the mortality, disability, and cost associated with fragility fractures, identifying the factors that contribute to fracture risk has become increasingly important for improved diagnosis, treatment, and prevention.[2] Peak bone mass, which is defined by genetic and environmental factors,[3] has been postulated to be an important risk factor. However, measures of bone mass and bone density have been inconsistent predictors of fracture risk.[4–8] Although altered bone quality has been recognized as an additional determinant of fracture risk,[9–11] the genetic and environmental contributions to variations in bone quality, the correlation between bone quality and fracture risk, and the relationship between bone quality and bone mass are poorly understood.

The objectives of this study were to determine if bone quality varies with genetic background. Bone quality is a generic term that refers to a wide spectrum of tissue mechanical properties such as elastic modulus, strength, tough-

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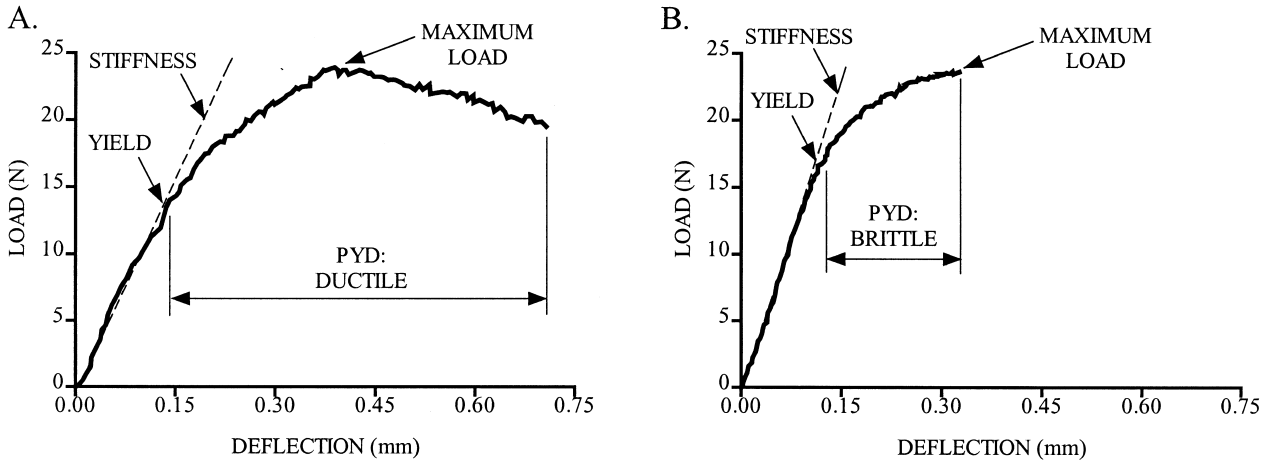

FIG. 1. Load-deflection diagrams illustrating the differences in the mechanical properties of (A) ductile and (B) brittle materials. The graphs show that stiffness and maximum load can be similar for ductile and brittle materials. The most dramatic difference between the two materials is that brittle materials fail almost immediately after reaching the yield point and, therefore, exhibit a significantly reduced postyield deflection (PYD). The decreased PYD results in a dramatic decrease in the amount of work or energy the material can absorb before failure. Work to fracture is determined by calculating the area under the graph and this property can be used only as a measure of brittleness if no differences in stiffness and maximum load exist between the two materials.

ness, creep, and brittleness. The specific tissue mechanical properties that contribute to bone fragility are not well understood. Based on the observation that the energy absorbing capacity of bone changes with age and shows greater interindividual variability than other mechanical properties,[12] we and others have postulated that the way bone fails (i.e., ductile failure vs. brittle failure) contributes to fracture risk.[13–15] Brittle failures tend to be catastrophic, like chalk, and require less energy for fracture. This reduced energy to fracture is manifest as a dramatic loss in postyield deflection compared with ductile materials (Fig. 1). The magnitude of postyield deformation can be used as a simple mechanical phenotype in genetic analyses.[14] It is important to note that ductile and brittle materials may exhibit similar stiffness and strength and, therefore, cannot be differentiated easily from these properties.[16]

Inbred mouse strains represent an important tool to study the effects of genetic background on bone morphology,[17–21] density,[22–23] and mechanical properties.[24–26] The influence of genetic background on bone brittleness has not been investigated fully. Kaye and Kusy[24] reported that the A/J and C57BL/6J inbred strains exhibit significant differences in whole bone ductility and fracture toughness, suggesting that these two inbred strains are potential models to study the effects of genetic background on bone brittleness. In this study, we conducted further mechanical, morphological, and compositional tests of long bones from the A/J and C57BL/6J inbred mouse strains to determine how genetic background affects a mechanical property like brittleness. Unlike previous studies examining bone mineral density (BMD),[22] the phenotypic marker used in this study was postyield deflection. Given that brittleness is associated also with altered tissue damageability,[13–15] the damage accumulation behavior was assessed for the two inbred strains as a second measure of brittleness. Differences in whole bone mechanical properties between A/J and C57BL/6J femurs were explained further by quantifying cross-sectional geometry, microhardness, ash content, collagen content, and tissue microstructure.

MATERIALS AND METHODS

Animals

Male A/J and C57BL/6J mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA) at 4–6 weeks of age. All mice were killed at 14 weeks of age so the long bones were at peak bone mass.[27] Mice were fed a standard mouse chow (Teklad 8664; Harlan, Indianapolis, IN, USA) ad libitum, kept on a 12-h light/dark cycle, and housed with 5 mice per cage. The handling and treatment of all mice was approved by the Committee on Animal Care at Case Western Reserve University (Cleveland, OH, USA). Immediately after death, the right and left femurs were harvested; cleaned of soft tissue; and segregated into biomechanical, histological, and compositional test groups. Femurs used in biomechanical and compositional analyses were stored frozen at −20°C before testing. Femurs used in histological analyses were prepared for embedding immediately after death. The number of independent samples (n) is noted for each test. No femur was used in more than one mechanical test.

Whole bone monotonic tests

To test for differences in whole bone failure properties between inbred strains, one femur from each mouse (A/J, $n = 19$; C57BL/6J, $n = 20$) was loaded to failure in four-point bending at 0.05mm/s, similar to that described previously.[14] All whole bone tests were conducted by loading the femurs in the posterior to anterior direction, such that the anterior quadrant was subjected to tensile loads. Contralateral femurs were used to assess cross-sectional geometry. The widths of the lower and upper

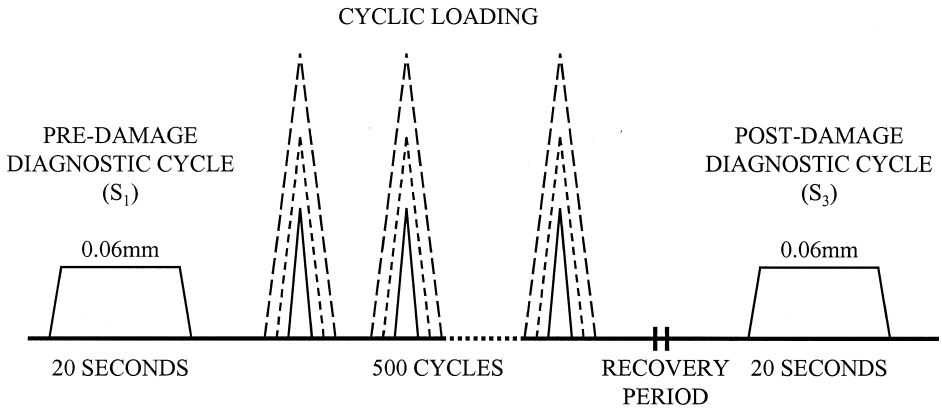

FIG. 2. Schematic illustration of the loading protocol used to quantify degradation in whole mouse femurs. Damage was induced during cyclic loading and degradation was quantified by comparing the loading stiffness of the predamage diagnostic cycle (S_1) and the loading stiffness of the postdamage diagnostic cycle (S_3). A 4-minute recovery period was introduced to allow time for transient effects to dissipate.

supports of the four-point bending apparatus were 6.35 mm and 2.2 mm, respectively. The load-deflection curves were analyzed for postyield deflection, which was defined as the deflection at failure minus the deflection at yield. Yield was defined as a 10% reduction in the secant stiffness (load range normalized for deflection range) relative to the initial tangent stiffness. The stiffness, maximum load, and work to fracture were reported also. Femurs were tested at room temperature and kept moist with phosphate-buffered saline (PBS).

Whole bone damage tests

To test whether the variations in bone brittleness were associated with altered tissue damageability, femurs from A/J ($n = 9$) and C57BL/6J ($n = 10$) mice were subjected to a damage accumulation protocol (Fig. 2) similar to that described previously.[28] Damage was induced by loading femurs in four-point bending for 500 cycles using a 1-Hz triangular waveform. All loading and unloading rates were 0.5 mm/s. Deflection magnitudes ranged from 0.06 (prevailed) to 0.25 mm (postyield) and these were based on the average monotonic yield deflection determined from an independent series of monotonic failure tests conducted at 0.5 mm/s for 14-week-old male A/J ($n = 7$) and C57BL/6J ($n = 7$) femurs. Degradation was determined by conducting two diagnostic relaxation cycles, one before the fatigue test and one after the fatigue test. Each diagnostic test consisted of loading the femur to a preyield deflection level of 0.06 mm at 0.5 mm/s, holding for 20 s, and then unloading at 0.5 mm/s. Stiffness degradation, a measure of damage accumulation, was calculated by comparing the stiffness of the postfatigue (S_3) and prefatigue (S_1) diagnostic tests, such that

$$D = 1 - S_3/S_1. \quad (1)$$

After the damage tests, the femurs were fixed in 40% ethanol, bulk-stained in 1% basic fuchsin,[29] and embedded in polymethylmethacrylate. The area fractions of linear micro-cracks and diffuse damage were assessed under green epifluorescence[30] for three transverse middiaphyseal sections and averaged. The observer quantifying the area fraction of damage was blind to the stiffness degradation results. To minimize geometry effects and to determine how damage accumulation related to monotonic yielding, stiffness degradation and the area fraction of damage were plotted against the applied displacement level normalized for the average monotonic yield deflection determined for the femurs loaded to failure at 0.5 mm/s.

Microhardness tests

To test for differences in tissue mechanical properties between A/J ($n = 5$) and C57BL/6J ($n = 5$) femurs, microhardness measures were conducted on cortical bone from the middiaphyses. The distal metaphysis of each femur was potted in a quick setting cement and the middiaphysis was sectioned transversely. The exposed cortical surface was polished to a 1-μm diamond finish. The Vickers hardness number (VHN) was determined by conducting eight microhardness measurements around the femoral cortex using a Vickers diamond indenter with a 50-g load and a 10-s dwell time. The Vickers microindenter has a pyramidal shape and the size of the indentation varied between 35 and 40 μm. Samples were kept wet with PBS at all times.

Geometry and microstructure

To explain observed differences in whole bone mechanical properties, the cross-sectional geometry was quantified for the femoral middiaphyses of A/J ($n = 21$) and C57BL/6J ($n = 21$) mice. The A/J and C57BL/6J femurs analyzed for geometry included most of the contralateral femurs from the four-point bending group plus several age- and gender-matched femurs from an independent mechanical test group. Femurs were fixed in 40% ethanol and embedded in polymethylmethacrylate. The middiaphyses were sectioned transversely and surface-stained with toluidine blue. The cross-sectional area, cortical thickness, rectangular moments of inertia, and polar moment of inertia were quantified using public domain image analysis software (Scion Corp., Frederick, MD, USA). The rectangular moment of inertia reported in this study (I_{ML}) represents the geometric resistance to bending loads applied in the anterior-posterior direction, consistent with the direction of loading used during whole bone testing. The polar moment of inertia (J) was

TABLE 1. WHOLE BONE MECHANICAL PROPERTIES WERE DETERMINED FOR THE FEMURS OF 14-WEEK-OLD MALE A/J ($n = 19$) AND C57BL/6J ($n = 20$) MICE

Strain	*Mass (g)*	*Stiffness (N/mm)*	P_{MAX} *(N)*	*PYD (mm)*	*Work (N/mm)*
A/J	25.2 ± 1.8	146.4 ± 16.6	24.8 ± 2.3	0.172 ± 0.092	10.7 ± 3.8
C57BL/6J	27.4 ± 1.9	125.6 ± 36.3	24.5 ± 2.8	0.560 ± 0.168	21.1 ± 5.5
p Value	$p < 0.0007$	$p < 0.03$	$p < 0.72$	$p < 0.0001$	$p < 0.0001$

Femurs were loaded to failure in four-point bending and the stiffness, maximum load (P_{MAX}), postyield deflection (PYD), and work to fracture (work) were calculated from the load-deflection curves. The significant differences in work to fracture and PYD indicated that A/J femurs failed in a significantly more brittle manner compared with C57BL/6J femurs. The average body mass and the whole bone mechanical properties are shown as mean ± SD. Differences between inbred strains were determined using a Student's *t*-test with Welch's correction for unequal variances.

determined by summing the rectangular moments of inertia, such that $J = I_{ML} + I_{AP}$.

Differences in microstructural organization were determined for the A/J ($n = 17$) and C5BL6/J ($n = 17$) femurs using the same cross-sections. The area fraction of lamellar and nonlamellar tissues was determined for three transverse sections using standard point counting and then averaged.[14]

Composition

The density, ash content, and water content were determined for the diaphyseal cortical bone of A/J ($n = 10$) and C57BL/6J ($n = 10$) femurs. These composition measures were performed on the broken femurs retrieved from the whole bone bending tests. The metaphyses were removed and the diaphyses were cleaned under a stereomicroscope to remove all soft tissue. Specimen volume, submerged weight, hydrated weight, dry weight, and ash weight were determined using Archimede's principal as described previously.[14] Collagen content was determined for A/J ($n = 7$) and C57BL/6J ($n = 7$) femurs using the hydroxyproline method described previously.[31] Ash, water, and collagen content measures were expressed as a percentage of the hydrated tissue weight.

Statistical analysis

Differences in mechanical, geometric, and compositional properties between A/J and C57BL/6J femurs were determined using a Student's *t*-test with Welch's correction for unequal variances (GraphPad Prism 3.0; GraphPad, Inc., San Diego, CA, USA).

RESULTS

Whole bone monotonic tests

Weekly monitoring of body mass revealed that the A/J and C57BL/6J inbred mouse strains exhibited similar growth curves (data not shown). By 14 weeks of age, a small (≈8.5%) but significant difference in body mass was observed between the two strains (Table 1). The femurs loaded in four-point bending failed within the inner posts of the bending apparatus. The four-point bending tests revealed that the stiffness of A/J femurs was 17% greater than the C57BL/6J femurs ($p < 0.03$, *t*-test). No difference in maximum load was observed between the two strains. The most striking difference in whole bone properties was a 70% reduction in postyield deflection ($p < 0.0001$, *t*-test) and a 49% reduction in work to fracture ($p < 0.0001$, *t*-test) of the A/J femurs compared with C57BL/6J (Table 1). These differences in postyield deflection and work to fracture indicated that A/J femurs were significantly more brittle compared with C57BL/6J femurs. The 25th and 75th percentiles for postyield deflection ranged from 0.114 to 0.247 mm for A/J and from 0.426 to 0.683 mm for C57BL/6J (Fig. 3). These results indicated that postyield deflection was an adequate trait to discriminate between phenotypes.

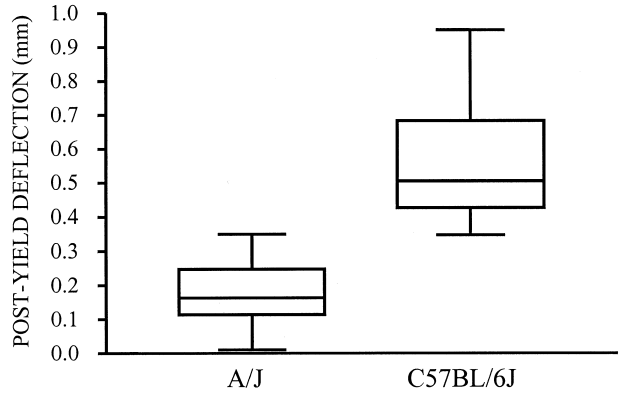

FIG. 3. Differences in postyield deflection for the femurs of 14-week-old male A/J ($n = 19$) and C57BL/6J ($n = 20$) mice loaded to failure in four-point bending are represented as box and whiskers plots. The boxes represent the differences in the 25th and 75th percentiles, the bars represent the median or 50th percentile, and the whiskers represent the range within the sampled data for the two inbred mouse strains. Postyield deflection is a simple measure of bone brittleness and appears to be an adequate phenotypic marker to discriminate between the failure modes of A/J and C57BL/6J femurs.

Whole bone damage tests

The damage accumulation tests revealed that the A/J femurs exhibited altered tissue damageability. Stiffness degradation (Fig. 4A) increased in a nonlinear manner with increased cyclic displacement level for both the A/J and the C57BL/6J femurs. A comparison of power law regressions

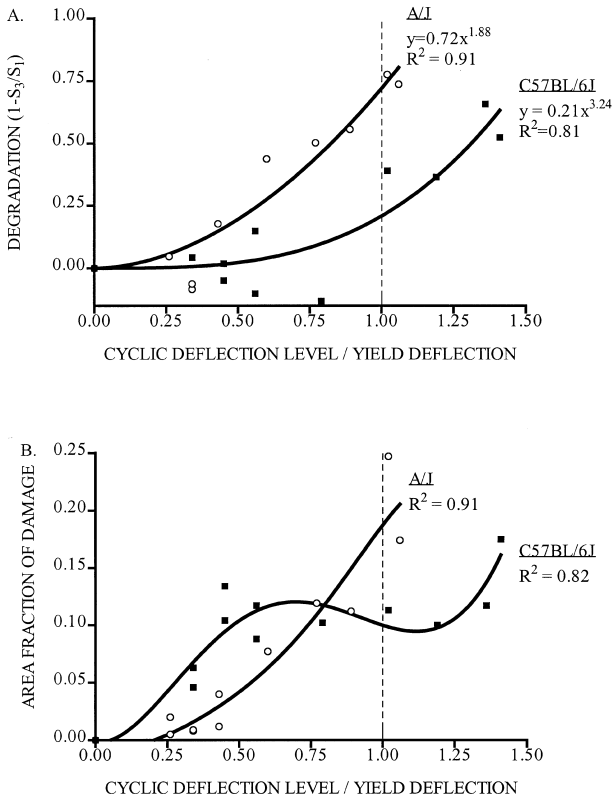

FIG. 4. The results of the whole bone damage accumulation tests revealed significant differences in damageability between A/J and C57BL/6J femurs. (A) Stiffness degradation was determined by comparing the stiffness before and after loading the samples to a specified deflection level for 500 cycles and plotted against the applied displacement level normalized for the average monotonic yield deflection. The stiffness degradation curves were fit to power law regressions ($y = Ax^B$). A comparison of power law regressions revealed a significant increase in the constant A for the degradation curve of the A/J femurs compared with C57BL/6J ($p < 0.0001$, t-test). Negative values represent repeatability errors in measuring stiffness degradation when little to no damage has been induced. (B) The total area fraction of damage after 500 cycles of loading was plotted against the applied cyclic deflection level normalized for the average monotonic yield deflection. Both curves were fitted to a fifth-order polynomial. This regression captured the power law behavior of the A/J femurs but did not quite capture the behavior of C57BL/6J.

($y = Ax^B$) revealed a significant difference in the constant A between A/J and C57BL/6J stiffness degradation curves ($p < 0.0001$, t-test). These results indicated that the magnitude of stiffness degradation increased in a more dramatic manner in the A/J femurs as the applied cyclic deflection level approached the average monotonic yield deflection. Substantial qualitative differences were evident between the two inbred strains for the plots of area fraction of damage versus applied deflection level (Fig. 4B). However, no statistical comparison could be made because the curve for C57BL/6J was complex and could not be modeled using a simple power law regression. The area fraction of damage in the fatigue-loaded C57BL/6J femurs appeared to saturate as deflection levels approached the monotonic yield deflection and then did not increase until cyclic deflection levels reached postyield magnitudes. These results indicated that C57BL/6J femurs were able to sustain cyclic loads well into the postyield region. In contrast, the total area fraction of damage in the A/J femurs increased in a power law manner as the applied deflection levels approached the average monotonic yield deflection, consistent with the stiffness degradation results. These results indicated that damage accumulated very quickly in the A/J femurs as the applied load approached the average monotonic yield level. In a monotonic test, this increased damage accumulation would be expected to lead to the premature formation and propagation of the fatal crack and, therefore, a reduction in postyield deflection.

Microhardness tests

The microhardness tests revealed that the differences in whole bone mechanical properties were associated also with significant differences in tissue mechanical properties. The average VHN of the A/J femurs (VHN = 62.7 ± 3.0) was 10% greater than C57BL/6J (VHN = 57.0 ± 4.1) and this difference was significant ($p < 0.03$, t-test).

Geometry and microstructure

Cross-sectional geometry was assessed to further explain the differences in whole bone mechanical properties. The cortical area, bending moment of inertia, and polar moment of inertia of the A/J femurs were 16, 47, and 53% lower, respectively, than the C57BL/6J femurs (Table 2). These differences remained significant even when normalizing for the differences in body mass (data not shown). The significant differences in cross-sectional geometry between the A/J and C57BL/6J femurs (Fig. 5) were surprising given that the stiffness and maximum load of A/J femurs were the same or greater than C57BL/6J femurs (Table 1). These results indicated that the material stiffness of A/J femurs was greater than C57BL/6J to compensate for the reduced cross-sectional geometry. The significant increase in microhardness of A/J cortical bone is consistent with this interpretation. The tissue architecture results revealed that the brittle phenotype of A/J femurs was not a consequence of excess woven or pathological tissue. No significant difference in the total area fraction of lamellar tissue was observed between A/J (79.5 ± 3.7%) and C57BL/6J (75.9 ± 6.9%) femurs ($p < 0.07$, t-test).

Composition

Tissue density, ash content, water content, and collagen content were assessed to determine whether the disparity observed between whole bone mechanical properties and cross-sectional geometry could be explained by variations in tissue composition. The composition assays revealed no significant differences in wet tissue density or water content between A/J and C57BL/6J femurs (Table 3). However, the ash content of A/J femurs was 4.5% greater than C57BL/6J ($p < 0.0001$, t-test). The increased ash content of A/J femurs was consistent with the increased microhardness and likely contributed to the increased material stiffness to compensate for the smaller cross-sectional geometry. Finally,

TABLE 2. CROSS-SECTIONAL GEOMETRIC MEASURES WERE DETERMINED FOR THE FEMORAL MIDDIAPHYSES OF A/J ($n = 21$) AND C57BL/6J ($n = 21$) MICE

Strain	*Area (mm^2)*	*I_{ML} (mm^4)*	*J (mm^4)*	*CT (mm)*
A/J	0.72 ± 0.05	0.075 ± 0.009	0.208 ± 0.027	0.22 ± 0.01
C57BL/6J	0.86 ± 0.10	0.141 ± 0.030	0.440 ± 0.091	0.19 ± 0.01
p Value	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$

The cortical area (area), bending moment of inertia (I_{ML}), polar moment of inertia (J), and the cortical thickness (CT) were determined and presented as mean ± SD. The moment of inertia (I_{ML}) reflects the geometric resistance to bending in the anterior-posterior direction, consistent with the direction of loading conducted during the whole bone mechanical tests. The polar moment of inertia was calculated as $I_{ML} + I_{AP}$ and represents the geometric resistance to torsional loading. The significant differences in area and moment of inertia indicated that the A/J femurs were significantly more slender than the C57BL/6J femurs. Differences between inbred strains were determined using a Student's *t*-test with Welch's correction for unequal variances.

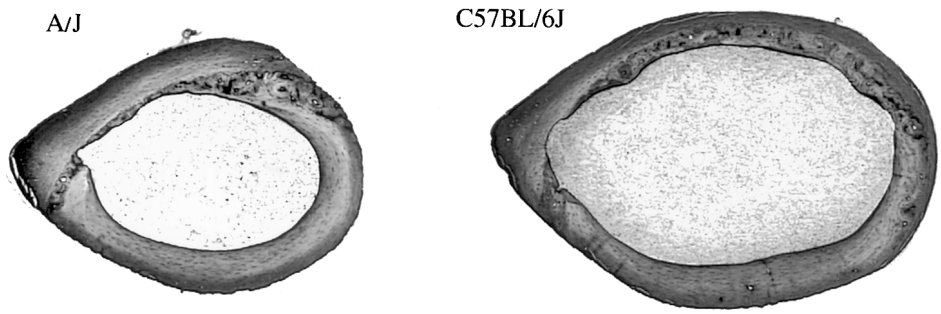

FIG. 5. Middiaphyseal cross-sections representative of the average cortical area, moment of inertia, and cortical thickness for the femurs of 14-week-old male A/J and C57BL/6J mice. Sections were obtained immediately distal to the linea aspera and were imaged at the same magnification. Note the significant differences in cortical area and the distribution of bone mass (moment of inertia) between the two inbred strains.

the collagen content of A/J femurs was 11.8% lower compared with C57BL/6J femurs ($p < 0.0003$, *t*-test), revealing further differences in matrix composition between the two inbred strains.

DISCUSSION

Bone brittleness as a genetic trait

The two factors contributing to the fracture resistance of bone are geometry and material properties. Although genetic background plays a large role in determining peak bone mass,[3] inheritance of low bone mass does not necessarily imply inheritance of increased fracture risk.[4–8] One of the reasons why measures of bone mass or bone density have been inconsistent predictors of fracture risk is that these measures do not consider variations in bone material properties.[9–11] Ex vivo biomechanical testing revealed that not all bones are constructed in the same manner and that variations in matrix construction have a significant impact on bone mechanical properties.[32–33] Recent studies indicate that variations in bone material properties have a genetic basis. Variations in quantitative ultrasound have been correlated with fracture risk[34–37] and exhibit a moderate to strong genetic component.[38–40] Ultrasound measures have been related to the architecture[41] and stiffness[42] of cancellous bone. Moderate to strong heritability of failure load has been observed for the tibia and humeri of egg-laying hens.[43] Hens selected for increased whole bone failure load exhibited a lower incidence of fractures. Significant differences in whole bone stiffness, failure load, and ductility have been observed between inbred mouse strains[24–26]; however, the heritability of these mechanical property variations has not been investigated.

Given that the A/J and C57BL/6J inbred mouse strains were raised under similar environmental conditions, the current results provide additional evidence that genetic background not only influences bone mass, but genetic background also significantly affected a mechanical property that is relevant to fracture risk. Tissue brittleness will contribute to whole bone fracture risk because brittle materials require less energy to failure and accumulate more damage during fatigue loading. Mice were analyzed at 14 weeks of age so the results reflected biomechanical differences at peak bone mass[27] and not transient effects because of growth or aging. Differences in the genetic backgrounds of A/J and C57BL/6J mice resulted in the construction of two different bone materials, one that failed in a brittle manner and one that failed in a ductile manner. The brittle phenotype of A/J femurs was consistent with the decreased ductility and toughness of 11-week-old male mice reported by Kaye and Kusy.[24] Kaye and Kusy[24] interpreted the differences in mechanical properties relative to whole tissue weight instead of cross-sectional geometry and did not provide a mechanism explaining the differences in toughness between the two strains. The altered tissue damageability of A/J femurs observed in this study provided new

TABLE 3. TISSUE DENSITY, ASH CONTENT, AND WATER CONTENT WERE DETERMINED FOR THE DIAPHYSES OF A/J ($n = 10$) AND C57BL/6J ($n = 10$) FEMURS USING ARCHIMEDE'S PRINCIPAL

Strain	*Wet density (g/cc)*	*Ash content*	*Water content*	*Collagen content*
A/J	2.19 ± 0.17	68.6 ± 0.8	10.6 ± 1.8	13.4 ± 0.65
C57BL/6J	2.20 ± 0.14	66.0 ± 1.2	11.9 ± 2.9	15.2 ± 0.67
p Value	$p < 0.89$	$p < 0.0001$	$p < 0.40$	$p < 0.0003$

In addition, the total collagen content was determined for the diaphyses of A/J ($n = 7$) and C57BL/6J ($n = 7$) femurs. Ash content, water content, and collagen content are expressed as percent of hydrated tissue weight. Data are presented as mean ± SD. A/J femurs exhibited significantly greater ash content and significantly reduced collagen content compared with C57BL/6J femurs. Differences between inbred strains were determined using a Student's *t*-test with Welch's correction for unequal variances.

insight into a material-based mechanism explaining the reduced postyield deflection.[44]

The damage accumulation results suggested that A/J femurs were brittle because variations in matrix construction interfered with normal damage accumulation mechanisms. The fact that the altered damageability of A/J femurs was not a consequence of increased woven or pathological tissue indicated that the lamellar microstructure of A/J femurs was not effective in stopping, deterring, or isolating microcracks during loading. The reduced postyield deflection of A/J femurs can be explained on the basis that a rapid increase in damage during yielding leads to premature failure. These results are consistent with the hypothesis that bone brittleness is a consequence of constitutive changes within the extracellular matrix that interfere with the ability of internal interfaces (e.g., lamellae) to stop or deter microcracks to delay the formation of the fatal crack.[13–14] Altered tissue damageability represents a rational mechanism linking variations in matrix composition and/or organization with whole bone brittleness.[44] Therefore, understanding the factors regulating matrix assembly may provide new insight into the material basis of skeletal fragility. Matrix assembly involves the temporal expression of specific genes encoding macromolecules involved in matrix synthesis, collagen fibrillogenesis, and matrix mineralization.[45] The range in the timing and magnitude of matrix gene expression within the human population and the contribution of allelic variants on fracture risk are not understood fully.[8] Osteogenesis imperfecta represents an extreme example of how genetic mutations involving type I collagen result in severe bone tissue fragility.[46] Prockop[47] postulated that individuals within the general population will be predisposed to increased fracture risk because of more subtle differences in genetic background that affect matrix assembly. The results of this study suggested that the genetic basis of bone brittleness may be manifest through altered tissue damageability arising from variations in matrix construction. Both the increased ash content and the reduced collagen content of A/J femurs may represent important matrix variations contributing to the brittle phenotype.[28,44,48] Additional biomechanical analyses are required to identify other matrix variations and further genetic analyses will test for inheritance of specific matrix variations contributing to the brittle A/J phenotype.

Genetics of a complex trait

The biomechanical analyses of A/J and C57BL/6J femurs revealed an important reciprocal relationship between bone mass and bone material properties. Whole bone mechanical properties depend on the amount (area) and distribution (moment of inertia) of bone as well as tissue mechanical properties. In this study, we found that the differences in whole bone properties between A/J and C57BL/6J femurs were a result of differences in both geometry and material properties. If the material properties were similar for A/J and C57BL/6J femurs, then the whole bone stiffness and failure load should have been significantly reduced for the A/J femurs given the significantly smaller cross-sectional moment of inertia. However, the observation that whole bone failure load was similar for A/J and C57BL/6J femurs despite the significant variations in cross-sectional moment of inertia is consistent with the idea that bone geometry and bone material properties are comodulated within the context of body mass to satisfy global mechanical demands.[49–51] The genetic background of C57BL/6J mice favored an efficient structural design, taking advantage of a large moment of inertia and a small tissue stiffness to accommodate mechanical demands. In contrast, the A/J genetic background favored a more slender whole bone design, such that the smaller moment of inertia was compensated by a 4.5% increase in ash content to increase tissue stiffness.[32,48] However, the increased tissue stiffness of A/J femurs appeared to compensate for the smaller geometry at the expense of tissue damageability.[48] A smaller bending moment of inertia has been identified as an important risk factor for stress fractures in military recruits.[52,53] These studies assumed that the mechanical properties of cortical bone were similar between individuals and attributed the increased risk of stress fractures to increased applied strains engendered during military training. The current results suggest that a genetic background that favors a more slender whole bone design may put individuals at increased risk of fracture because of associated material property variations that result in increased tissue damageability.

The relationship between tissue stiffness and whole bone geometry observed for A/J and C57BL/6J mice is consistent with that reported previously for the Mov13 transgenic mouse.[54] However, the specific matrix variations contributing to the brittle phenotypes may be different for the A/J and the Mov13 mouse strains; the brittleness of A/J femurs

was associated with increased tissue stiffness, whereas the brittleness of Mov13 femurs was associated with reduced tissue stiffness and strength.[44] Taken together, these results indicate that tissue fragility can arise from multiple matrix variations. The observation that is consistent between the two studies is that the matrix variations appeared to increase bone brittleness by compromising tissue damageability.[44]

The observation of a reciprocal relationship between bone geometry and bone material properties may be important because it implies that variations in bone mass are not independent of tissue mechanical properties. This observation has important implications when considering prior bone density studies between inbred mouse strains.[21–23] Bone mass, as a quantitative trait, may be a poor predictor of bone strength or bone fracture risk if material or structural properties are not considered. For example, Turner et al.[25] showed that the high bone mass of C3H/HeJ femurs resulted in greater stiffness and failure load compared with the low bone mass of C57BL/6J femurs. However, the femoral neck and vertebrate of C3H/HeJ mice failed in a more brittle manner compared with C57BL/6J. Therefore, studies of bone density measures alone may not identify genetic loci that strongly influence bone properties related to fracture risk.

Limitations

Phenotypic markers used in genetic analyses of inbred mice have included BMD[22,23]; cortical thickness[21]; and whole bone stiffness, failure load, and ductility.[24–26] In this study, the phenotypic marker was postyield deflection. Although postyield deflection is a simple measure of brittleness, this particular whole bone mechanical property can exhibit large SDs because bone failure is a complex process involving the initiation, accumulation, and coalescence of matrix damage. Therefore, the complexity of this process requires large samples sizes to discriminate between ductile and brittle phenotypes. Finally, brittleness, as defined in this study, was based on ex vivo whole bone mechanical tests. No evidence of in vivo fractures has been observed in A/J mice.

The results of this study contributed to our understanding of the genetic basis of bone fragility because they showed that bone brittleness varied with genetic background and was a result of altered tissue damageability associated with variations in matrix construction. These results suggest that the expression of genes involved in matrix synthesis, organization, and mineralization may vary with genetic background and that these variations have a profound impact on whole bone fracture behavior. The possibility that genetic variations in tissue brittleness exist in the human population suggests that individuals with normal bone mass may be at increased risk of fracture because of a material property defect. These results may help explain why bone density has been an inconsistent predictor of fracture risk. The underlying mechanism(s) responsible for the differences in bone brittleness between A/J and C57BL/6J femurs is not yet known, but may be a result of differences in activity level,[24] muscle mass,[24] or the number of glucocorticoid receptors.[55] The results of this study indicated that the A/J and C57BL/6J inbred mice represent feasible models to further investigate the relationship between genetic background and bone brittleness and to study how bone brittleness is inherited in F1 and F2 crosses.

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