Genetic Variation in Mouse Femoral Tissue-Level Mineral Content Underlies Differences in Whole Bone Mechanical Properties

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Key Words

Bone fracture · Genetics · Mechanical properties · Tissue quality · Mouse

Abstract

A/J mice, as compared to C57BL/6J (B6) mice, have a significantly greater total femoral mineral (ash) content which correlates with an increased femoral stiffness (resistance to deformation), but also with an increased brittleness (catastrophic failure). To determine if this whole bone variation in mineral content is indicative of significant mineral and/or matrix variation at the tissue level, femora from 16-week-old female A/J and B6 mice were isolated, embedded in PMMA, sectioned and mounted on barium fluoride infrared windows for FTIR analyses. In addition, preliminary studies of femora from C3H/HeJ (C3H) mice were conducted, since they have an ash content intermediate to A/J and B6. Mean values for mineral-to-matrix ratio were significantly different for A/J (8.4 ± 0.8) and B6 (7.5 ± 0.4), as were values for collagen cross-link maturity (1.8 ± 0.05 and 3.2 ± 0.1 , respectively). C3H mice appeared to have a mineral-to-matrix ratio intermediate of A/J and B6, and cross-link maturity greater than both A/J and B6. B6 femora had similar carbonate-to-amide ratios, carbonate-to-mineral ratios and acid phosphate levels. Thus, whole bone differences in mineral content are concurrent with tissue-level variation in mineral

content and collagen maturity in inbred mice. The greater stiffness and brittleness of A/J femora are likely due to differential biological control (osteoblast activity) of the amount of mineral.

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Introduction

Osteoporosis, a condition characterized by low bone mass and deterioration of bone microstructure, affects millions of people and costs the health care profession billions of dollars annually [Eddy et al., 1998]. Osteoporosis-induced skeletal fragility is an unusually complex pathology because the endpoint of disease (fracture) is a mechanical event. Both the morphology (size and shape) and quality (composition and tissue-level structure) of a bone determine its susceptibility to fracture. Although fracture susceptibility is known to be heritable, the genetic variation of bone compositional traits and the rela-

Abbreviations used in this paper

B6	C57BL/6J
C3H	C3H/HeJ

Fig. 1. Representative FTIRI of mineral-to-matrix ratio for A/J, C3H and B6 femora. Regions shown represent an approximately 25- μm -tall portion of the anterior-medial quadrant of right femora from 16-week-old females. The color bar indicates increasing levels of mineral-to-matrix ratios from 2 to 8.

tionship between this variation and bone mechanical properties remains unclear.

Inbred mouse strains are a useful model for studying skeletal fragility because they have significant genetic homology to humans [Nadeau, 1989], and previous work has demonstrated that genes and gene products characterized in mice are of relevance in humans [Rizzoli et al., 2001]. In addition, inbred mice of a given strain are genetically identical, thereby minimizing the phenotypic variation among individuals and permitting trait comparisons among different cohorts. Research in our laboratory using 2 inbred strains of mice has demonstrated significant variation in whole bone mineral (ash) content and whole bone mechanical properties. Specifically, A/J mice have slender femora, which would tend to make them mechanically weaker than the larger femora of C57BL/6J (B6) mice [Price et al., 2005]. However, A/J mice have a greater total femoral ash content which correlates with an increased femoral stiffness (resistance to deformation), one that is comparable to that of B6 [Jepsen et al., 2001]. Interestingly, C3H/HeJ (C3H) femora have a greater stiffness than both A/J and B6 femora, even though they have an intermediate ash content. Thus, femora from inbred mice are able to achieve functionality for daily activity through varying combinations of morphological and compositional traits. What is not known is whether observed whole bone variation in mineral content is indicative of significant mineral and/or matrix variation at the tissue level. To determine if mouse femora with differing genetic backgrounds and whole bone mechanical properties will also exhibit tissue-level mineral and matrix variation, we analyzed A/J, B6 and C3H femora using FTIR microspectroscopy.

Materials and Methods

Femora from 16-week-old female A/J, B6 and C3H mice were isolated, embedded in PMMA, sectioned (coronal, 2 μm thick) and mounted on barium fluoride infrared windows for FTIRI analyses. Two to three replicate femoral sections were taken from each of 2–3 animals/strain (A/J and B6) and 1 animal/strain (C3H). Each section was analyzed using a Perkin Elmer Spotlight Imaging System. The spectral resolution was 4 cm^{-1} and regions of 25 $\times$ 500 μm were analyzed at approximately 7 μm spatial resolution. Mineral-to-matrix area ratio [(916–1,180 cm^{-1})/(1,588–1,712 cm^{-1})], carbonate-to-matrix area ratio [(840–890 cm^{-1})/(1,588–1,712 cm^{-1})], carbonate-to-mineral area ratio [(840–890 cm^{-1})/(916–1,180 cm^{-1})], collagen cross-linking (1,660/1,690 cm^{-1}), crystallinity (1,030/1,020 cm^{-1}) and acid phosphate (1,106/960 cm^{-1}) parameters were calculated using ISYS Chemical Imaging Software. Significant differences in mean A/J and B6 FTIRI parameters were assessed using a t test with a significance threshold of $p < 0.05$.

Results

Mean mineral-to-matrix ratios for A/J and B6 were 8.7 ± 1.0 and 7.5 ± 0.3 , respectively (fig. 1). Mean collagen cross-linking values were 1.8 ± 0.05 and 3.2 ± 0.1 for A/J and B6, respectively. Both mineral-to-matrix and collagen cross-linking values were significantly different ($p < 0.05$). A/J and B6 femora had similar carbonate-to-amide ratios (0.084 ± 0.004 and 0.075 ± 0.008 , respectively) and carbonate-to-mineral ratios (0.010 ± 0.001 and 0.009 ± 0.001 , respectively), revealing no difference in the amount of carbonate-substituted mineral. Mineral crystallinity was also found to be similar between A/J (1.18 ± 0.03) and B6 (1.19 ± 0.04) as were values for acid phosphate (6.4 ± 0.9 and 6.6 ± 1.2 , respectively). Preliminary data from C3H femora indicated a mineral-to-

Fig. 2. Representative pixel population histograms of mineral-to-matrix ratios for A/J, C3H and B6 femora. Data represent values from an approximately 25- μ m-tall portion of the anterior-medial quadrant of right femora from 16-week-old females.

matrix ratio that is intermediate to A/J and B6 (fig. 1) and collagen cross-linking that is greater than both A/J and B6. Preliminary data also suggested differences in carbonate substitution in C3H femora compared to A/J and B6. Although mean values of mineral-to-matrix were different among the 3 strains, mineral-to-matrix distribution did not appear to differ (fig. 2).

Discussion

The fact that no significant differences were found in carbonate-to-amide ratios and carbonate-to-mineral ratios between A/J and B6 femora indicates that carbonate substitution did not vary during growth or, if variations

do exist during growth, that carbonate substitutions reached a common endpoint for these 2 strains by adulthood. A similar explanation can be applied to crystallinity and acid phosphate content, since neither parameter was significantly different between our 2 strains at 16 weeks of age. Although mineral composition did not vary between strains, the amount of mineral packed into the bone did. Specifically, results of this investigation demonstrated that differences in whole-bone ash content ($A/J > B6$) are associated with significant tissue-level variation in mineral content. In addition, C3H femora, with an ash content intermediate to A/J and B6, were found to have an intermediate mineral-to-matrix ratio. These findings are in agreement with previous research which revealed a strong correlation between ash weight and spectroscopically determined mineral-to-matrix ratio in human biopsies [Faibish et al., 2005].

From an engineering perspective, A/J femora have a whole bone stiffness that is greater than expected based on their slender morphology and this has been attributed to a larger mineral (ash) content [Jepsen et al., 2001]. Here we show that mineral variation also exists at the tissue level and is likely a result of differential biological control (osteoblast activity) of the amount of mineral packed into the matrix. The increased mineral-to-matrix ratio of A/J femora may impart a mechanical advantage at the tissue level, contributing to a larger than expected whole bone stiffness. However, given that increased mineral content is known to correlate with decreased post-yield behavior (increased brittleness) at the whole bone level, the increased tissue-level mineral content may similarly reduce tissue-level post-yield deformation.

Collagen cross-linking was found to be different among femora of A/J, B6 and C3H mice. Compared to A/J femora, B6 femora were found to have more mature cross-links relative to immature cross-links. Although it is tempting to suggest that mature cross-links and tissue-level mineral content may be inversely related, thereby imparting a mechanical advantage by increasing tissue stiffness, preliminary data from C3H mice indicate that they have an intermediate tissue-level mineral content and yet more mature cross-links than both A/J and B6 mice. Thus, how whole bone mechanical properties arise from tissue-level variation in mineral and collagen appears complex. It is currently unknown if greater collagen maturity in B6 and C3H is a result of faster matrix maturation in these strains or greater tissue turnover in A/J. Longitudinal studies are necessary to determine if mineral-to-matrix ratio and collagen maturity differences between A/J, B6 and C3H exist throughout growth and

development or if they manifest only at adulthood. In addition, future studies are necessary to determine if increases in mineral content and collagen maturity also contribute to increases in tissue-level mechanical properties such as stiffness and toughness.

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