BONE MASS DOES NOT ADEQUATELY PREDICT VARIATIONS IN BONE FRAGILITY: A GENETICS APPROACH

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Introduction: Two important factors contributing to osteoporosis and age-related bone fragility include failure to attain high peak bone mass and poor bone material properties (bone quality). How these factors interact to contribute to skeletal fragility is not well understood. In particular, the relationship between bone mass and bone quality is not known. Genetic analyses using human twins demonstrated that bone mass is largely genetically determined [1]. We postulate that variations in bone material properties also have a genetic origin: that is, some individuals are genetically predisposed to increased fracture risk because of variations in the way the extracellular matrix is constructed. Using genetically defined and genetically distinct inbred mouse strains, we demonstrated previously that bone brittleness varied significantly between two inbred strains [2], providing initial evidence that bone quality varies with genetic background. In the current study, we included a larger cohort of inbred strains to obtain a wider range in bone mass. The goals of the current study were 1) to determine whether genetic variations in bone quality will hold for a larger number of inbred strains and 2) to determine whether genetic variations in bone quality are predictable from geometric measures.

Methods: Female mice from 5 genetically distinct inbred mouse strains were purchased from Jackson Laboratory at 8 weeks of age and sacrificed at 15 weeks of age. The strains included C57BL/6J (n=11), AJ (n=10), DBA/2J (n=10), BALB/CByJ (n=10), and C3H/HeJ (n=10). Mice were housed under identical conditions and handled according to institutional guidelines. Whole bone mechanical properties were determined by loading left femurs to failure in 4-point bending at a rate of 0.05mm/sec. The stiffness, failure load, work to fracture, and post-yield deflection were determined. Post-yield deflection (PYD) was defined as the deflection at failure minus the deflection at yield. Ash content was determined for the diaphyses of the femurs failed in 4-point bending (n=5/group) [3]. To assess cross-sectional geometry, right femurs (n=5-6/group) were embedded in poly-methyl methacrylate, sectioned, and stained with toluidine blue. The area, bending moments of inertia, and cortical thickness were averaged over three mid-diaphyseal cross-sections. Since femurs were loaded in the anterior to posterior direction, the moment of inertia about the medial-lateral axis (I_ML) was reported. Differences between groups were determined using a one-way ANOVA with a Tukey post-hoc test.

Results: Significant differences in bone mass (cross-sectional area) were observed among the 5 inbred mouse strains (Fig 1). However, the bending moment of inertia (I_ML) did not follow this variation in bone mass, indicating that the distribution of bone mass also varied with genetic background. In particular, the I_ML of C57BL/6J femurs [low bone mass] were similar to C3H/HeJ [high bone mass]. The 4-point bending tests revealed significant differences in whole bone mechanical properties among the 5 inbred strains (Fig 2, 3). The variations in failure load and stiffness (not shown) followed the variations in bone mass closely. In contrast, the variations in work to fracture followed neither geometric parameter. Linear regression analysis revealed significant correlations between stiffness (R^2=0.42), maximum load (R^2=0.34), and work to fracture (R^2=0.36) with moment of inertia (p<0.05). However, I_ML explained only 35-42% of the variability in these whole bone properties, indicating that more than 60% of the variability was a result of variations in material properties. Further, post-yield deflection (Fig 3) did not correlate with I_ML, indicating there were important tissue level differences contributing to whole bone failure behavior. Femurs from C3H/HeJ mice [high bone mass] exhibited significantly lower post-yield deflection (i.e. were more brittle) compared to C57BL/6J femurs [low bone mass]. The differences in whole bone properties may be partly explained by the significant variation in ash content (Fig 3). Despite the small variations in ash content, a linear regression revealed that more than 90% of the variability in the average post-yield deflection of the 5 inbred strains was explained by ash content (p<0.05). These results indicated that genetic variations in ash content may be contributing to the differences in brittleness between the inbred strains.

Discussion: By expanding our initial study to include a larger number of inbred mouse strains, we demonstrated that certain key failure characteristics of bone varied significantly among inbred mouse strains and were not reliably predicted by variations in bone mass. Post-yield deflection, a measure of bone brittleness, relates to the ability of bone tissue to sustain damage prior to fracture. The fact that PYD was independent of bone mass is important because it suggests that tissue damageability varied among the inbred strains and that these matrix level differences affected whole bone brittleness [2]. Preliminary findings suggested that genetic variations in mineral content may contribute to the variations in post-yield deflection [4]. Since the inbred strains had similar whole body weights, similar femur lengths, and were raised under similar environmental conditions, these differences in tissue fragility can be attributed to differences in genetic background. These results provide further evidence that genetic background not only influences bone mass [5], but profoundly influences the failure behavior of bone, possibly through variations in the organization and composition of the extracellular matrix. These results have important implications for understanding why bone mass and bone density are inconsistent predictors of fracture risk [6].

The observation that bone mass and bone material properties vary with genetic background has important implications regarding the selection of phenotypic markers used in genetic analyses to identify candidate genes that promote a favorable trait. Identifying genes that promote high bone mass may have deleterious consequences if the tissue is mechanically fragile. To search for genes that promote both high bone mass and favorable material properties (e.g. high strength and toughness), it will be necessary to take bone mass, whole bone mechanical properties, and tissue properties into consideration.

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