INTRODUCTION. Application of cyclic strains lower than those required to fracture normal bone in a single cycle may result in a partial or complete fatigue or stress fracture. Site-specific fatigue fractures are common in elderly osteoporotic human beings and in running athletes. Despite the fatigue resistance of bone material [1], the prevalence of these pathological fractures suggests that the structural properties of whole bones and the biological response of bone to cyclic load are important factors affecting development of fatigue fractures. Cyclic loading of the skeleton of tA, ulnar animals leads to the formation of bone microdamage due to fatigue failure at the microscopic level [2].

Microdamage accumulation and coalescence may be an important factor leading to pathological skeletal weakening. Although fatigue fractures are known to propagate through adapted bone [3], the relative effects of microdamage and reparative remodeling on bone strength are unclear. When bone loses 15% or greater stiffness, microdamage accumulation is exponential [4], but the effect of this damage on structural properties is unclear. We, therefore, hypothesized that fatigue loading of whole bones ex-vivo beyond 15% loss of stiffness would have significant effects on both microdamage and whole bone structural properties. Because of the irregular shape and larger size of whole bones, it is likely that effects from the geometry and stressed volume of the specimen will influence initiation and growth of microcracks into a fracture [5].

METHODS. Experimental Design. We used an ex-vivo end-loading ulnar bending model for fatigue testing [6] by applying cyclic axial loads to the external surface of the antebraehium of adult male Sprague-Dawley rats. We also used this loading system to perform monotonic biomechanical testing of isolated ulnae. Experimental Groups. Rats were randomly assigned to one of three test groups (n = 7 rats/group). All fatigue tests were performed with an initial surface strain on the caudal medial surface of the ulna of – 6,000µsn. In Groups 1 to 3 ulna pairs were loaded to 0% control, 20% and 40% loss of stiffness respectively. One randomly selected ulna was then monotonically loaded to failure, and the contralateral bone was bulk-stained in basic fuchsin for quantification of cross-sectional geometry and bone microdamage. Using peripheral quantitative computed tomography (pQCT), volumetric bone mineral density (vBMD, mg/cm^3) of all isolated ulnae was determined before monotonic biomechanical testing.

Cyclic Biomechanical Testing. All fatigue tests were performed after application of a single rosette strain gauge. Mechanical testing was performed using a servohydraulic materials testing machine (Model 8800 DynaMight; Instron, Canton MA). The antebraehium was initially loaded in a static fashion to – 6,000µsn, to determine the peak load required for fatigue loading, and was then loaded cyclically at 4Hz in load control using a haversine with peak load set to the level to produce 6,000µsn. The development of fatigue was determined by measuring the increase in displacement amplitude. Monotonic Biomechanical Testing. Isolated ulnae were placed lengthwise between the opposing loading cups. The ulnae were loaded monotonically to failure in the axial direction using a cross speed of 0.2mm/sec. From the load-deformation curve, ultimate force (FMAX) and B.Ar, and between FMAX and B.Ar was determined.

Histology and Morphometric Analysis. Isolated ulnae were bulk-stained for microdamage in 1% basic fuchsin in a graded series of alcohols (80%, 90%, 100%) under a vacuum of 20 mm Hg for a total of 32 hours, before being embedded in methylmethacrylate. Transverse sections, 150 µm thick, were made in the proximal, mid and distal ulna at 25%, 50%, and 75% of diaphyseal bone length. The following histomorphometric variables were measured: bone cortical area (B.Ar), microcrack density (Cr.Dn, #/mm^3), and maximum second moment of inertia (I_h). Data were collected by a single observer (SAC).

STATISTICAL ANALYSIS. Parametric and non-parametric ANOVA tests were used to determine the effect of fatigue (0% control, 20%, or 40% loss of stiffness) on F, vBMD, B.Ar, and Cr.Dn. Least squares regression was used to determine the relationship between F and B.Ar, and F and I_h. Results were considered significant at p<0.05.

RESULTS. There was no significant difference in B.Ar and I_h between groups. Further, there were no significant differences in vBMD between left and right bones or among groups. F was decreased in both the 20% and 40% fatigue groups when compared to the 0% control group (p = 0.03). Cr.Dn was increased in both the 20% and 40% fatigue groups compared to the 0% control group (p = 0.009). Degradation of structural properties after fatigue loading was most apparent in the ulnae of individual rats with smaller bones. There was significant correlation between F and B.Ar, and between F and I_h in the 40% group (B.Ar: R^2 = 0.85, p < 0.003), but not the 20% group (B.Ar: R^2 = 0.046, p = 0.64), or the 0% group (B.Ar: R^2 = 0.025, p = 0.73).

DISCUSSION. Using the rat ulna bone end-loading model, we found that fatigue had significant effects on structural properties with progressive reduction in ultimate strength occurring as fatigue developed. We have also identified a biologically significant individual animal effect in a homogeneous population of rats, as the ulna of some rats were much more resistant to developing fracture after fatigue at high strain. The rats with lower risk of fatigue fracture had larger B.Ar and I_h values. These data imply that individuals with larger bones would have a lower fracture risk in-vivo after fatigue loading. Minor variation in cross-sectional bone geometry seems to be a key variable that allows whole bones to be much more resistant to cyclic loading. The mechanism by which bones with larger B.Ar values resist structural failure after induction of fatigue microdamage is unclear. A larger stressed volume may influence the risk of propagation of a critical flaw during a structural test. In a heterogeneous population, these effects are likely to be even greater, and bone size is likely to be a critical determinant of fatigue fracture risk. The bone-loading model described in this study will be a useful tool for further investigation of fatigue fracture in bone. This model will also facilitate study of the skeletal effects of adaptive remodeling and new drugs, such as bisphosphonates, that modulate bone remodeling, as loading can easily be performed in-vivo.


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