INTRODUCTION Bone has the ability to alter its mass and structure with changing mechanical usage. A single bout of low-cycle loading that does not produce intracortical microdamage has been shown to induce lamellar bone formation in the rat tibia. Yet little is known about the osteogenic response of bone tissue to a single bout of high-cycle loading that causes fatigue. Intracortical resorption has been observed in the rat ulnar cortex in association with microcannulation.8,9 Ten days after fatigue loading9, indicating that fatigue loading triggers a remodeling response. However, it is not known how fatigue loading affects bone mechanical properties by strain adaptive modeling and/or microdamage induced remodeling. It is possible that remodeling of bone following a period of fatigue loading may cause a reduction in the mechanical properties of bone, followed by an increase in mechanical properties due to new bone formation. Our objective was to investigate the mechanical and osteogenic response of the rat ulna to a single bout of in vivo fatigue loading followed by recovery periods ranging from 0 to 18 days.

METHODS Forty-nine adult, female Fisher rats (8 mo; 203 ± 16g) were used for this study. Six rats were used for determination of the relationship between applied load and mechanical strain on the ulna, 7 rats for characterizing the ex vivo fatigue behavior of the ulna, and 36 rats for measurement of ulnar densitometric, geometric, and mechanical properties and new bone formation in response to fatigue loading. Axial, compressive loading was applied as a haversian waveform under load control to the rat ulna across the flexed carpus and olecranon process10 using a servohydraulic loading machine (Instron 8500R). Bending is induced due to the natural curvature of the ulna, resulting in compression on the medial surface and tension on the lateral surface.11,12 Our institutional Animal Care and Use Committee approved all the procedures performed in the present study.

Load-Strain Calibration Following euthanasia, the midshaft of the right ulna was excised and the bone surface and strain gauges were bonded to the medial and lateral ulnar surfaces at the ulna midpoint.13 Strains were measured for loading at a frequency of 2 Hz with peak loads of 4, 8, 12, 16, and 20 N.

Ex Vivo Fatigue Behavior of the Rat Ulna The right and left forelimbs of each rat were loaded at 1 Hz. The left ulna served as contralateral control. Intraperitoneal injections of calcein (7 mg/kg) were given to the rats on days 0 and 1 day before sacrifice. Rats were sacrificed on days 0 (n=12), 6 (n=8), 12 (n=8), and 18 (n=8) following loading. The ulnae were scanned by pQCT (Stratec XCT Research M, Norland) for determination of densitometric and geometric properties. Specimens were then loaded to failure at the midshaft in 3-point bending (Instron 8500R) to determine the whole bone structural properties. Material properties were estimated from beam theory. New bone formation was quantified using double-label histomorphometry for the transverse section obtained at the midshaft.

Statistical Analysis Paired t-tests were performed for each experimental group to determine significant differences in new bone formation and densitometric, geometric, and biomechanical property parameters between right (loaded) and left (control) ulnae. One-way ANOVA and Fisher’s tests were used to determine the effects of recovery time following loading on the measured parameters. Data are presented as means and SD.

RESULTS The ulnar load-strain relationships were linear throughout the loading range (r² = 0.93 for compression; r² = 0.85 for tension). The logarithm of loading cycles to failure ex vivo was highly correlated with peak applied load (r² = 0.94), consistent with classic S-N behavior for bone tissue. Fatigue loading resulted in a significant decrease in the ultimate force and stiffness of the loaded ulnae compared to the unloaded controls on days 0 and 6, followed by a significant increase in ultimate force and stiffness back to normal levels on days 12 and 18 (Figure 1A). Unlike the recovery of whole bone structural properties by day 12, the material properties remained reduced through day 18 (Figure 1B). The osteogenic response to fatigue loading on the periosteal surface was exclusively woven bone with significantly lower bone mineral density (BMD) than the original cortical bone, although the BMD of the new bone increased from 6-18 days (Table 1). Fatigue loading also caused a significant increase in cross-sectional area and moment of inertia of the bone tissue and the BMD of the original cortical bone with time.

DISCUSSION In contrast to increased lamellar bone formation in the rat tibia after a single period of low-cycle loading,14 woven bone formation was observed on the periosteal surface of the ulnar diaphysis after fatigue loading. The amount of new bone increased with recovery time, resulting in an increase in bone tissue cross-sectional area and moment of inertia. In addition, the BMD of the original cortical bone increased with time, suggesting that fatigue loading also induced intracortical remodeling. The initial time-zero decrease in ultimate force and stiffness is consistent with acute fatigue response resulting from the cyclic loading history. We found no evidence of a further reduction in whole bone stiffness or strength beyond the initial time-zero reduction, suggesting that any reductions in intracortical strength due to fatigue-induced remodeling are more than compensated structurally by rapid periosteal new bone formation.