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FATIGUE-LOADING INCREASES INTRACORTICAL RESORPTION IN RAT ULNAE INDEPENDENT OF LOAD-BEARING STRAINS

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Introduction: Fatigue-loading of bone results in tissue-level damage prior to complete fracture and has been shown to have a detrimental effect on bone mechanics. Microdamage in cadaveric human and animal bone is, however, relatively rare and is often only observed in elderly individuals or animals exposed to extreme loading conditions. This may be because bone remodeling processes effectively remove damaged tissue in young healthy individuals. In vivo models in both rats (1) and dogs (2) have demonstrated that intracortical remodeling is increased following fatigue-loading. It has been hypothesized that the increase in remodeling activity functions as a targeted repair mechanism. The mechanisms responsible for this increase in remodeling activity are unknown, but may be mediated by osteocyte apoptosis (3,4). Investigators have hypothesized that the local strain field is altered near a microcrack which may serve as a signal for osteocyte apoptosis and targeted bone resorption. This study was designed to investigate the role of load-bearing strains in the repair of fatigue damage. Rat ulnae were fatigue-loaded and immediately immobilized, thus eliminating regional variations in bone strain. Control rats were allowed to resume normal load-bearing following fatigue-loading.

Materials and Methods: Forty Sprague-Dawley female retired breeder rats were obtained and housed in standard vivarium cages. Rats were sorted by weight into four groups: fatigue (n=13), fatigue/immobilized (n=13), sham (n=7), and sham/immobilized (n=7). Fatigue-loaded rats were anesthetized with sodium pentobarbitol and placed in a custom-built loading device. An 18 N load was cyclically (5 Hz) applied to the ends of the right ulna at the olecranon and the flexed carpus. The limb was loaded until a 25% increase in whole bone compliance was observed. While still under anesthesia, the rat was either returned to its cage or the right forelimb was immobilized with an elastic bandage. Sham loaded rats were similarly anesthetized, placed in the loading device, and a small static load (3 N) was applied to the ulna. Sham rats were either immobilized or allowed to ambulate following loading. Immobilized rats were checked daily to ensure adequate immobilization throughout the experiment. Ten days after loading, rats were euthanized by CO_2 inhalation. The right ulnae were removed, fixed in formalin for 48 hours, stained en bloc in basic fuchsin, and embedded in methyl methacrylate following published protocols. Three cross-sections were cut from each bone with a low-speed bone saw, ground to 70 micron thickness, and mounted on glass slides. Specimens were examined under both brightfield and epifluorescent microscopy. All specimens were examined with the investigator blinded to the experimental treatment. All animal protocols were approved by the University of Utah Institutional Animal Care and Utilization Committee.

Results: Rats were loaded an average of 5618 cycles. There were no significant differences between the ambulatory (5728 ± 3871 cycles) or immobilized (5508 ± 5235 cycles) groups, but there were extremely large variations in number of loading cycles that did not appear to be related to rat weight. Small, non-significant differences in cortical bone area (Ct.Ar) were observed among the four groups (Table 1). Immobilized rats had significantly ($p<0.05$) larger medullary canals (Me.Ar) than ambulatory rats, but no differences were observed between fatigue- and sham-loading groups. In contrast, a woven bone response (Ps.Wo.B.Ar) was observed on the periosteal surface in fatigue loaded rats irrespective of ambulatory status. No woven bone was observed on any sham loaded animals. Number of resorption spaces (Rs.N/Ct.Ar) and resorption space area (Rs.Ar/Ct.Ar) were determined for each section and normalized to cortical bone area. Significantly ($p<0.05$) more resorption spaces were observed in fatigue loaded rats, independent of load-bearing activity (Figure 1). Similarly, resorption area was increased ($p<0.05$) in fatigue loaded rats with or without immobilization (Table 1).

Table 1.	Fatigue	Fatigue Immobilized	Sham	Sham Immobilized
Ct.Ar (mm^2)	5.38 ± 0.14	5.31 ± 0.29	4.67 ± 0.21	5.49 ± 0.34
Me.Ar (mm^2)	0.018 ± 0.004	0.040 ± 0.013	0.013 ± 0.007	0.039 ± 0.020
Ps.Wo.B.Ar (mm^2)	0.25 ± 0.08	0.18 ± 0.05	0 ± 0	0 ± 0
Rs.Ar/ Ct.Ar x 10^3	0.69 ± 0.40	0.85 ± 0.51	0.10 ± 0.10	0.11 ± 0.08

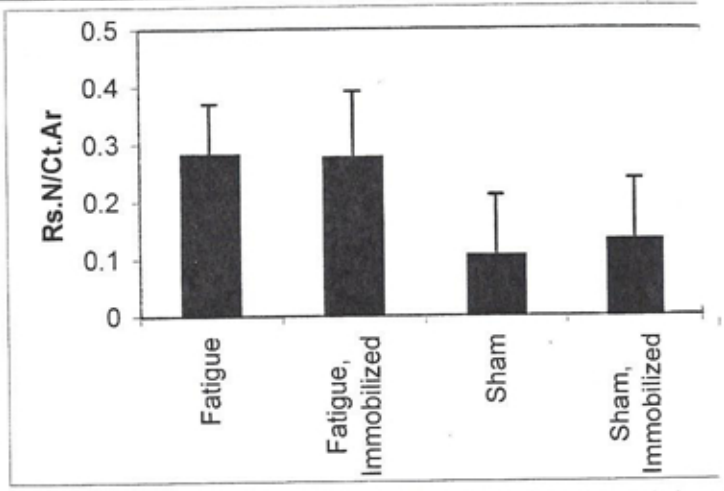

Figure 1. Effects of fatigue loading and immobilization on intracortical remodeling. Fatigue loading increased intracortical resorption in rat ulnae independent of subsequent load bearing strains.

Discussion: Increases in endocortical erosion and medullary size are typical of immobilization studies in rats. Similarly, periosteal woven bone formation indicates hyper-physiological loading of the ulnae and has been reported in other studies of applied loading (5,6).

In addition to the endocortical and periosteal responses, fatigue-loading induced a consistent intracortical response, irrespective of subsequent load-bearing activity. Immobilization reduces strains throughout the cortex, effectively eliminating regional variations in the strain field. Because immobilization did not alter the response to fatigue-loading, it strongly suggests that local variations in the strain field during load-bearing activity do not serve as signals for osteoclastic resorption. Rather, these data establish that the initial damage event initiates a cellular response, presumably from the osteocytes near the damaged tissue. It is not clear, however, what signaling pathways are responsible for this response.

References:
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