Activation of Bone Remodeling After Fatigue: Differential Response to Linear Microcracks and Diffuse Damage

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Introduction: Numerous studies demonstrate that bone microdamage is associated with activation of resorption [1,2] since this relationship was first posited by Frost [3]. However, recent experiments point to two predominant forms of microdamage: linear microcracks and diffuse damage [1,4], the physiological relevance of which in activating bone remodeling is not known. Microdamage in the form of linear microcracks, 30-100 μm long, is of the type first reported by Frost [5]. Diffuse damage (due to diffuse basic fuchsin staining) is comprised of sublamellar size microcracks that occurs in clusters. Whether all fatigue-induced microdamage evokes the same biological response has not been evaluated. The purpose of this study was to vary microdamage amount and type to determine which of the two types of microdamage control activation of resorption. Understanding these characteristics of in vivo fatigue loading will help provide insight as to which type of “wear and tear” injury in bone evokes a biological response.

Materials and Methods: All tests were conducted in vivo on anesthetized animals and were approved by Mount Sinai IACUC. Ulnae from adult female Sprague-Dawley rats (4-5 months old, n=22) were fatigued in end-load bending [1] for either 1500, 3000, or 4500 loading cycles under load control to vary damage content and type. Cyclic loading was applied at 2 Hz with mean maximum loads of 16.6 N corresponding to a peak initial strain of 4000 με. We found that each bone damaged differently (i.e. content of linear versus diffuse microdamage) and used this variation in damage content to segregate the remodeling response based on damage type. After loading (“experimental bones”), rats were allowed to recover from anesthesia and resume normal cage activity. At day 14 after fatigue loading, contralateral ulnae (“baseline fatigued control bones”) were loaded identically to experimental bones, after which rats were immediately sacrificed and bones harvested. Baseline fatigued control bones were used to estimate damage incurred in experimental bones due to fatigue [1]. Both experimental and baseline fatigued control bones were compared to non-loaded controls. Basic Fuchsin staining was used to visualize microdamage [1]. After staining and embedding, cross-sections were cut, polished to 70 μm and examined using fluorescence microscopy. Specimens were analyzed for intracortical remodeling (Rs.N/B.Ar). Linear microdamage and sublamellar microdamage (“diffuse damage”) were quantified by determining area fraction of bone occupied by microdamage (Lin.Dx.Ar/B.Ar and Diff.Dx.Ar/B.Ar, respectively) using an eyepiece grid reticule at 40x magnification. Statistical analyses were performed using ANOVA regression analysis and Chi-Square analysis with equations for three-dimensional contingency analysis [6]. Significance is reported at p<0.05.

Results: Non-loaded bones did not show any microdamage or intracortical remodeling. Linear microdamage content and resorption space density were strongly, linearly related (Fig 1A, R² = 0.85, p<0.0001). In contrast, there was no relationship between resorption space density and sublamellar microdamage in the 6 ulnae that showed only sublamellar microdamage (Fig 1B). In addition, Fig 1A illustrates that there is no relationship between resorption space density and duration of loading. Contingency analysis of resorption, linear microdamage, and duration of loading (number of cycles or time) shows that activation of resorption depends on the presence of linear microdamage but not on duration of loading (Overall: Chi-Square = 25.9, p<0.001; Activation of Resorption Vs. Duration of Loading: Chi-Square = 2.41, p=0.3; Activation of Resorption Vs. Presence of Linear Microdamage: p<0.001, Fisher’s Exact Test)

Discussion: These data indicate that the remodeling response occurs only in bone with linear-type microcracks. Bone containing only diffuse damage had no activation of intracortical remodeling. Moreover, our results show that the response observed is due to linear microdamage and not to loading itself. These data support with anecdotal reports [1] showing that despite several thousands of cycles of loading, if there is no linear microdamage, no intracortical remodeling will occur. A previous study examined diffuse damage and activation of resorption [1], but did not distinguish bones that contained only sublamellar damage versus bones that contained both linear and sublamellar microdamage. Here, by varying cycles, we generated bones that contained only sublamellar microdamage, thus uncoupling the two forms of microdamage. Intracortical remodeling that results from microdamage is associated with osteocyte apoptosis [4] and that osteocyte apoptosis is necessary for intracortical remodeling to be activated [7]. Linear microcracks impair local canalicular fluid transport between osteocytes [8]. Loss of canalicular fluid transport due to linear microdamage may lead to osteocyte apoptosis by causing a metabolic stress on cells [9]. Alternatively, linear microcracks that directly transect osteocytes likely cause necrosis of those few cells which may play a role in apoptosis of adjacent osteocytes and downstream signaling that activates remodeling. Sublamellar damage may not effectively impair fluid transport to evoke a metabolic stress on the osteocytes. Another possibility is that sublamellar damage may not effectively cause cells to undergo necrosis to activate a focal injury response. In conclusion, linear microdamage is the major determinant of activation of intracortical remodeling after fatigue loading.


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Figure 1(A): Resorption Space Density Depends on Linear Damage Area Fraction

Figure 1(B): Intracortical Remodeling Does Not Occur When All Damage Accumulated Is Diffuse