Introduction:
Apoptosis of osteocytes near fatigue microcracks in bone precedes the site-specific recruitment of osteoclasts that remodel the damaged tissue.1,2 Apoptosis is an essential, controlling step in the initiation of damage-induced osteoclastic resorption; inhibiting caspase activity after fatigue not only prevents osteocyte apoptosis but also completely blocks the subsequent activation of osteoclastic resorption.3 In other localized injuries (e.g., focal ischemia) apoptosis that occurs within a very short time after damage is crucial to determining the magnitude and extent of the subsequent cytokine burst, inflammation and phagocytosis and tissue remodeling that comprise the focal injury response.4 In the current studies we tested whether osteocyte apoptosis that immediately follows fatigue microdamage plays a similarly pivotal role in regulating the remodeling response of bone to focal injury.

Methods:
Adult female Sprague-Dawley rats (16 weeks old, N=10) underwent in vivo fatigue loading of the ulna to initiate intracortical resorption.1,2 Briefly, ulnae were cyclically loaded in vivo (18N load, 4 Hz) to a predetermined fatigue damage state, as reflected by decrease in whole bone stiffness.

To determine whether osteocyte apoptosis that immediately follows fatigue microdamage regulates the initiation of the bone remodeling response, a pan-caspase inhibitor (QVD-OPh, MP Biomedical, Livermore, CA, 20mg/kg/d) was administered for 2 days beginning 2 hours before fatigue loading. Five animals received caspase inhibitor (FAT+Casp Inh) which has been previously shown to prevent osteocyte apoptosis in vivo;7 the remaining animals received vehicle only (FAT+Veh). Non-loaded ulnae from the contralateral forelimbs were examined as controls. Ulnae were harvested on day 14 after fatigue loading. Numbers of resorption spaces and apoptotic and normal-appearing osteocytes (based on measurement of pyknotic cells in the same sections) around microcracks were determined from diaphyseal cross-sections stained with basic fuchsin; apoptosis was confirmed by immunostaining for cleaved caspase-3 and H2AX in parallel tissue samples. Morphometric data were collected by a single observer. All procedures were conducted with approval from the Institutional Animal Care and Use Committee. Differences in numbers of resorption spaces and apoptotic osteocytes were evaluated using ANOVA with Fisher PLSD for post-hoc testing.

Results:
Osteocyte apoptosis was virtually absent in control bones. However, it was dramatically increased around microcracks in fatigued, vehicle-treated bones (p<0.01, Fig 1). Treatment with caspase inhibitor only during the acute period after loading attenuated this increase by nearly 70 percent.

Discussion:
In previous studies, continuous, long-term administration of a caspase inhibitor in vivo prevented osteocyte apoptosis and completely blocked bone resorption in response to fatigue-induced microdamage.8 The current studies reveal that short-term suppression of caspase activity results in a substantial inhibition of osteocyte apoptosis and a comparable reduction in the activation of bone resorption. Thus, key signaling events stemming from osteocyte death, and necessary to activate bone resorption, occur early after acute microinjury. This scenario parallels the events following focal ischemia perfusion–reperfusion injury in brain, myocardium and transplants.9 In those circumstances the acute local injury also leads to apoptotic cell death followed by inflammation, phagocytosis and tissue remodeling, and pharmacological inhibition of the initial apoptosis in focal ischemic injury has also been shown to attenuate the later tissue responses.6,10 Consequently, these instances of focal injury and repair in non-skeletal tissue may provide insights into the signaling pathways that link osteocyte death to osteoclast recruitment.

Conclusion:
These studies demonstrate that osteocyte apoptosis occurring immediately after fatigue-induced microdamage is critical for activation of the remodeling response in bone. These data suggest that certain signals necessary to activate bone resorption are produced only during a critical early time period following microinjury.

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References:
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THE IMPORTANCE OF EARLY APOPTOTIC EVENTS FOLLOWING MICRODAMAGE IN TRIGGERING FATIGUE INDUCED BONE REMODELING
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