INTRODUCTION

Microdamage has been cited as an important component of bone quality [1]. Microdamage could affect fracture risk in bone by providing fracture initiation sites, or by decreasing the mechanical integrity of the trabecular network. However, the effects of microdamage are difficult to detect in large populations, because they are much smaller than those of density and architecture [2]. The majority of fractures occur during falls [3], where the principal stresses are off-axis [4]. Multiaxial and off-axis damage mechanics of trabecular bone have not been extensively studied, and would provide insight into fracture progression.

The goal of this study was to investigate the formation of microdamage in human femoral trabecular bone on the apparent loading mode, and initial microdamage levels. Specifically the aims were to: 1) determine how the density and lengths of microcracks depend on the applied loading mode and pre-existing damage and 2) to determine the dependence of modulus loss on microdamage.

METHODS

Three groups of eight human femoral necks, four male and four female, were studied. Bones were obtained from a national tissue bank (NDR) with informed consent, and stored at -20 °C until testing. The mean age was 71.4 ± 12.1 yrs, and did not differ between groups. Cylindrical trabecular bone specimens were not prepared with the axis aligned with the principal mechanical axis. Micro-CT (Scanco μCT-80, Brüttisellen, Switzerland) images were obtained at 20 μm resolution to quantify architecture and density parameters (IPL V6.0, Scanco).

Samples in group I were subjected to torsional overloading to 4% shear strain, which is equivalent to 2% principal compressive and tensile strain. Group II was subjected to compressive overloading to 2% strain (2% and 0% compressive and tensile principal strains), and group III was subjected to combined compressive and torsional overloading to 1.236% compressive and 2.472% torsional strain (2% compressive and 0.764% tensile principal strains). The Young’s and shear modulus of each sample were measured before and after overloading.

Damage was quantified by differential fluorescent staining [5]. Prior to testing, in vivo damage was labeled with 0.5 mM alizarin complexone. Following overloading induced damage was labeled with 0.5 mM calcein. Specimens were dehydrated and embedded in poly (methyl methacrylate). A 200 μm section was prepared, fixed to a glass slide, and polished. The sections were viewed under epifluorescence microscopy at 100x magnification (Fig. 1a). The number (Cr.N.) and length (Cr.Ln.) of in vivo and overloading induced microcracks was quantified using ImageJ. Damage was quantified separately in three longitudinal regions to account for the variable strain applied in torsional loading. Crack density (Cr.Dn.) was defined as Cr.Dn./Bone Area. Crack surface density (Cr.S.Dn.) was defined as Cr.S.Dn./Bone Area.

RESULTS

Within the outer 1/3 of the sample, where the apparent principal strain was similar for all groups, Cr.Dn was twice as high for multiaxial loading as for compression alone (p < 0.005, Fig. 1b). Both the Cr.Dn. and Cr.S.Dn. due to overloading were positively correlated with in vivo Cr.Dn. and Cr.S.Dn. for all loading modes (p < 0.03; Fig. 2). The Cr.Dn. in group II and III increased with increasing structure model index (SMI) (p < 0.05), while the Cr.Ln. was independent of architecture (p > 0.41).

In vivo Cr.Dn. increased with increasing SMI for all groups (p < 0.001).

On average, Young’s modulus decreased by 17.1% ± 2.3% in group III, compared to 12.1% ± 2.5% in group II and 9.6% ± 2.4% in group I (p < 0.05). The relative decrease in Young’s modulus was correlated to the density of cracks formed during overloading (p = 0.005, Fig. 3).

DISCUSSION

Microdamage formation in trabecular bone may play a role in increasing bone fragility. This study demonstrated that combined shear and compressive overloading resulted in more microcracks, which was correlated to a greater axial stiffness decrease than shear or compressive overloading alone at the same principal strain level. Moreover, the density of induced microdamage depended on the density of pre-existing microdamage. Taken together, the results indicate that microdamage burden may contribute to osteoporotic fractures by increasing the risk of further damage formation, which decreases modulus and strength.

The results are consistent with previous studies that found that damage is prone to propagation under differing loading modes, even at low strain [6], and the correlation of crack density with SMI is consistent with results for both human [7] and bovine [8] bone. Hence, the increased damage susceptibility may be due to deteriorated microarchitecture, or to propagation of existing microdamage [8].

SIGNIFICANCE

Microdamage in trabecular bone may increase fracture risk due to its detrimental effect on mechanical properties. Bone with pre-existing damage is particularly susceptible to further damage when subjected to multiaxial loads.

ACKNOWLEDGEMENTS

NIH AR052008, NDR

REFERENCES