INTRODUCTION:
Osteoporosis is a condition characterized by low bone mass and microarchitectural deterioration of bone tissue which leads to bone fragility and susceptibility to fracture (1). While bone mineral density (BMD) is the most common way to diagnose and monitor osteoporosis, there is a component of bone fragility that is independent of mass and determined by bone quality. Bone quality may be defined by bone geometry, microarchitecture, bone turnover, nature of collagen/mineral matrix and microdamage (2). We used an ovariectomized sheep model to simulate postmenopausal osteoporosis. This model was chosen because of the suitable hormone profile and also because of the commensurate bone remodeling between sheep and humans, both of which are 2-3 months. The specific aim of this study was to investigate fatigue properties in OVX and control bone and to analyze microdamage in terms of morphology, location and behavior.

METHODS:
Thirty-four aged ewes were divided into two groups; ovariectomy (OVX; n=16) and control (n=18). All animals were intravenously administered a different colored fluorochrome dye at 3 month intervals following surgery to label bone turnover. Animals were sacrificed 12 months post surgery. Rectangular beams were taken from the anterior quadrant of the right metatarsal using a diamond saw (Accutom-50, Struers, Ballerup, Denmark). The final dimensions (2 x 2 x 36 mm) were attained using a slow speed grinding wheel (DP10, Struers, Ballerup, Denmark). Specimens were fatigue loaded in three-point bending on a pneumatic testing machine (MTS Tytron 250, USA). Loading was applied such that compression would be induced on the endosteal surface and tension on the periosteal surface. Specimens were fatigue at a stress range of 110 MPa with a frequency of 3 Hz and were tested to outright failure. Following failure, specimens were stained en bloc with basic fuchsin. Histological sections were examined at X1 magnification to measure bone area (Olympus IX51, Hamburg, Germany) and then were examined at X10 magnification to identify microdamage. The measurement area was divided at the midpoint into two regions; compressive and tensile. Linear microcracks and diffusse damage were identified using standard criteria (3, 4). Microdamage was classified in terms of morphology and region, also any interaction between microdamage and local microstructural features, specifically fluorochrome labeled osteons, was recorded.

RESULTS:
Figure 1 shows the S-N curve for beams of cortical bone from both groups. The average number of cycles to failure (Nf) in the OVX group was less than the controls, but the difference was not significant (Fig.1).

![S-N Curve](image)

**Fig. 1:** S/N curve from beams which were fatigued in 3-point bending.

Linear microcrack density (Cr.Dn) was lower in the OVX group compared to controls, but the difference was not significant. In the control group Cr.Dn was lower in the compressive region compared to the tensile region (0.738±0.67 and 0.285±0.20 #/mm² respectively, p<0.05). In the OVX group, a similar trend was present in Cr.Dn between the compressive and tensile regions (0.679±0.32 and 0.192±0.09 #/mm² respectively, p<0.05). While short microcracks (~150µm in length) which encountered osteons were observed to stop at the cement line, longer microcracks (150-300µm) tended to be deflected around the osteon and some large cracks (>300µm) penetrated into unlabeled osteons. However, fluorochrome labeled osteons were rarely penetrated by linear microcracks, regardless of their length (Fig.2). In contrast to linear microcracks, diffuse damage was higher in the tensile region compared to the compressive region in the control group (0.025±0.01 and 0.004±0.005 (damage area/mm²)) and also in the OVX group (0.015±0.09 and 0.002±0.003 (damage area/mm²)) (p<0.05). Unlike linear microcracks diffuse damage was observed to be present within fluorochrome labeled osteons.

![Image](image)

**Fig.2:** Two long (>300 µm) linear microcracks arrested at a fluorochrome labeled osteon. Sample was stained en bloc with basic fuchsin and viewed using green epifluorescence at X10 magnification.

DISCUSSION:
During fatigue loading of bone, damage occurs within the tissue matrix. Microdamage is a parameter which influences bone quality, and thus fracture risk. In this study, we measured the fatigue properties of compact bone and analyzed the behavior of fatigue induced microdamage in relation to local microstructural features. Average Nf was lower in the OVX group, but the difference was not significant. While considerable scatter was present in the data, it compared well with other data in the literature. Linear microcracks were found mainly in the compressive region and diffuse damage was mainly in the tensile region, this agrees with findings by other workers (3). These linear microcracks initiated in interstitial bone and if, during propagation, an osteon was encountered, cracks would not become arrested at the cement line, deflected around it, or penetrate through it, depending on crack length. This phenomenon was previously described by O’Brien et al. (4). However, it was noted that when a crack encountered a fluorochrome labeled (and thus formed during the experimental period) osteon, it would not break through the cement line, regardless of crack length (Fig.2). This may be because newer osteons are still in the primary stage of mineralization. Diffuse damage was observed mainly in the tensile region. However, in contrast to linear cracks, areas of diffuse damage were present within labeled osteons. This may be a result of higher strain experienced by less mineralized, and thus less stiff, osteons. Preliminary nonindentation testing showed that labeled osteons were significantly less stiff than surrounding tissue. The results of this study show that the pre-fracture behavior of microdamage in bone is a complex and multifactorial phenomenon. Increasing our understanding of this phenomenon will be important in order to create a better definition of bone quality.

REFERENCES:

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