INTRODUCTION: Bone loss associated with estrogen depletion increases fracture risk in humans [1]. BMD via DTA is an insufficient estimate of fracture risk in metabolic bone diseases such as osteoporosis; overlap exists in BMD measurements between those who suffer from osteoporotic fractures and those who have no history of fracture [2,3]. Recent clinical diagnostic instrumentation advances could provide an in vivo means to test patients’ bone time dependent mechanical properties, which have been generally correlated with fracture properties [4]. A significant relationship between stiffness and yield strength has been demonstrated in bovine cortical bone [5] leading to the current study rationale of exploring cortical bone quality as an issue of bone material defined by the material time dependent mechanical property, storage modulus (E1), and the material fracture mechanics, fracture toughness (Kf) and maximum load (Pmax). Are the relationships maintained in the face of hormonal perturbation? The storage modulus of a material can be measured by the application of a sub-yield oscillatory stress. Fracture toughness as measured by the stress intensity factor, Kf, is a single value measure of material resistance to crack growth. The maximum load, Pmax, achieved during a fracture test is a measure of material strength. We hypothesized that: (1) time dependent mechanical properties predict the fracture toughness and strength of bone tissue, (2) time dependent mechanical properties of ovine bone change after ovariectomy (OVX), correlating with a decrease in the strength and fracture toughness for rates of loading associated with falls, (3) after degradation caused by OVX, the time dependent mechanical and fracture properties of ovine bone can recover after estrogen replacement therapy (ERT).

METHODS: Under local IACUC approval, 5-6yo Columbia-Rabouillette ewes were subjected to: an abdominal sham surgery (Sham N=10), an ovariectomy (OVX N=8), or an OVX for 24 months followed with periodic subcutaneous estrogen replacement therapy (ERT) for 24 months (ERT N=10). Upon sacrifice, the left radius was harvested and stored in saline-soaked towels at -20°C until testing. The mid-diaphysis from each radius was cut under cold-water irrigation (Exakt, Oklahoma City, OK) into 1.75x1.75x19mm beams, oriented with long axis in the proximodistal direction. For this study, 2 beams from the cranial (convex, tensile [6]) side and 2 from the caudal (concave, compressive) side were used. Dynamic Mechanical Analysis: All beams were prepared nondestructively in 3-point bending (DMA7e, PerkinElmer; force control 1-20Hz @2Hz increments; 15mm span, cranial side in tension, normal saline at 37°C, static load 550mN, dynamic load 500mN). E1 (storage modulus, analogous to Elastic Modulus) was calculated at each frequency. E1@1Hz and E1@20Hz were the outcome measures for this study. Fracture Mechanics: A dental cutter (150μm kerf, 22mm diameter, SummaDisk, Shofu Dental Corp.) was attached to a CNC milling machine (Micromill 2000, Denford, Inc.). Each beam was notched on the cranial tensile side under saline irrigation such that the ratio of notch length to beam width (a/W) was 0.3. The beam was then loaded beyond failure in 3-point bending (Enduratec BOSE Inc., displacement control, 15mm span, normal saline at 37°C). A cranial and caudal beam from each ewe was randomly selected for fracture under a low and high displacement rate. Displacement rates were set for each beam by calculating the strain rate achieved during DMA testing (the low displacement rate reproduced strain rate achieved @1Hz during DMA testing, high displacement rate @20Hz [7]). Mean strain rates were 0.001 sec⁻¹ and 0.011 sec⁻¹ for low and high displacement rates, respectively. The outcome measures, K and Pmax, were calculated from the load-displacement curves as described elsewhere [8]. Analysis: E1@1Hz and E1@20Hz were examined as functions of treatment (OVX vs. Sham vs. ERT), side (cranial vs. caudal), and fracture test speed (slow or fast) with a 3-way Mixed Model ANOVA (α = 0.05). Fracture properties were examined as functions of treatment and side with 2-way Repeated Measures ANOVA (α = 0.05). Pearson Product Moment Correlations (α = 0.05) were used to test the correlations between fracture properties and the time dependent mechanical property by treatment and speed of fracture.

RESULTS: ANOVAe (Table 1): Storage Modulus (E1): There is significant cranial-caudal variability at both 1Hz and 20Hz (cranial-caudal). There were no demonstrable main effect or interaction involving treatment. Fracture Properties: There were no demonstrable main effects or interactions involving treatment or side at either fracture speed for K or Pmax. Pearson Product Moment Correlation (Table 2): At the slow fracture speed (Fig.1), there was a significant positive correlation between K and storage modulus regardless of hormonal status. At the fast fracture speed (Fig.2), the significant correlation between K and storage modulus which exists for Sham was lost for OVX and recovered with ERT. The only demonstrable correlation involving Pmax and storage modulus involved the Sham animals at the slow fracture speed. This relationship was lost with OVX and not recovered with ERT.

DISCUSSION: In this long-term ovariectomy model, material stiffness was not a reliable predictor of fracture toughness or strength of bone tissue; nor were there significant main effects of OVX found on mechanical properties. The significant relationships between fracture properties and storage modulus varied with hormonal status and speed of fracture. The hypotheses that the main effects of degradation caused by OVX and recovery with ERT on time dependent mechanical and fracture properties were not supported by this data set. However, it was demonstrated that ERT recovered the relationship lost with OVX between storage modulus and fracture toughness at high loading rates. Although the strength-stiffness paradigm existed for normal bone, this data set suggests it may not be extended to an estrogen-depleted population. The lack of relationship between stiffness and strength in estrogen depleted compact bone may become problematic when using nondestructive oscillatory mechanical tests as a clinical screening tool for fracture risk.

SIGNIFICANCE: Fracture risk may be unpredictable using nondestructive tests of time dependent mechanical properties for the population most susceptible to fracture, the estrogen-depleted.

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