INTRODUCTION:
The issue of whether, and to what extent, bone materials and fracture characteristics changes in humans with the seasons is complicated by matters of differential seasonal risk factors, nutritional opportunities, and social interaction. Studies examining seasonal bone turnover, density, and fracture risk have been hindered by wide intersubject variation and contradictory results. If seasonal variation in bone materials properties exists in the human (and the current data are ambiguous on this point), then the use of an animal model with known seasonality in other aspects of its physiology may be of some use in determining mechanisms for these phenomena.

The sheep has been for many years a convenient and robust large animal model of bone material and structural properties, but most breeds used in research are seasonal breeders (experiencing a winter anestrus). Some seasonal variation in ovine cancellous bone histomorphometry has been noted, but a systematic examination of compact bone material properties has not, to our knowledge, been attempted. In this study, we set out to determine whether significant seasonal variation exists in ovine compact bone viscoelastic properties, parameters that are known to be quite labile with perturbations such as estrogen depletion, unloading, and ischemia. We hypothesized that in the normal sheep, significant seasonal variability in the compact bone viscoelastic material properties, above and beyond those normally seen as a matter of anatomic variation, would be observed.

METHODS:
Under local IACUC approval, 56 5-year-old female Columbia-Rambouillet sheep were anesthetized and subjected to a sham-ovariectomy (abdomen entered, ovaries handled but left in place) as part of another study. 14 animals began the study in the summer, and 14 each in the following autumn, winter, and spring, respectively. Animals were kept outside in a dry lot on a grass-hay diet, with access to shelter, for 3 or 12 months at a location 1524m above sea level, 41°N latitude, USDA climate zone 5/6. They were shorn twice a year. Thus, each seasonal sacrifice group consisted of members of two purchase cohorts, and except for the summer sacrifice group, each seasonal sacrifice group represented samples collected during two successive years. At sacrifice, the left radius/ulna (the two bones fuse early in life in this species), was wrapped in saline-soaked towels and kept at -20°C until further processing. 18-25 1.75 x 1.75 x 19mm beams were cut from the mid-radial diaphysis of each specimen, with the long axis of the beam parallel to the long axis of the bone, and the short sides oriented in the cranio-caudal and lateromedial planes. One beam from each of six radial sectors (craniomedial, cranial, craniolateral, caudomedial, caudal, and caudolateral) was sampled for dynamic mechanical analysis. Beams were tested in force control in cranio-caudal 3-point bending (15mm between outer supports) in normal saline at 37°C, in a PerkinElmer DMA7e. Non-destructive testing consisted of a frequency scan of 1-20Hz at 0.2Hz increments, with a static load of 550mN and a dynamic load of 500mN. E1 (storage modulus, analogous to Elastic Modulus), and tangent delta (the tangent of the phase angle between applied force and resulting strain, a measure of damping), were calculated at each frequency. E1 data were fit to the curve $E_1 = a \cdot \text{frequency}^b$, where a represents $E_1$ at Frequency=1Hz (quasistatic material stiffness), and the exponent b represents a measure of stress-rate sensitivity. After testing, the beam was cut in half: the proximal half was subjected to drying at 100°C and subsequent ashing at 800°C, and an ash density (g/cc) and % mineralization (100*ash mass/dry mass) were calculated. Analysis: All output variables (a,b, tan-delta at 1,3,6,9,12,15,18, and 20Hz; ash density, %min) were analyzed in a 2-way repeated-measures ANOVA (sector and sacrifice season), with post-hoc Fishers LSD tests. Alpha=0.05 for main effects, alpha=0.10 for interactions with subsequent post-hoc alpha set to 0.05.

RESULTS:
Sacrifice season had no demonstrable effect on ash density, a, or tan-delta(1Hz) (0.051<p<0.32). Exponent b (a measure of stress-rate sensitivity) was significantly greater in the summer sacrifice group than in the other three groups (Fig.1). At applied oscillations > 1Hz, tangent-delta was consistently greater for the summer and spring groups than for the winter and autumn sacrifice groups (Fig.2). There were no demonstrable interactions (0.137<p<0.946) between season and sector for a,b, or any tangent-delta (that is, the anatomic variation in these parameters, with high damping characteristics caudally and lower damping cranially, did not change with the seasons). % min, on the other hand, is altered in its anatomic distribution with the seasons, as well as its overall levels (Fig.3), resulting in low values in all sectors in the spring, while the season of maximum values varies with the location.

DISCUSSION:
Normal ovine compact bone material from a known seasonal breeding strain is most responsive to changes in strain rate in the summer, and undergoes more efficient damping for a given oscillation in the spring and summer. There appears to be no change in the normal anatomic distribution of the time-dependent mechanical properties that we measured, more of a concentric increase and decrease with the seasons. Ash density, a function of both material and architecture, did not change significantly through the year, though % mineralization, a subtle but important pure-material property, not only was altered in its overall values but was changed in its anatomic distribution. Since the bending that occurs in normal structural bone service is likely a function of both geometry and material distribution, this change could conceivably alter the bending characteristics of the structure enough to alter fracture risk in certain seasons under normal loading conditions.

Whether or not this is a serviceable model for seasonal variation (should it exist) in the human, it is apparent from these data that seasonality of compact bone material properties should be considered in any long-term study involving the sheep.