Introduction: Osteoporosis is a disease characterised by reduced bone mass, microarchitectural deterioration and an increased risk of fragility fractures. Compact bone makes up approximately 80% of the human skeleton by mass, with the remainder made up by trabecular bone. There is a substantial volume of literature available on the effect of osteoporosis on trabecular bone, however relatively few investigators have examined compact bone. We used an ovine model of osteoporosis to examine the effects of estrogen deficiency over a 31 month period. The specific aims of the study to investigate the effect of ovariectomy on bone turnover and microarchitecture in compact bone; and to assess their effect on bone strength.

Materials and Methods: Twenty seven skeletally mature sheep were randomly divided into two groups: ovariectomy (OVX; n=11) and control (CON; n=16). Control and ovariectomised animals were administered five different coloured fluorochromes over the ensuing twenty four month period (0, 12, 16, 20 and 24 months) to label sites of bone turnover. Animals were sacrificed at 31 months following surgery.

Compact bone samples (11mm length) were harvested from mid-diaphysis of the left metatarsal 4cm proximal to the metatarsal-phalangeal joint using a low speed diamond saw (Accutom 50, Struers, Ballerup, Denmark). For histological analysis, thin sections (150-200μm) were prepared. Each section was initially examined using brightfield microscopy (Olympus 1X51, Hamburg, Germany). Cortical area was measured using an image analysis system by measuring area enclosed by the perisoteal surface and subtracting the area of the medullary canal (analySIS, Soft Imaging systems, Munster, Germany). Sections were then examined using epifluorescence microscopy at 10X magnification. The number of resorption spaces at time of sacrifice was recorded, and the amount of intracortical bone turnover at each time point was assessed by the ratio of area to the number of resorption spaces at time of sacrifice was recorded, and the amount of intracortical porosity cannot reliably predict histological evidence of sustained increased turnover over the study period.

The remainder of the metatarsal bone sample (10mm length) was scanned using microCT (Scanco,μCT-40, Basserdorf, Switzerland). The scan time for each sample was less than thirty minutes with a resolution of 8μm. Image Processing Language (IPL, Scanco, Basserdorf, Switzerland) was using to calculate the intracortical porosity. After scanning samples were subjected to unconfined compression testing on the long axis of the bone on a materials testing machine (Instron 8501, Bucks, UK) with a cross-head speed of 0.01mm/s. The compressive strength was calculated by divided the maximum force by the average cross-sectional area of the sample.

Results: All animals tolerated surgery and administration of fluorochrome dyes without complication. There was no significant difference between mean control and OVX carcass weight at sacrifice. Cortical area of samples did not differ significantly between groups. The number of resorption spaces was significantly increased in the OVX relative to controls (0.252±0.056/mm2 and 0.111±0.202/mm2 respectively) (p=0.02). At 0 months there was no significant difference in labelled osteons/mm2 between control and OVX groups. At 12, 20 and 24 months the labelled osteons/mm2 were significantly increased in the OVX group compared to controls. At 16 months both groups had low numbers of labelled osteons, and although the OVX group was greater than control, this was not statistically significant. Intracortical porosity, measured by microCT, was not significantly increased in the OVX group compared to controls (2.53±0.002% and 2.20±0.001% respectively) and the compressive strength was not significantly different between controls and OVX (121.43±0.006 MPa and 131.12±0.007MPa respectively)

Discussion: It has previously been shown by this group that intracortical bone turnover is increased in ovine compact bone 12 months post-ovariectomy (1). The current study demonstrates that this trend continues at 31 months and is evident even at times of relatively low bone turnover as seen at 24 months. In contrast data on compressive strength and porosity did not demonstrate significant differences between the groups. These findings are unexpected when we have previously shown a significant increase in porosity at 12 month and a reduction in compressive strength. In contrast literature available on trabecular bone demonstrates progressive diminution of mechanical strength and increased porosity in osteoporosis models (2,3). Our results indicate that compressive strength or Micro CT analysis of porosity cannot reliably predict histological evidence of sustained increased turnover over the study period.


Acknowledgements: This project was funded by the Health Research Board of Ireland and the Higher Education Authority in Ireland under the PRTLI Cycle 3.