

## Further characterisation of an ovine model of steroid-induced osteopaenia

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**INTRODUCTION:** Bone loss and increased bone fragility following long-term steroid therapy may result in painful vertebral fractures. A large animal model is needed to fully understand the pathogenesis of steroid-induced osteoporosis and to test potential implants. The sheep is a valid model since bone remodelling is similar to humans and the vertebral dimensions permit easy surgical manipulation. This study was undertaken to fully characterise the sheep as a model of steroid-induced vertebral osteoporosis.

**METHODS:** Osteoporosis was induced in ten 8-year-old lactating ewes by oophorectomy, weekly injection of 54 mg dexamethasone and a diet containing 0.2% calcium for up to 6 months. Iliac crest biopsies were taken at the start and end of treatment to measure a range of bone histomorphometric indices. Control animals were neither ovariectomised nor treated with steroids. Bone mineral density (BMD) in the lumbar spine (L2-L5) was measured by dual energy X-ray absorptiometry (DXA) after 0, 3 and 6 months of treatment. At each time interval sheep were killed and the entire lumbar spine (L1-L6) and distal and proximal femora were processed for histology, quantitative histomorphometry, mechanical testing, micro-CT (computed tomography) and for expression of a range of molecular markers using real-time PCR analysis.

**RESULTS:** After six months of treatment BMD in the lumbar spine decreased by 29.5% from baseline (Figure 1). Trabecular bone volume (BV/TV) of L2, L3 and L4 vertebrae (pooled) decreased by 31.4% and trabecular thickness (TbTh) decreased by 33.9%;  $p < 0.05$  (Figure 2). Cortical bone thickness decreased by 43.9%;  $p < 0.05$ . BV/TV in the distal and proximal femora decreased by 48.6% and 42.5% respectively ( $p < 0.05$ ) and trabecular thickness decreased by 35.2% and 29.5%. The average load at which L1 yielded decreased by 67.4%. Static measurements of bone formation

decreased by 68.3% and bone resorption increased tenfold. Coll-1 gene expression was significantly down regulated in the vertebral bodies.

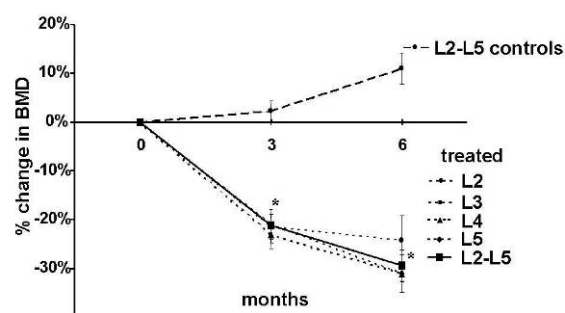


Figure 1: Percent change in lumbar spine BMD over 6 months of continuous steroid treatment

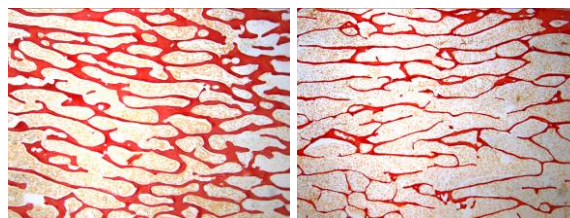


Figure 2: Vertebral trabecular architecture of control (left) and steroid-treated (right) sheep

**DISCUSSION & CONCLUSIONS:** Using DXA, cancellous bone histomorphometry and mechanical testing, this study has demonstrated significant trabecular and cortical bone loss in the sheep lumbar spine up to six months after ovariectomy and continuous steroid treatment. Significant loss of trabecular bone was also seen in the femora. These changes are the result of increased resorption and decreased formation of bone. This is supported by the molecular analyses. This ovine model is suitable for pharmacological trials and in-vivo assessment of vertebral augmentation procedures and orthopaedic devices.