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Short communication

Alterations in trabecular bone microarchitecture in the ovine spine and distal femur following ovariectomy

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ABSTRACT

Osteoporosis is a bone disease resulting in increased fracture risk as a result of alterations in both quantity and quality of bone. Bone quality is a combination of metabolic and microarchitectural properties of bone that can help to explain the increased susceptibility to fracture. Translational animal models are essential to understanding the pathology and for evaluating potential treatments of this disease. Large animals, such as the ovariectomized sheep, have been used as models for post-menopausal osteoporosis. However, long-term studies have not been carried out to observe the effects of ovariectomy after more than one year. This study employed micro-computed tomography to quantify changes in microarchitectural and mechanical parameters in femoral condyles and vertebral bodies of sheep that were sacrificed one or two years following ovariectomy. In the vertebral body, microarchitectural characteristics were significantly degraded following one year of ovariectomy in comparison to controls. The mechanical anisotropy, determined from micro-scale finite element models, was also greater in the ovariectomized groups, although the fabric tensor anisotropy was similar. There was no greater architectural degradation following two years of ovariectomy compared to one. Ovariectomy had minimal effects on the trabecular architecture of the distal femur even after two years. These results indicate that the vertebral body is the preferred anatomic site for studying bone from the ovariectomized sheep model, and that architectural changes stabilize after the first year.

1. Introduction

Animal models are essential for studying the mechanisms and potential treatments of osteoporosis. Osteoporosis is a bone disease characterized by an increase in bone fragility that leads to an increased risk of fracture (NIH, 2001). Clinically, the primary diagnostic criteria for osteoporosis are based on decreased bone mineral density (BMD) (WHO, 1993). However, BMD is neither sufficiently specific nor sensitive as a predictor for future fracture risk (Schuit et al., 2004). As such, bone quality has been suggested as an additional measure of bone fragility (Turner, 2002; Bouxsein, 2003; Seeman, 2003). In trabecular bone, microarchitectural parameters such as bone volume fraction (BV/TV), structural model index (SMI), trabecular thickness (Tb.Th.), and trabecular spacing (Tb.Sp.) play an important role in bone quality (Fazzalari et al., 1998; Hernandez and Keaveny ,2006).

Ovariectomized rats have commonly been used as a model for post-menopausal osteoporosis (Mosekilde et al., 1993; Bagi et al., 1997). However, rats lack Haversian systems in cortical bone and basic multicellular unit remodeling in trabecular bone (Wronski et al., 1989). Biomechanical effects of ovariectomy on trabecular bone mechanics are also difficult to assess in rats because their bones are too small to prepare adequate test samples. However, trabecular bone plays a major role in fractures because it represents over half of the bone mass in proximal femur and in lumbar vertebrae, where most osteoporotic fractures occur (van Staa et al., 2001).

Sheep have been used as a model for osteoporosis research owing to their docile nature, lack of confounding dietary and lifestyle factors, and a bone macrostructure that resembles humans (Turner, 2001; Pearce et al., 2007). Sheep are also large enough to provide tissue samples from various anatomic sites that are suitable for mechanical testing (Lill et al., 2002). Sheep have also been demonstrated to be a suitable model for secondary osteoporosis (Lill et al., 2002; Schorlemmer et al., 2003; Zarrinkalam et al., 2009; Ding et al., 2010). Ovariectomy (Newton et al., 2004), glucocorticoids (Ding et al., 2010), and metabolic

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acidosis (Macleay et al., 2004) alone or in combination can be used to induce bone loss in sheep. However, ovariectomy has complex effects on the sheep, with some studies showing sustained architectural degradation (Newton et al., 2004), and others showing an initial degradation that returns to baseline values (Sigrist et al., 2007). Long-term studies observing microarchitectural effects of ovariectomy have only been carried out for six months or one year (Turner et al., 1995; Lill et al., 2002; Schorlemmer et al., 2003; Newton et al., 2004; Zarrinkalam et al., 2009). The objective of this study was to determine the effects of long-term ovariectomy on microarchitectural and mechanical parameters of vertebral bodies and femoral condyles of sheep 12 months and 24 months after ovariectomy.

2. Materials and methods

2.1. Animal model

This study was approved by the Institutional Animal and Care and Use Committees of the Colorado State University and the University of Notre Dame. Thirteen skeletally mature female sheep underwent bilateral ovariectomy under general anesthesia. The sheep were then returned to pasture until they were sacrificed one year (OVX-1) or two years (OVX-2) following the ovariectomy. Six control samples were taken from sheep of similar age sacrificed for other studies or due to age. Bone tissues were harvested immediately, stripped of soft tissue, and stored at −20 °C until they were prepared for testing.

2.2. Microarchitecture and mechanics quantification

The L3 vertebra was harvested from seven sheep in the control group and OVX-1 group, and from six sheep in the OVX-2 group. Spinous and transverse processes were removed at the pedicles, leaving only the vertebral body. Two cylindrical cores with a diameter of 8 mm and a length of 30 mm were taken from each medial and lateral femoral condyles, resulting in 14 samples from control and OVX-1 groups, and 12 from the OVX-2 group.

The vertebrae were scanned at 20 μm isotropic resolution at 70 kVp with 1000 projections at 350 ms per projection. Specimens were kept hydrated with buffered saline solution for the duration of the 2 h scan. The microarchitecture of the femoral condyles was quantified by scanning each core at 20 μm isotropic resolution at 70 kVp with 500 projections at 210 ms per projection. Specimens were kept hydrated in buffered saline for the duration of the 1 h scan. Architectural measures for all samples were quantified by a model-free method (μCT evaluation program V4.3, Scanco Medical AG, Brüttisellen, Switzerland). Images were Gaussian filtered with width of 0.8 and a support of 2 voxels. Bone mineral density (BMD) and tissue mineral density (TMD) were quantified using the scanner's calibration phantom using a constant segmentation threshold of 200, which corresponded to 407.3 mg-HA/cc on the scanner. Representative scans for each group at each anatomic site are shown in Fig. 1.

BV/TV, SMI, Tb.Th., Tb.Sp., BMD, TMD, and degree of anisotropy (DA) were quantified and compared between OVX group and anatomic site.

The mechanical anisotropy was quantified for the vertebral bodies and a subset of the medial femoral samples. The elasticity tensor for each sample was calculated using detailed finite element models of the microstructure (Van Rietbergen et al., 1996). Briefly, six models with uniaxial strain boundary conditions were solved, each providing one column of the elasticity matrix. An optimization procedure was used to align the elasticity tensor to its nearest orthotropic form (Van Rietbergen et al., 1996). The ratios of the largest to smallest elastic moduli, largest to smallest shear moduli, and the largest elastic modulus to the mean of two shear moduli in planes parallel to the largest elastic modulus were calculated. Note that these ratios are independent of the tissue modulus for the model, but do assume that the tissue modulus is homogeneous.

Statistical analysis was performed using JMP IN 5.1 (SAS Institute Inc., Cary, NC) with a significance level of 0.05. ANOVA with a Tukey's post-hoc test was used to determine difference between groups. A Wilcoxon Test was used for mechanical anisotropy tests due to the non-normal distribution of the data. Measurements were not compared between different anatomic sites.

3. Results

In the vertebral body, both OVX-1 and OVX-2 groups had lower BV/TV, Tb.Th., and BMD, and higher SMI and Tb.Sp. than the CTL group ($p < 0.05$, Table 1, Fig. 2). In contrast, OVX-1 and OVX-2 groups were not significantly different ($p > 0.05$, Table 1, Fig. 2).

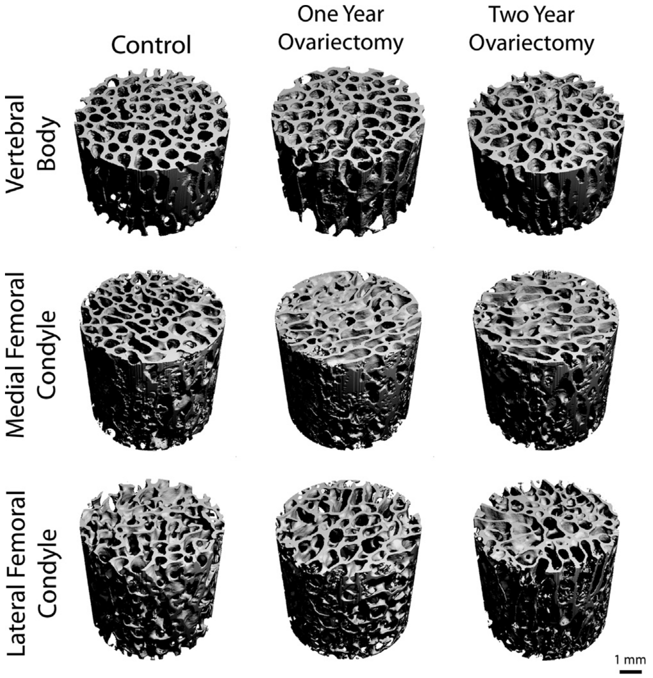

Fig. 1. Three-dimensional renderings of μ-CT scans of representative samples for each group at each anatomic site.

Ovariectomy resulted in few architectural changes in the femoral condyles. In the medial condyle, the BV/TV, BMD, and TMD were higher in the OVX-1 group than in controls ($p < 0.05$), but were similar in OVX-2 and control groups (Table 1, Fig. 2). The lateral condyle had lower DA in the OVX-1 group compared to controls ($p < 0.05$), but the OVX-2 group did not differ from controls and was higher than OVX-1. The TMD was higher in the ovariectomized groups than in controls ($p < 0.05$, Fig. 2).

Mechanical anisotropy significantly increased in the vertebral body for both elastic and shear modulus ratios ($p < 0.05$, Table 2). There were no significant differences in anisotropy between groups for the medial condyle samples ($p > 0.05$, Table 2).

4. Discussion

Large animal models of osteoporosis are important, because they provide a source of tissue that is amenable to mechanical testing. Sheep can be raised at relatively low cost, respond to ovariectomy, and exhibit osteonal remodeling in the cortical bone. These results demonstrate that the architecture and mechanical behavior of the vertebral body are significantly altered following one year of ovariectomy. Notably, changes in SMI, Tb.Th., and Tb.Sp. were found that, in combination with decreased BV/TV, are known to contribute to decreased mechanical integrity of bone (Mittra et al., 2005; Teo et al., 2006; Garrison et al., 2009; Wegrzyn et al., 2010; Wu et al., 2013). Similar changes were observed in animals followed for two years after ovariectomy, suggesting that architectural degradation occurs within the first year, but does not progress. Changes in the femoral bone were small, and not associated with increased fracture risk. Hence, the distal femur may provide a suitable site to study changes in trabecular bone mechanical behavior that are associated with ovariectomy status but independent of the architecture, such as tissue modulus, toughness, or composition.