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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 the 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 evaluating potential treatments of this disease. Large animal models, such as the ovariectomized sheep, have been used as a model 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 the 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 the architectural changes stabilize after the first year.

Keywords

Microarchitecture; Ovariectomy; Osteoporosis; Sheep; Vertebrae; Femur

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Conflict of Interest Statement

The authors have no conflict of interest

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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 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 the proximal femur and in lumbar vertebrae, where most osteoporotic fractures occur (van Staa 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 2009; Ding et al. 2010). Ovariectomy (Newton 2004), glucocorticoids (Ding et al. 2010), and metabolic 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 2004), while others showed 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 2004; Zarrinkalam 2009). The objective of this study was to determine the effects of long-term ovariectomy on microarchitectural and mechanical parameters of the 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. The 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 in the control and OVX-1 groups, and 12 from the OVX-2 group.

The vertebrae were scanned at $20\text{ }\mu\text{m}$ 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 hr. scan. The microarchitecture of the femoral condyles was quantified by scanning each core at $20\text{ }\mu\text{m}$ 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 hr. 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. 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 stage and anatomic site.

The mechanical anisotropy of the vertebral bodies and a subset of the medial femoral samples was quantified. 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 most nearly 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 the two shear moduli in the planes parallel to the largest elastic modulus were calculated. Note that these ratios are independent of the assumed 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, the OVX-1 and OVX-2 groups both 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, the OVX-1 and OVX-2 groups were not significantly different ($p > 0.05$, Table 1, Fig. 2).

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 the controls ($p < 0.05$), but were similar between the 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 the 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 the mechanical behavior of the vertebral body is 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 2006; Garrison et al. 2009; Wegrzyn et al. 2010; Wu 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.

The mechanical anisotropy of the bone increased in the vertebral body for both elastic and shear loading. This suggests that microarchitectural changes resulting from ovariectomy are preferentially in the transverse plane. The mechanical anisotropy would be expected to coincide with changes in the architectural degree of anisotropy (Cowin 1985; Zysset and Curnier 1996; Zysset et al. 1998), but the latter were much smaller and nonsignificant. No effects were seen in elastic or shear moduli ratios following ovariectomy in the medial condyle. The lack of changes in ratios in the medial condyles suggests that changes in mechanical anisotropy are primarily dependent on architectural changes.

The results from the study in the vertebral body are in agreement with previous studies, which found decreased BV/TV and Tb.Th. and increased Tb.Sp. in ovariectomized ewes through histomorphometric analysis of the lumbar vertebrae (Giavaresi et al. 2001) and the iliac crest (Newton 2004). Similar results have been reported for the femoral neck, with decreases in BV/TV, SMI, and Tb.Th. one year after ovariectomy (Wu et al. 2008; Holland

et al. 2011). However, the complex morphology of the femoral neck may complicate preparation of mechanically aligned trabecular bone samples for testing. In contrast, the distal femur has a large volume of bone for preparation of samples, but exhibits little architectural change in ovariectomized animals. The difference in anatomic site is likely the cause of the discrepancy in the results.

The architecture and density of the ovine vertebral body may not be representative of human bone. Even after ovariectomy the BV/TV was 0.36 and the Tb.Th. was 190 μm , which are slightly higher than those seen in healthy human trabecular bone at the proximal femur (Krug et al. 2005; Perilli et al. 2008). Meanwhile, the distal femoral bone was more representative of human trabecular bone, but underwent little change as a result of ovariectomy. As such, ovariectomy may not be sufficient to induce microarchitectural changes relevant to the biomechanical evaluation of osteoporosis in humans. In addition to ovariectomy, a glucocorticoid regimen, or a highly anionic diet to induce metabolic acidosis, may induce further degradation in the trabecular microarchitecture to be relevant for mechanical studies of osteoporosis in the sheep model (MacLeay et al., 2004).

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

Figure 2.

Both BV/TV (a) and BMD (b) of the vertebral bodies were lower in the ovariectomized groups than in controls, while in the medial condyle, both were higher in the one year ovariectomy group than in controls. In the lateral condyle, TMD (c) was higher in both ovariectomized groups compared to controls, while in the medial condyle TMD was only higher in the one year ovariectomy group compared to the two year and control groups (* $p < 0.05$, ANOVA within each anatomic site).

Table 1

Microarchitectural degradation was seen primarily in the vertebral body. Data are mean (S.D.)

	Vertebral Body			Medial Condyle			Lateral Condyle		
	CTL	OVX-1	OVX-2	CTL	OVX-1	OVX-2	CTL	OVX-1	OVX-2
SMI	-1.72 (0.04)	-0.97 (0.40)*	-1.19 (0.24)*	-0.086 (0.13)	-0.35 (0.47)	-0.20 (0.27)	0.12 (0.26)	0.19 (0.26)	0.29 (0.17)
Tb.Sp. (mm)	0.44 (0.05)	0.52 (0.05)*	0.55 (0.03)*	0.58 (0.03)	0.56 (0.07)	0.56 (0.05)	0.58 (0.03)	0.58 (0.05)	0.62 (0.04)
Tb.Th. (mm)	0.22 (0.03)	0.19 (0.02)*	0.19 (0.01)*	0.18 (0.02)	0.21 (0.03)	0.19 (0.02)	0.18 (0.01)	0.19 (0.01)	0.17 (0.01)
DA	2.45 (0.11)	2.49±0 (0.12)	2.55 (0.08)	2.21 (0.22)	2.03 (0.12)	2.20 (0.14)	2.07 (0.15)	1.84 (0.12)*	1.88 (0.15)

* p < 0.05 vs CTL, ANOVA within anatomic site. No differences were detected between OVX-1 and OVX-2 groups

Table 2

Mechanical anisotropy depended on ovariectomy status in the vertebral body, but not in the medial femoral condyle. Data are mean (S.D.)

	Vertebral Body			Medial Condyle		
	CTL	OVX-1	OVX-2	CTL	OVX-1	OVX-2
Elastic Modulus Ratio	2.06 (0.15)	2.50 (0.31)*	2.50 (0.24)*	2.79 (0.50)	2.38 (0.50)	2.49 (0.50)
Shear Modulus Ratio	1.51 (0.07)	1.80 (0.19)*	1.79 (0.13)*	2.51 (0.45)	2.19 (0.37)	2.20 (0.36)
Elastic to Shear Modulus Ratio	3.58 (0.09)	3.90 (0.21)*	3.82 (0.16)*	3.91 (0.27)	3.66 (0.24)	3.86 (0.12)

* p < 0.05 within each anatomic site, N=7. No differences were detected between OVX-1 and OVX-2 groups.