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Variations in nanomechanical properties and tissue composition within trabeculae from an ovine model of osteoporosis and treatment

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ABSTRACT

Osteoporosis and treatment may affect both composition and nanomechanical properties and their spatial distributions within the individual trabeculae of cancellous bone at length scales that cannot be captured by bulk measurements. This study utilized 25 mature adult ewes divided into 5 treatment groups. Four treatment groups were given a dietary model for human high-turnover osteoporosis, and two of these were treated with antiresorptive drugs, either zoledronate (ZOL) or raloxifene (RAL), to examine their effects on bulk tissue properties and nanoscale tissue composition and mechanical properties within trabeculae. Treatment effects were most pronounced at the nanoscale, where RAL increased indentation modulus and hardness throughout trabeculae by 10% relative to the osteoporosis model. In comparison, ZOL increased these properties exclusively at the surfaces of trabeculae (indentation modulus +12%, hardness +16%). Nanomechanical alterations correlated with changes in tissue mineralization, carbonate substitution, crystallinity, and aligned collagen. Despite only minimal changes in bulk tissue tBMD, the nanomechanical improvements within trabeculae with both treatments greatly improved the predicted theoretical bending stiffness of individual trabeculae when idealized as cylindrical struts. Hence, small tissue-level alterations in critical locations for resisting trabecular failure could account for some of the discrepancy between the large reductions in fracture risk and the only modest changes in BMD with antiresorptive treatments.

1. Introduction

One in three women and one in five men over the age of fifty will experience an osteoporotic fracture in their lifetime [1–3]. The risk for additional fractures increases markedly after experiencing the first fracture [4]. Antiresorptive agents and selective estrogen receptor modulators (SERMs) reduce fracture risk from 30–50%, but increase areal BMD only slightly by 0–8% [5–10], suggesting that BMD alone is unable to fully capture skeletal alterations with treatment. BMD reflects the total amount of bone mineral (and the overall level of mineralization) but cannot capture effects at smaller length scales where failure initiates. At these smaller length scales, skeletal fracture risk depends on tissue microarchitecture and properties of mineral and matrix constituents that can vary spatially within individual bone microstructures due to the remodeling process [11–18]. These spatial distributions are particularly important because small tissue property changes in critical locations for resisting trabecular failure can profoundly affect skeletal fracture resistance. Imbalances or alterations in the remodeling process with osteoporosis and treatment may alter levels and spatial distributions of mineral and matrix properties within bone microstructures and could play a crucial role in our understanding of fracture risk and our ability to develop and evaluate successful therapies.

The mineralization gradient within trabeculae of healthy human bone is positive from surface to center and produces a positive gradient in tissue stiffness [15,19–23]. The manner in which individual trabeculae bear load is influenced by these tissue-level properties and

Abbreviations: BMD, bone mineral density; SERM, selective estrogen receptor modulator; MA, metabolic acidosis; CONT, control; RAL, raloxifene; ZOL, zoledronate; Sup, Superficial; Int, Intermediate; Ctr, Central; HA, hydroxyapatite; SHG, second harmonic generation; FTIR, Fourier transform infrared spectroscopy; mineral:matrix, mineral-to-matrix ratio; XLR, collagen cross-linking network maturity; BV/TV, bone volume fraction; tBMD, tissue mineral density; Tb.Th, trabecular thickness; Tb.Sp, trabecular separation.

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their arrangement within trabeculae. In postmenopausal women, osteoporosis reduces the overall heterogeneity of both tissue mineralization and mineral crystallinity [22,24,25], and bisphosphonate treatment further reduces heterogeneity in these properties [26–30]. However, the effects of osteoporosis and treatments on specific spatial distributions of compositional properties within trabeculae are not well-documented and may depend on treatment type. Mineral and matrix composition ultimately determine tissue mechanical function. Thus, any heterogeneity in tissue compositional properties results in nanomechanical heterogeneity that alters profiles of stress and strain within trabeculae and possibly affects the bone composite's ability to dissipate energy [14,17,18]. In healthy tissue, nanomechanical properties follow changes in mineralization and aligned collagen content [19,20,31]. Relationships between spatial compositional changes and the consequential nanomechanical alterations with osteoporosis and treatment have not yet been determined.

The goal of the present study was to compare spatial compositional and nanomechanical alterations in trabeculae with a large-animal model of osteoporosis and treatment. Two drugs were compared in this study. The first was a selective estrogen receptor modulator (SERM) that reduces turnover by acting through natural estrogen receptor pathways in bone and mimicking the effects of estrogen. The second treatment was from the widely-used bisphosphonate class of drugs that bind to bone matrix and directly inhibit osteoclastic activity upon resorption. SERMs and bisphosphonates reduce bone turnover to varying degrees yet produce similar reductions in fracture risk [32,33]. In a previous study, bisphosphonates altered collagen cross-linking in healthy female beagle vertebrae whereas SERMs did not [34]. SERMs improved vertebral mechanical performance independently of BMD in these same animals, suggesting that other changes in tissue composition might play an important role in fracture resistance [10,35].

Our hypotheses were that tissue stiffness and hardness would decrease in an ovine model of osteoporosis relative to healthy ewes due to alterations in tissue composition. Conversely, treatment with either raloxifene (SERM) or zoledronate (bisphosphonate) would restore tissue nanomechanical properties through further alterations in tissue composition. Greatest effects were expected near surfaces of trabeculae in regions of active remodeling and bone formation. Additionally, nanoscale rather than bulk tissue measures were predicted to better capture these changes with treatment because of their better spatial resolution. As tissue age and the amount of turnover influence tissue properties [21,22], zoledronate was expected to alter these properties more markedly than raloxifene relative to the ovine model for osteoporosis because bisphosphonates reduce bone turnover to a greater degree than SERMs [33,36]. However, raloxifene treatment was expected to better restore cancellous tissue properties to those present in healthy, ovary intact female sheep, since SERMs act through natural estrogen receptor pathways [37].

2. Materials and methods

The present study utilized a dietary-induced ovine model of osteoporosis and treatment to examine alterations in bone tissue compositional and nanomechanical properties, and changes in the spatial arrangements of these properties within trabeculae. The microstructure of sheep bone is similar to that of humans [38–42]. In a previous study, dietary-induced metabolic acidosis (MA) in sheep increased bone turnover and reduced bone mineral and whole bone strength similar to postmenopausal osteoporosis in humans [38,41,43]. In contrast, the ovine ovariectomy (OVX) model decreased bone turnover and caused osteomalacia [43], making MA the appropriate model choice for post-menopausal, high-turnover osteoporosis. Two different antiresorptive treatments were examined in this study: the SERM, raloxifene (RAL), and the bisphosphonate, zoledronate (ZOL).

2.1. Samples and specimen preparation

Femora were obtained from 25 mature adult Swiss-Ramboulet ewes (6–7 years old at the start of the experiment) from 2 experiments (approved by the Colorado State University Institutional Animal Care and Use Committee). Originally, the bisphosphonate and SERM treatments were planned as a single experiment; however, due to difficulty administering alendronate in the first study, the bisphosphonate study was repeated with zoledronate and an additional MA group for comparison. The first experiment had two groups. Both groups were fed a diet that induced compensated metabolic acidosis (MA) via the consumption anionic salts. Previously, this diet increased bone remodeling and decreased bone density as assessed by dual-energy x-ray absorptiometry [38,41,43] After an initial 6 months on the MA diet, the ewes continued this diet for another 6 months while receiving either vehicle (MA1, $n=5$) or a clinically equivalent dose of raloxifene (RAL, 0.80 mg/kg daily, $n=4$) administered daily through abomasal cannulae [41,43]. Both treatment groups started with $n=6$ sheep, based on a power analysis for nanoscale measurements in a pilot study plus one additional sheep per treatment group in accordance with standard procedure for ovine experiments at Colorado State University. Two RAL sheep were euthanized early due to cannula pull-out and one MA sheep was euthanized due to injuries unrelated to the experiment. A group of healthy animals fed a normal diet (CONT, $n=5$) were housed in the same facility and euthanized in the same season (summer) as Experiment 1. The second experiment had two treatment groups. Both groups received 8 months of MA rather than 6 months due to a delay in receiving the medication for the study. Subsequently, one group continued on the MA diet for 6 months with no treatment (MA2, $n=6$) and one group continued on the MA for 6 months with a clinically equivalent dose of zoledronate (ZOL, 5 mg/sheep, $n=6$) at the beginning of the treatment period. Both groups were euthanized in the winter. The second experiment included a MA group to compare the effects of zoledronate treatment with the ovine osteoporosis model, but did not include a normal diet control group. After euthanasia, femora were stored at −20 °C prior to specimen preparation.

2.2. Bulk cancellous tissue characterization

A single cylindrical core was taken from the medial-caudal quadrant of the distal femur from each sheep to characterize morphology and mechanical properties. Cores were excised with a diamond core drill (5 mm ID, Starlight Industries, Rosemont, PA) under constant irrigation with physiological saline, wrapped in saline-soaked gauze, and stored at −20 °C prior to scanning.

2.2.1. Micro-computed tomography (microCT)

Tissue architecture within each cancellous core was quantified by microCT (55 kVp, 145 mA, 600 ms integration time, μCT 35, Scanco Medical, Bruttisellen, Switzerland). The axial central third of each core was scanned at 15 μm isotropic resolution. A calibrated HA standard was used to convert the linear attenuation for each voxel to g HA/cm^3. A 0.5 mm aluminum filter reduced the effects of beam hardening. A global threshold was chosen for all specimens. Outcomes included bone volume fraction (BV/TV), tissue mineral density (tBMD, mg/cm^3), and trabecular thickness and separation (Tb.Th and Tb.Sp, mm).

2.2.2. Compression testing and ashing

Bulk cancellous mechanical properties were assessed by compression testing of the bone cores. Prior to testing, press-fit brass end-caps were bonded to the ends of each core to minimize end artifacts [44]. The average gage length and diameter of the exposed core between endcaps were measured and used for subsequent stress and strain calculations. The load function consisted of 5 preconditioning cycles of 0 to 0.1% compressive strain before monotonically loading to 3%