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The Effect of Osteoporosis Treatments on Fatigue Properties of Cortical Bone Tissue

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Abstract

Bisphosphonates are commonly prescribed for treatment of osteoporosis. Long-term use of bisphosphonates has been correlated to atypical femoral fractures (AFF). AFFs arise from fatigue damage to bone tissue that cannot be repaired due to pharmacologic treatments. Despite fatigue being the primary damage mechanism of AFFs, the effects of osteoporosis treatments on fatigue properties of cortical bone are unknown. To examine if fatigue-life differences occur in bone tissue after different pharmacologic treatments for osteoporosis, we tested bone tissue from the femurs of sheep given a metabolic acidosis diet to induce osteoporosis, followed by treatment with a selective estrogen reception modulator (raloxifene), a bisphosphonate (alendronate or zoledronate), or parathyroid hormone (teriparatide, PTH). Beams of cortical bone tissue were

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created and tested in four-point bending fatigue to failure. Tissues treated with alendronate had reduced fatigue life and less modulus loss at failure compared to other treatments, while tissue treated with PTH had a prolonged fatigue life. No loss of fatigue life occurred with zoledronate treatment despite its greater binding affinity and potency compared to alendronate. Tissue mineralization measured by microCT did not explain the differences seen in fatigue behavior. Increased fatigue life with PTH suggests that current treatment methods for AFF could have beneficial effects for restoring fatigue life. These results indicate that fatigue life differs with each type of osteoporosis treatment.

Introduction

Osteoporotic fractures are a substantial public health concern with total fractures and associated costs estimated to continue to rise through 2025[1]. Bisphosphonates are a commonly prescribed class of anti-resorptive drug that increase bone mineral density between 0–8% while reducing the risk of fracture by up to 50% in osteoporotic patients[2,3]. The large decrease in fracture risk despite the modest increase in bone mineral density suggests a material property change in bisphosphonate-treated tissue. Suppression of bone remodeling with bisphosphonates has led to concern over inability to repair damaged and older tissue[4]. To fully understand the reduction in fracture risk, all fracture properties and mechanisms should be examined.

Fracture of osteoporotic bone typically occurs through one of two mechanisms, a single overload (traumatic failure), or repetitive sub-fracture loads (fatigue failure; Figure 1). Typical osteoporotic hip fractures are due to mechanical overload, in which the femoral head and neck are subjected to loads that the bone cannot withstand due to reduced bone mass. Fatigue loads are repetitive, sub-failure forces applied to the tissue. Activities of daily living create fatigue loads that in turn create microdamage in the tissue[5]. Healthy individuals are unlikely to experience fatigue fractures under normal loading conditions since damage to the bone is typically repaired before fracture can occur. However, tissue properties may be altered in individuals using anti-resorptive treatments[6–9]. Knowledge of fatigue on bone tissue has been primarily gained from testing of machined sections of bones and has shown fatigue dependence with temperature, stress amplitude, and bone microstructure[10–12]. Studies examining fatigue of osteoporotic and treated tissue have focused on microdamage accumulation rather than the material properties of the tissue[4].

Bisphosphonates act through osteoclast inhibition, which leads to reduced bone turnover, increased bone mass and increased mineralization[13]. However, injury within tissue cannot be remodeled leading to an accumulation of microdamage[14–17]. Reduced bone turnover with bisphosphonate treatment increases mineralization and collagen maturity in bone tissue as measured by Fourier transform infrared spectroscopy (FTIR)[18]. Tests on whole bones after bisphosphonate therapy indicate an increase in monotonic strength and stiffness at corticocancellous sites without concomitant changes to the tissue-level modulus or ultimate strength[4,17]. A loss of toughness and energy dissipation in cortical and cancellous tissue has been found with bisphosphonate treatment[4]. Fatigue properties are likely altered with bisphosphonate treatment; however, minimal data regarding these properties have been

published[4]. Increased microdamage in both cortical and cancellous tissue with bisphosphonate treatment may reflect an inability to repair damage within the tissue[14–17]. Alendronate reduced the fatigue life in beams created from rib bones from healthy canines; however, the dosing was supraphysiological and osteoporosis was not induced prior to treatment[19].

Long-term bisphosphonate use is associated with atypical femoral fractures (AFF)[20,21]. AFF incidence with bisphosphonate use is relatively low, but is associated with considerable morbidity[22]. The mechanics of these fractures indicate critical differences from typical osteoporotic fractures[23,24]. Association with low loads indicates AFFs result from repetitive (fatigue) loading rather than a single traumatic incident. The transverse nature of the fractures suggests altered material properties with tissue becoming more brittle.

Bisphosphonates are the most common therapy prescribed for osteoporosis treatment, but other treatments exist. Selective Estrogen Receptor Modulators (SERM) reduce osteoporotic vertebral fracture risk by 30–50%[25]. SERMs bind to the estrogen receptors with an affinity similar to estradiol[26]. Teriparatide (PTH) has been beneficial in patients who experience AFFs by inducing increased bone remodeling, removal of older more fully mineralized tissue and replacement with new less fully mineralized tissue[27]. Mechanical property data for SERM and PTH treatments of bone have focused on monotonic failure properties and have not included fatigue.

The purpose of this study was to examine the fatigue and fracture properties of bone tissue after different osteoporosis treatments using a sheep model of osteopenia to determine if a correlation exists between fatigue life and treatment type. Osteopenia was induced in sheep and followed by an osteoporosis treatment or vehicle. Beams of known geometry created from the femoral diaphysis of these sheep were loaded in four-point bending fatigue to failure. Given the inhibition of remodeling, and increased mineralization and collagen maturity reported with bisphosphonate treatment, we theorized that a shorter fatigue life will occur with bisphosphonate treatment.

Materials and Methods

Animal Model

Samples used in this study were from remaining femur tissue from previously published and in progress studies[28]. For all studies we fed a metabolic acidosis (MA) diet to skeletally mature sheep to induce osteopenia[29]. In the first study, sheep fed a normal diet served as healthy controls for the experiment (C, n=6). In the second study, sheep were fed the MA diet for 12 months and given Alendronate (ALN; n=2), Raloxifene (RAL; n=2) or a vehicle (MA1; n=3) treatment during months 7–12. The low sample sizes were not planned and reflect factors beyond our control in the experiment. To further examine bisphosphonate treatment, a third experiment was performed with sheep fed a MA diet for 8 months followed by 6 months of treatment with Zoledronate (Reclast, ZOL; n=6) or vehicle (MA2; n=6) while continuing the MA diet. The longer initial MA term in experiment three was due a delay in procuring the zoledronate. In the second study, alendronate (0.15 mg/kg) and raloxifene (0.8 mg/kg) were administered daily via a cannula placed into the duodenum,

whereas zoledronate (5 mg/sheep) was administered as a single intravenous injection. This schedule replicates the clinical dosing in which alendronate is taken orally daily or weekly and zoledronate is administered intravenously once a year[30,31]. All animal procedures were reviewed and approved by the Colorado State University IACUC and the Hospital for Special Surgery IACUC.

A fourth set of skeletally mature sheep were fed an MA diet for one year after ovariectomy, which has been shown to induce osteopenia[32]. The sheep were then maintained on the MA diet and were administered teriparatide (PTH, n=6) or vehicle (MA3+OVX, n=6) for one year. Treatment was administered daily via subcutaneous injection (5 mcg/kg). All animal procedures were reviewed and approved by the University of Minnesota IACUC and the Hospital for Special Surgery IACUC.

Sample Preparation

Sheep were euthanized at the end of the specified treatment period. Femurs were removed and stored at −20°C in saline-soaked gauze until the time of sample preparation. Beams were cut out of the medial diaphysis of the femurs using a low speed diamond saw (Buehler Isomet; Lake Bluff, IL, USA). Beams were then polished using 15, 5 and 1 micron lapping films with ethylene glycol used as a lubricant to prevent mineral leaching[33,34]. Samples were polished to a final size of 2 × 2 × 25 mm. After polishing, samples were stored at −20°C in hydroxyapatite-buffered saline-soaked gauze until testing.

Fatigue Testing

Beams were tested in four-point bending fatigue[35,36] (Bose Electroforce LM-1, Eden Prairie, Minnesota, USA). The bottom supports were placed 20 mm apart, and the top loading points placed 5 mm apart (Figure 2). These positions created a constant bending moment between the loading points and limited the effects of crushing at the load points[37]. Preconditioning was completed by 20 cycles of loading from 2 to 20 N. These values were chosen through preliminary testing that demonstrated that these loads induced normal surface strains below the 2500 με necessary to create microdamage and alter fatigue life[10]. The initial flexural modulus was measured from the 10^{th} cycle and calculated using the assumptions of linear elastic beam theory. Initial modulus values were used to calculate the force necessary to achieve desired values of strain of 400 to 4000 με on the tensile and compressive surfaces. Samples were loaded in force control from 400 to 4000 με (R=0.1) to failure with peak-to-peak force and displacement measured at each cycle. Cycles–to-failure, N_f, was defined as the number of cycles experienced before the sample broke. Modulus loss at failure was defined as the percent change in modulus from the 10^{th} cycle to N_f, and was calculated with linear elastic beam theory[38]. All testing was completed at physiologic temperature (37°C) in hydroxyapatite-buffered PBS (1g HA added per 1L PBS and allowed to sit overnight until solution was supersaturated) with temperature monitored continuously.

Microcomputed Tomography (microCT)

Tissue mineral density (TMD) was measured with microCT at a 50 micron voxel size (eXplore CT 120, GE Healthcare, Waukesha, WI, USA). A mineral phantom was used for

calibration with analysis completed in Microview (version ABA 2.2, GE Healthcare, Waukesha, WI, USA).

Statistical Analysis

The purpose of the experiment was to determine differences in N_f among the different treatment groups and correlate the differences to TMD data. A standard least squares analysis was used to compare each group to the grand mean of the data. The four separate experiments limited the ability to compare data across experiments. A log transform was performed on the cycles-to-failure data to meet the assumption of equal variance between groups.

Different treatments and durations of MA controls can influence the results. Low sample sizes also limited comparisons among groups. For comparison of the MA1, raloxifene and alendronate groups, a Students t-test was used to compare each group with a Bonferroni post hoc correction applied. A Students t-test was completed also for the MA2 and zoledronate data, MA3+OVX data and PTH. The Bonferroni correction and t-test comparisons were necessary due to low sample sizes and not meeting the assumptions for an ANOVA.

Results

An increase in the initial flexural modulus was seen in the MA3+OVX and PTH groups as compared to the grand mean of all groups (Figure 3). Samples treated with alendronate had a significantly lower N_f compared to the grand mean of all groups ($p<0.01$), while PTH samples had significantly greater N_f compared to the grand mean ($p<0.01$; Figure 4). A loss of fatigue life occurred between alendronate (ALN) and its metabolic acidosis control (MA1; $p<0.01$). Modulus loss at failure was significantly lower in the alendronate-treated groups compared to the grand mean ($p<0.05$; Figure 5).

Mineralization measures (TMD) did not account for the differences in fatigue behavior. Control samples had a lower TMD compared to the grand mean, while raloxifene raised the TMD above the grand mean ($p<0.05$; Figure 6).

Discussion and Conclusions

Fatigue properties were examined in cortical bone tissue from sheep treated by anti-resorptive drugs after induction of osteoporosis. Four-point bending fatigue testing to failure was completed at physiologic temperature on bone beams created from the femoral diaphysis. Osteoporosis treatments had differing effects on the fatigue life of cortical bone tissue. Alendronate treatment caused a significant loss in fatigue life as compared to the grand mean and its MA control; however, zoledronate-treated specimens did not experience any change in fatigue life from the grand mean or MA control. Greater changes might be expected with zoledronate than alendronate given zoledronate's greater binding affinity and potency[39]. Alendronate has a relative potency of $1–2\times10^3$, whereas zoledronate has a relative potency of 10^4 compared to etidronate[40]. Raloxifene did not change the fatigue life of the tissue while PTH increased fatigue life over the grand mean of the data. Differences in

the fatigue life indicate material property changes caused by binding affinity, dosing, chemical structure or collagen changes.

Differences in the administration of the bisphosphonates may contribute to the altered fatigue properties. Alendronate was administered daily via cannula while zoledronate was given once over the course of the experiment via intravenous injection following clinical dosing regimens[30,31]. With daily dosing of alendronate the bisphosphonate is present in the serum continuously affecting biomarkers of bone turnover, whereas a single dose of zoledronate may allow the serum biomarker levels to return to pre-treatment homeostasis. Serum CTX is known to be reduced with bisphosphonate dosing and increase with time since last administration[41,42]. Increased collagen maturity occurs with bisphosphonate treatment, and suppression of serum biomarkers such as CTX may indicate differences between the two bisphosphonate types.

Bisphosphonate molecular structure and distribution throughout the tissue are also theorized to have an effect on the tissue properties. Regions of higher mineralization were surface-based on trabeculae with zoledronate treatment[28], which supports the idea that zoledronate has a more surface-based effect. Distribution of bisphosphonates throughout cortical bone tissue has only been reported with the use of ibandronate and differences in distribution between proximal and distal cortices were noted[43]. Higher-affinity bisphosphonates have less diffusion into the bone, which could cause differences between alendronate and zoledronate[44].

Alendronate-treated samples had lower modulus loss at failure indicating a more brittle material. Microdamage quantities in these samples were not analyzed, so differences in this parameter among groups are unknown. Microdamage is associated with fatigue loading, and increased microdamage is correlated to loss of modulus in trabecular bone[45]. Greater microdamage created by activities of daily living occurs in both cortical and cancellous tissues with alendronate treatment compared to untreated control tissues[14,16].

In this study, applied loads created maximum normal strains from 400 to 4000 με. In laboratory fatigue conditions in bending, damage creation starts at 2500 με in regions under tension; however, greater than 4000 με is necessary in the regions under compression[10]. The 4000 με applied in our study would, therefore, create damage in the tensile region with the compressive region receiving little damage. Greater damage in the tensile region is similar to AFF progression, in which the stress fracture develops from the lateral cortex that is under tension during normal weight-bearing activities.

Results of this study are limited by several factors including the underpowered sample sizes for both alendronate and raloxifene treatments. As previously stated, small sample sizes were unplanned and due to factors beyond our control in the experiment; however, recent studies in a different animal model have also demonstrated a reduction in fatigue life with alendronate treatment[19]. The lack of fatigue life change with raloxifene treatment may indicate fatigue life preservation; however, this result may be due to lack of power from a small sample size. SERM therapies also have the side effect of increased risk of thromboembolic problems[46] indicating that an increased fatigue life alone may not make

this therapy more appropriate. Four-point bending fatigue is not a typical method for fatigue measurements. Tensile and compressive fatigue are more commonly used for material characterization[10,11]; however, limited tissue from repurposed samples prevented the possibility of analyzing tissues by this method. Finally, although having samples from four separate studies enhanced our ability to make comparisons, this situation was less than ideal as sheep are known to experience seasonal differences in BMD[47]. Nevertheless, we did include untreated control samples to compare with treatment group samples.

In this study fatigue life differences with osteoporosis treatments depended on both the class of treatment, type of drug, and mode of delivery. Alendronate caused a reduction in bone tissue fatigue life while PTH caused an increase in fatigue life. Raloxifene and zoledronate did not change fatigue life. Material property alterations may be due to differences in chemical structure, mechanisms of actions of these drugs, or dosing regimens by which the drugs are administered. Under the confines of this study, drug uptake or the effect of dosing regimen were not possible to examine; however, these variables are avenues for future research that may help explain the occurrence of AFF.

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Highlights

- Cortical bone beams from sheep with osteoporosis treatments were tested in fatigue
- Alendronate lowered fatigue life while zoledronate did not alter fatigue life
- PTH treatment increased fatigue life of cortical bone tissue
- Effect on fatigue life differs by type of osteoporosis treatment in cortical bone

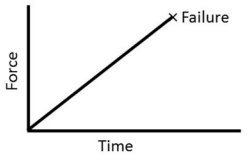
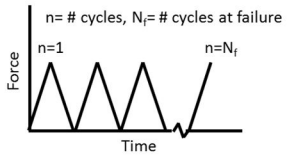
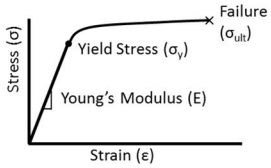
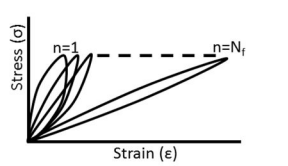
	Monotonic Loading	Fatigue Loading
Clinical Occurrence	Falls, blunt force trauma	Activities of daily living, including running
Load Levels	Single load applied to failure	Repetitive sub-failure loads applied
Force Applied over Time		
Stress - Strain		
Failure Mode	Failure when material is loaded to ultimate stress	Cracks (microdamage) formed during each cycle until a critical level is reached inducing failure

Figure 1.

Comparison of monotonic and fatigue loading. In monotonic loaded samples, force is increased until the sample fails. In fatigue, a repetitive sub-failure load is applied creating damage that eventually coalesces to cause failure.

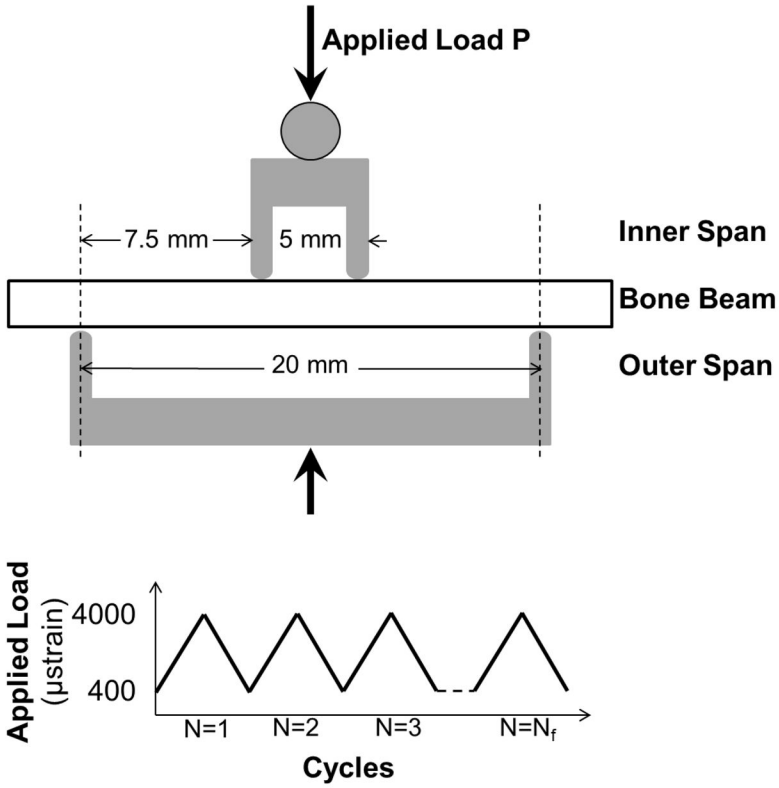


Figure 2.

Set up for four point bending fatigue loading. P = applied load. The span between the inner supports was 5 mm, and for the outer supports 20 mm. A cyclic load was applied to failure with strain levels between 400 and 4000 μstrain.

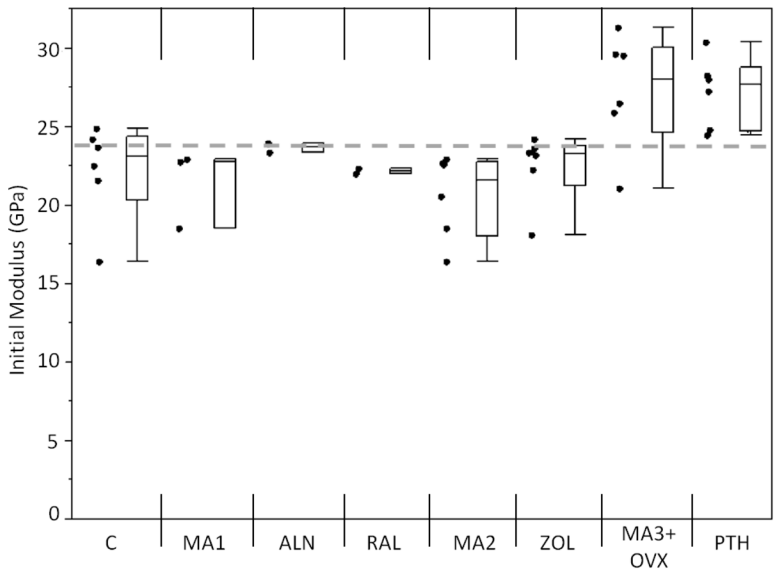


Figure 3.

Initial modulus values for each group. MA3+OVX and PTH had higher initial moduli than the other groups. Markers to the left represent individual sample data points. Box and whisker plots on right show the minimum, maximum, mean, and 25th and 75th quartiles for each group. Dashed line is the grand mean.

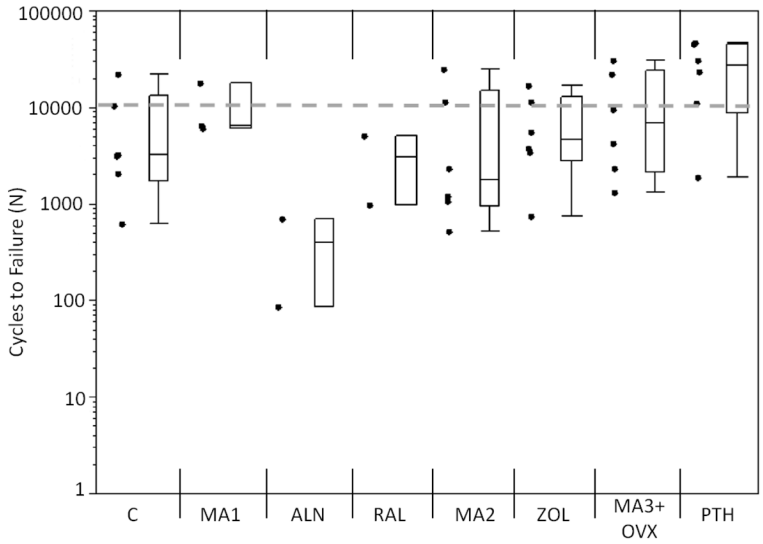


Figure 4.

Cycles to failure for each group. Alendronate (ALN) had fewer cycles to failure compared to the grand mean ($p<0.01$). Teriparatide (PTH) had more cycles to failure compared to the grand mean ($p<0.01$). Markers to the left represent individual sample data points. Box and whisker plots on the right show the minimum, maximum, mean, and 25^{th} and 75^{th} quartiles. Dashed line represents the grand mean.

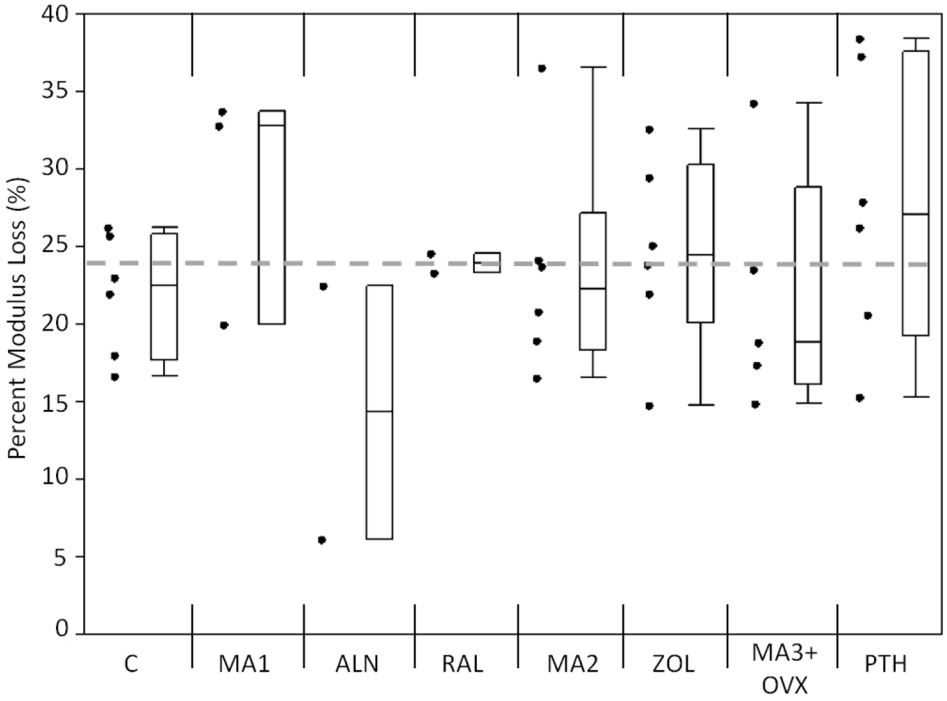


Figure 5.

Modulus loss at failure for each group. Alendronate had a lower modulus loss at failure compared to the grand mean ($p<0.05$). Markers to the left represent individual sample data points. Box and whisker plots on the right show the minimum, maximum, mean, and 25th and 75th quartiles. Dashed line represents the grand mean.

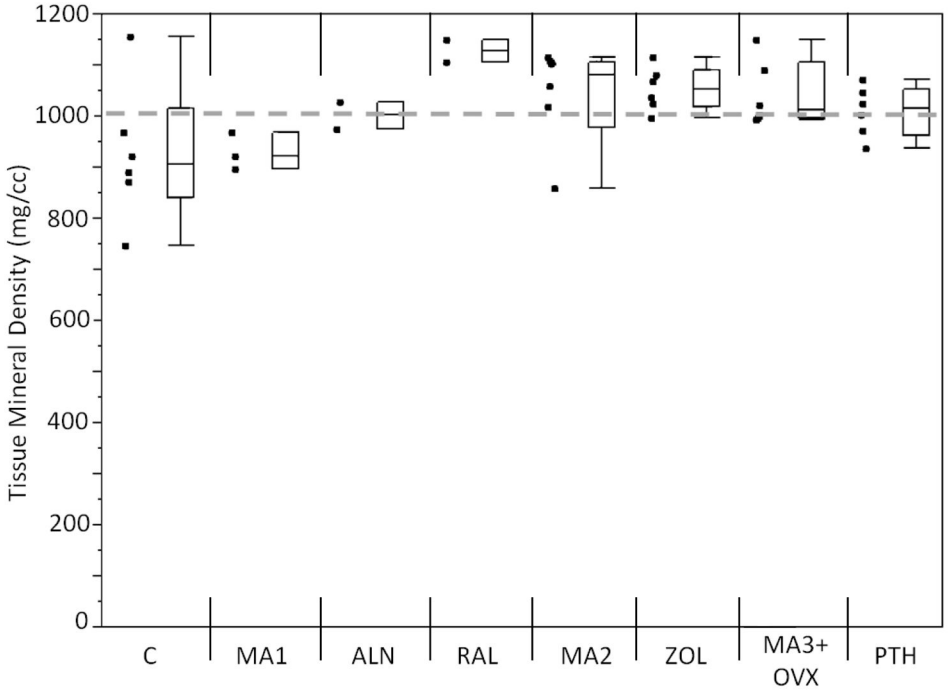


Figure 6.

Mineralization measure for each group. Control samples had a lower TMD as compared to the grand mean while raloxifene samples had increased TMD as compared to the grand mean ($p<0.05$). Markers to the left represent individual sample data points. Box and whisker plots on the right show the minimum, maximum, mean, and 25^{th} and 75^{th} quartiles. Dashed line represents the grand mean.

Table 1

Samples used were four different studies: [1] Age-matched control sheep fed a normal diet (Control); [2] Sheep were fed a metabolic acidosis (MA) diet for six months followed by the MA diet and twelve months of MA diet and treated by vehicle (MA1), raloxifene (RAL) or alendronate (ALN); [3] Sheep were fed an MA diet for eight months followed by six months of the MA diet combined with vehicle (MA2) or zoledronate (ZOL); [4] Sheep had an ovariectomy and were fed an MA diet for a year, followed by a year of the MA diet and vehicle (MA3+OVX) or parathyroid hormone (PTH). Control, MA1, RAL and ALN were euthanized after 12 months, MA2 and ZOL at 14 months and MA3+OVX and PTH at 24 months.

		Month																					
	n	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Control	6	Normal Diet																							
MA1	3	MA Diet							MA + Vehicle																
RAL	2	MA Diet							MA + Raloxifene																
ALN	2	MA Diet							MA + Alendronate																
MA2	6	MA Diet									MA + Vehicle														
ZOL	6	MA Diet									MA + Zoledronate														
MA3+OVX	6	MA Diet + OVX												MA + OVX + Vehicle											
PTH	6	MA Diet + OVX												MA + OVX + PTH											