INTRODUCTION:
Bisphosphonates (BPs) are used as anti-resorptive drugs to treat osteoporosis and other bone diseases. BPs increase bone mineral density in part by decreasing bone resorption. In a previous experiment, beagles were given BPs using doses at and above those used for treatment of osteoporosis. Microdamage accumulation and an increase in mineralization were observed. In the current report, we show that the microcrack density is negatively correlated to nanoindentation hardness of the vertebral tissue.

METHODS:
We used thirty L2 vertebral body specimens of dogs collected in a previous BP treatment study [1]. The dogs were treated daily for 1 year with Risedronate sodium (RIS, 0.10 mg/kg/day, n=10), Alendronate sodium (ALN, 0.20 mg/kg/day, n=12) or saline vehicle (VEH, n=8). The vertebrae were embedded in polymethylmethacrylate and sectioned along the midline. The cut surface of each specimen block was polished with successively finer grades of carborundum paper and polishing powders before nanoindentation.

We used a Nano Indenter XP system (MTS Systems, TN) to measure the Young’s modulus and the hardness (H<sub>0-P</sub>) of the vertebral tissue [2]. Two sites from each specimen were selected and one hundred tests were performed at each site using a 10x10 array pattern, with spacing of 15 μm in both horizontal and vertical directions. The 100 tests were averaged for each specimen. The indentation was under displacement control to a maximum depth of 500 nm at a speed of 10 nm/s. Modulus and hardness were calculated from the unloading portion of the nanoindentation data (Fig. 1).

RESULTS:
The hardness (H<sub>0-P</sub>) and crack density (Cr.Dn) were different between groups as was previously reported [1,2]. A multiple linear regression predicting Cr.Dn:

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\text{Cr.Dn} = 2.09 - 2.53H_{0-P} + 0.44RIS \times H_{0-P} + 0.51\text{ALN} \times H_{0-P}
\]

was significant at p<0.02 for all parameters with adjusted r<sup>2</sup>=0.33. RIS was 1 when the data was from the risedronate group and 0 otherwise. The quantity ALN similarly separated the alendronate group. Linear regression within each group was not significant.

DISCUSSION:
Treatment with risedronate or alendronate for one year at doses consistent with those used for treatment of osteoporosis, increased microcrack density (Cr.Dn) and mineralization [1] and also increased bone hardness, modulus and shear strength [2]. The current analysis adds the interesting observation that tissue hardness may be a negative predictor of in vivo microcrack density.

The possible significance of this observation is that increased hardness was associated with increased tissue cohesive shear strength in a previous report [2]. The simple prediction, therefore, is that microdamage should be negatively correlated to hardness (and cohesive shear strength) if the microdamage is caused by mechanical loading. Our conclusion, therefore, is that the crack density in canine vertebrae is at least in part a result of mechanical loading and that the rate of microcracking is in part governed by hard tissue mechanical properties.

The requirement to consider group as a variable in the regression was interesting because it suggests that the drugs affect the overall relationship of the variables. However, a negative relationship of Cr.Dn with hardness was maintained for the entire data set. It is reasonable that the slowed remodeling due to the bisphosphonate treatment shifts the relationship upward and to the right over time as the microdamage burden and mineralization increase (Fig. 2).

In previous research on the effects of bisphosphonates, it was noted that Cr.Dn and mineralization of the vertebrae were increased [1]. The resulting hypothesis was that the increase in mineralization might cause the increase in the bone microdamage burden. The current analysis does not eliminate that interpretation for the between groups effect but does change interpretation of the within group relationship for microdamage and tissue hardness. Within each group the animals with harder bone tissue had a lower microdamage burden.

In the context of fracture toughness, the current observation has two implications. The first is that we know that the ability to microcrack is associated with high bone toughness. Therefore, animals with harder tissue and a lower propensity to microcrack are predicted to have lower tissue toughness. The second observation is that existing microdamage is known to decrease the initiation and propagation toughness of bone. This is a complex but well known phenomenon. The resulting overall hypothesis, therefore, is that higher hardness (decreased microcracking) and increased microdamage burden (low remodeling) both decrease bone toughness.

REFERENCES:

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