

## Does Trabecular Bone Have the Capacity to Adapt at the Material/Compositional Level?

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## INTRODUCTION

Important biomechanical relationships between trabecular micro-architecture and tissue mineralization might reveal the potential for adaptive plasticity and interactions of mini-modeling and hemi-osteonal activities that have not been recognized. Studies of the simply-loaded deer calcaneus model should help to advance our understanding of the biomechanical functions that regional variations in specific trabecular architectural and material characteristics have in a habitual bending environment during growth and in the adult, and also the interplay that these characteristics have with regional loading modes. It has been shown that energy absorption to yield, and ultimate and yield strengths are nearly 30% lower for tension *vs.* compression loading in trabecular bone [1]. Differences in tissue mineralization and trabecular architecture might occur between the dorsal "compression" and plantar "tension" trabecular tracts in deer calcanei that accommodate this disparity. It has been suggested in both cortical and trabecular bone that some degree of mineralization heterogeneity can be beneficial because it creates interfaces and microscopic modulus mismatches that optimize toughness and strength by accommodating microcrack formation and restricting microcrack propagation. Experimental data in bovine trabecular bone demonstrating that microdamage accumulation is related to both trabecular architecture and loading history [2] also support the possibility that natural selection mechanisms would favor adapting individual trabeculae differently in habitual tension *vs.* compression environments. The main purpose of this study is to quantify the microscopic mineralization and architectural characteristics of the "compression" and "tension" trabecular regions of skeletally immature and mature deer calcanei. We hypothesize that: (1) the compression tract will have greater mineralization patterns and/or heterogeneity than the tension tract, and (2) these mineralization differences will be correlated with differences in trabecular architectural characteristics.

## METHODS

With IACUC approval, calcanei were obtained from two groups of Rocky Mountain mule deer: skeletally immature (1-2 years old) and skeletally mature (3-4 years old). A transversely cut segment was obtained from the midshaft of each of seven bones from each age group (n=7 immature bones; n=7 mature bones). These 14 segments were embedded in polymethyl methacrylate (PMMA) and examined using backscattered electron (BSE) images at 100X. Three BSE images were obtained in each trabecular tract in each bone, resulting in a total of 42 BSE images for each age group (21/dorsal tract, 21/plantar tract). Imaging sessions were calibrated using magnesium oxide (MgO) and aluminum oxide (Al<sub>2</sub>O<sub>3</sub>) standards [3]. Regional differences in mineralization were inferred from corresponding differences in the intensity of the image gray levels, where darker gray levels (lower numerical values) represent relatively lower mineralization [4]. Mean mineralization of the bone in each image was quantified as a weighted mean gray level (WMGL) [4]. Mineralization heterogeneity was measured as the full width at one-half of the maximum (FWHM) [5] of the bone mineral density distribution (BMDD) [6, 7]. Trabecular bone packet prevalence (TBPP) was determined from BSE images and is expressed as the number of packets divided by the area of the trabecular bone in each image. 'Packets' refer to the basic structural units (typically hemi-osteonal bone) within trabecular bone. Packets can be distinguished by being separated by cement lines and/or differences in the degree of lamellations (banding patterns) or mineralization [8]. Seven additional calcanei (unembedded) from each age group were analyzed using micro-computed tomography (micro-CT; 46.4  $\mu$ m resolution) in order to identify potential differences in the adaptation of specific architectural characteristics between the trabecular tracts. Regions of interest (ROI) (5 mm diameter cylinders) were selected in the dorsal and plantar aspects of the midshaft. Architectural characteristics analyzed include: degree of anisotropy (DA), bone volume fraction (BV), bone surface density (Bs/Bv), trabecular thickness (Tb.Th), trabecular spacing (Tb.Sp), trabecular number (Tb.N), connectivity density (Conn.D), and structural model index (SMI). Paired t-tests were used to evaluate paired comparisons within each group for statistical significance. Correlations were not conducted between Micro-CT and mineralization data because they were not obtained from the same bones.

## RESULTS

As shown in the table below, the mature bones show significant material/compositional differences that cannot be attributed to growth ((TBPP) and mineralization (WMGL)). By contrast, only 1 of 9 trabecular architectural characteristics differed between the dorsal "compression" and plantar "tension" tracts.

	Immature Bones			Mature Bones		
	Dorsal	Plantar	p value	Dorsal	Plantar	p value
<b>BSE Mineralization Data</b>						
WMGL	135.5 (3.2)	127.1 (3.5)	0.002	137.8 (3.7)	128.9 (3.3)	<0.001
BMDD	4.8 (0.1)	5.1 (0.2)	0.01	4.7 (0.1)	4.8 (0.1)	ns
Skewness	-1.8 (0.0)	-1.7 (0.0)	0.01	-1.8 (0.0)	-1.8 (0.0)	ns
Kurtosis	1.2 (0.1)	0.9 (0.1)	0.01	1.3 (0.1)	1.3 (0.1)	ns
TBPP	46.2 (3.6)*	44.9 (2.8)	ns	34.6 (2.4)*	48.3 (3.0)	0.006
<b>Micro-CT Architectural Characteristics</b>						
DA	7.38 (0.70)	4.05 (0.35)	0.01	6.75 (0.83)	3.88 (0.35)	0.02
BV	0.43 (0.03)	0.58 (0.05)	0.03	0.52 (0.06)	0.56 (0.04)	ns
Bs/Bv	11.20 (1.25)*	8.61 (0.85)*	0.07	6.00 (1.23)*	5.62 (0.59)*	ns
Tb.Th	0.20 (0.04)*	0.22 (0.03)*	ns	0.47 (0.06)*	0.45 (0.06)*	ns
Tb.Sp	0.33 (0.09)	0.23 (0.04)*	ns	0.84 (0.26)	0.54 (0.05)*	ns
Tb.N	2.31 (0.19)*	2.40 (0.14)*	ns	1.30 (0.09)*	1.50 (0.07)*	0.09
Conn.D	8.84 (1.63)*	7.77 (1.06)*	ns	1.83 (0.31)*	2.29 (0.15)*	ns
SMI	-0.40 (0.35)	-3.60 (1.01)	0.02	-2.78 (0.93)	-3.35 (0.68)	ns

\* significantly different between dorsal "compression" and plantar "tension" trabecular tracts.

\* significantly different from same characteristic and trabecular location in the other age group.

ns = not significant (p&gt;0.1)

## DISCUSSION

Differences in tissue mineralization and trabecular architecture between the dorsal "compression" and plantar "tension" trabecular tracts in deer calcanei may accommodate the disparity in mechanical properties in these loading modes: namely, 30% weaker in tension *vs.* compression loading. The significant differences shown between the dorsal "compression" and plantar "tension" trabecular tracts in microscopic mineralization (immature and mature bones) and mineralization heterogeneity (BMDD; immature bones) may be adaptations that accommodate microdamage formation/propagation in the differing mechanical environments between these regions. Additionally, in the mature bones, TBPP was significantly greater in the plantar tract. Bone packets are considered an important toughening mechanism in trabecular bone because they create interfaces (i.e., they are separated by cement lines) and potentially increase mineral heterogeneity as a consequence of differences in their mean tissue age [9]. The increased packet prevalence in the plantar tract could also possibly affect stiffness of the trabecular bone (tissue modulus) in addition to accommodating/resisting microdamage. While previous studies have shown that trabecular bone can readily accommodate regional differences in mechanical requirements through adjustments in various architectural characteristics via regional differences in the mini-modeling process, results of this study also clearly show that trabecular bone has the capacity to adapt at the material/compositional level. This explanation is also supported by our data showing little, if any, adaptation in the trabecular architecture of this bone.

## SIGNIFICANCE

A few studies have suggested that trabecular bone has the capacity to adapt at the material/compositional level by adjusting the organization and prevalence of 'packets'. Degradations in this capacity could occur with age and osteoporosis. The present study more definitively establishes this trabecular bone potential in loading environments that are clinically relevant (e.g., such as may occur in the human femoral neck and some vertebral bodies). Understanding how trabecular bone of the simply loaded deer calcaneus adapts to accommodate differences in mechanical requirements helps to understand the more complex loading environment of bones that are not amenable to *in vivo* strain analysis and less accessible to experimentation.

## REFERENCES

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