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Fast Fourier Transform (FFT) Analysis of Lamellations within Trabecular Bone Packets: Relationships with Animal Maturity, Strain Mode, and Mean Tissue Age

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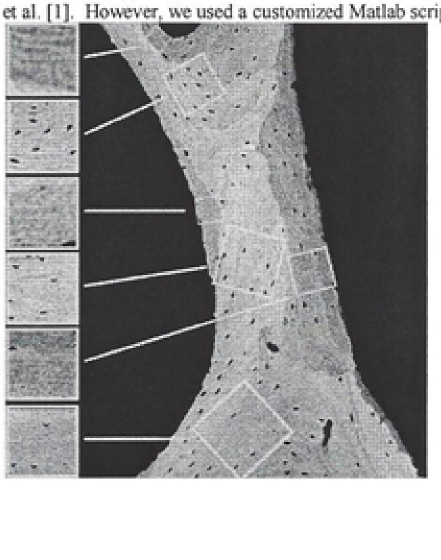
INTRODUCTION

'Packets' refer to the basic structural units (typically hemi-osteons) within trabecular bone [1]. Packets can be distinguished by being separated by cement lines and/or differences in the degree of lamellations (banding patterns) or mineralization; the latter is usually a function of mean tissue age (e.g., more highly mineralized bone correlates with age and/or reduced remodeling rate) [2]. Lamellations appear within trabecular packets and typically run parallel to a surface of the bone. Lamellations can slow microcrack propagation by dissipating energy and increasing bone toughness. In this study we investigated the degree of lamellations (i.e., how distinct/obvious they are) in the deer calcaneus model, which has trabecular regions that are thought to be habitually loaded in compression (dorsal tract) or tension (plantar tract). We hypothesize that: (1) mature bones will have more well developed lamellations that correlate with increased age, (2) the plantar "tension" tract of trabecular bone will have more packets and more well developed lamellations than the dorsal "compression" tract; likely because the mechanical properties of trabecular bone are relatively deficient in tension, and (3) there will be significant differences between superficial and deep packet lamellations as a function of mean tissue age (the older deeper packets will have greater lamellations).

METHODS

Specimens were obtained from immature (n=7) and mature (n=7) mule deer calcanei with IACUC approval. Preparation of the deer calcanei have been described previously [3]. Backscattered electron (BSE) images were obtained at 100X and 20 kV. The pixel resolution of the digital image was 1.2 square microns per pixel at 100X, with a resolution of 256 gray levels. 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_2O_3) standards [4]. Regional differences in mineralization were inferred from corresponding differences in image gray levels, where darker gray levels (lower numerical values) represent relatively lower mineralization [5]. Mean mineralization of the bone in each image was quantified as a weighted mean gray level (WMGL) [5]. Mineralization heterogeneity was measured as the full width at one-half of the maximum (FWHM) [6] of the bone mineral density distribution (BMDD = GL profile in each image) [7] Skewness and kurtosis of the BMDD were also measured. Trabecular bone packet prevalence (TBPP) was determined from BSE images and is expressed as the number of packets divided by the absolute area of the trabecular bone in each image. A region of each packet was cropped using a fixed pixel ratio of 1:1 and we ensured that lamellations/striations ran horizontally through the cropped ROI. In each cropped image, the degree of lamellations was determined using Fast Fourier transform (FFT) analysis in accordance with general methods of Ciarelli et al. [1]. However, we used a customized Matlab script available via email request) due to insufficient description in [1]. The strength/distinctness of lamellations was expressed as a FFT ratio where 1 = strongest lamellations, and < 1 = weaker lamellations. The FFT ratio is calculated as 1-(mean power of FFT/max power of FFT). Due to constraints of image pixel analysis the range in FFT ratio data is typically 1 to 0.3 (not 1 to 0 as reported by [1]). Although not shown in the Fig., osteocyte lacunae were not included in the cropped images that were analyzed in FFT. Paired t-tests were used for statistical analysis.



RESULTS

Significant differences were found between the mature and immature deer calcanei only in terms of WMGL (mineralization). In the immature bones, WMGL, BMDD, skewness, and kurtosis significantly differed between the dorsal and plantar tracts. WMGL, BMDD, skewness, and kurtosis were also significantly different in the mature bones. However, only in the mature bones was the plantar "tension" tract found to have significantly more trabecular bone packets and an FFT ratio that differed significantly (i.e., less distinct lamellations in the plantar tract). In the dorsal immature bones, there were also significant differences between superficial/deep packets, but only in terms of WMGL. By contrast, the mature bones showed differences between superficial/deep packets in FFT ratio, and all other measures. See table below for most results.

Lamellation and Mineralization Data [means (standard deviations)]

		FFT Ratio	WMGL	BMDD	Skewness	Kurtosis
Immature Deer Calcanei	Dorsal	0.76 (0.11)	132.7 (23.4)	42.7 (9.7)	1.83 (0.28)	5.49 (1.51)
	Superficial	0.76 (0.11)	**131.5 (22.8)**	43.1 (10.5)	1.82 (0.28)	5.45 (1.50)
	Deep	0.79 (0.08)	**140.6 (21.8)**	41.7 (7.2)	1.82 (0.25)	5.35 (1.23)
	Plantar	0.76 (0.11)	126.3 (24.7)	40.5 (8.3)	1.90 (0.29)	5.82 (1.60)
	Superficial	0.75 (0.12)	125.1 (26.1)	40.8 (8.3)	1.90 (0.29)	5.85 (1.62)
	Deep	0.77 (0.09)	130.4 (19.6)	40.1 (8.1)	1.88 (0.28)	5.76 (1.63)
Mature Deer Calcanei	Dorsal	0.77 (0.08)	129.4 (20.2)	42.6 (8.0)	1.78 (0.27)	5.28 (1.42)
	Superficial	**0.78 (0.08)**	**122.5 (20.8)**	**43.3 (7.7)**	**1.72 (0.24)**	**5.01 (1.19)**
	Deep	**0.75 (0.08)**	**142.9 (11.1)**	**40.9 (8.1)**	**1.91 (0.29)**	**5.87 (1.68)**
	Plantar	0.74 (0.10)	120.5 (21.2)	40.9 (9.7)	1.93 (0.33)	6.02 (1.87)
	Superficial	**0.75 (0.10)**	**112.2 (21.9)**	**42.4 (10.3)**	**1.85 (0.32)**	**5.65 (1.78)**
	Deep	**0.71 (0.10)**	**133.4 (11.6)**	**38.5 (8.3)**	**2.06 (0.30)**	**6.60 (1.87)**

[shaded] = significantly different ($p \leq 0.05$) from opposite trabecular tract (i.e., dorsal vs. plantar).
Bold Text = significantly different ($p \leq 0.05$) between superficial and deep packets.

DISCUSSION

Ciarelli et al. [1] reported that variations in the degree of lamellations may affect regional strength and stiffness of trabecular bone. In the perspective of their findings, we sought to determine if TBPP, the degree of lamellations (FFT ratio), and tissue mineralization differ between trabecular regions in a bone that: (1) is harshly loaded in bending, (2) has "tension" and "compression" trabecular tracts that resemble those in the human femoral neck, (3) will not show the effects of advanced aging, and (4) comes from a wild animal. For these reasons, the deer calcaneus is used as a "control bone" for adaptations for habitual bending in natural conditions [8]. The most provocative results in our study are found in the mature group. TBPP is relatively increased in the plantar "tension" tract while, contrary to our hypothesis, the FFT ratio is reduced in this tract. These differences may reflect plasticity in strain-mode-specific adaptations. Further studies are needed to understand the role of lamellations in toughening at the trabecular level and how they might be related to trabecular architectural changes, especially in bones where habitual bending is a common load history and fragility fractures become prevalent (e.g., human femoral neck and some vertebral bodies).

SIGNIFICANCE

The intensity and patterns of lamellations within trabecular bone packets are associated with increased fracture risk with aging and osteoporosis. Mineralization heterogeneity produced by lamellations, as well as cement lines, may be adaptations that help restrict microdamage propagation, and could be important in better understanding the degradation in bone "quality" with age and disease.

REFERENCES

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