Evidence of Strain-Mode-Related Ultrastructural Adaptation in Cortical Bone - Detected Using Circularly Polarized Light in Thin Sections

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INTRODUCTION Although investigated since the time of Wolff, the ultimate biomechanical objective(s) of the normal modeling and remodeling processes that mediate the functional adaptation of bone continue to evade orthopaedic researchers. Using the horse radius model, Riggs, Lanyon and co-workers (7) showed marked regional differences in collagen fiber orientation between the cranial "tension" cortex and caudal "compression" cortex at midshaft. They suggest that this regional difference in ultrastructural organization is ultimately aimed at achieving uniform safety factors to failure between cortical regions. Safety factors (Sfs) provide structures (e.g., bones, steel beams, etc.) with a margin of safety from potential failure (SF = ratio of yield (failure) stress to maximum in vivo physiologic stress). They based this conclusion on mechanical tests which showed that these ultrastructural differences significantly altered how the bone in the opposing cortices strains during physiologic loading. If their interpretation is correct and collagen fiber anisotropies are aimed at keeping peak strains at the same proportion of the yield point between these opposing cortices, then this has pervasive implications for bone remodeling theory since it reflects the apparent capacity of bone to organize its ultrastructure in accordance with local differences in yield behaviors of bone in habitual strain-related loading regimes while optimizing the structure as a whole.

However, these investigators did not examine control regions, where differences in collagen would not be expected. The present study further examines this idea by quantifying collagen fiber anisotropies in horse radii at multiple locations and in control regions: along the theoretical neutral axis of bending (medial vs. lateral cortices).

METHODS In vivo strain recordings at the midshaft of the horse radius show that the cortex located cranial to the medullary canal exhibits predominantly tension strains and the cortex located caudal to the canal exhibits predominantly compression strains (8). One radius was obtained from each of 11 skeletally mature standard breed horses. Each bone was cut into 5cm sections at 3 locations defined by percentages of overall length (proximal 65%, middle 50%, and distal 35%). After sections were embedded in polymethyl methacrylate, a one-millimeter-thick slice was obtained and milled to a high luster finish (Reichert/Jung Ultramiller) to a achieve a uniform overall thickness of 100 (+/- 5) microns. Milled sections were analyzed for collagen orientation using circularly polarized light (CPL) (3). Differences in collagen fiber orientation (anisotropy) are expressed in terms of the corresponding regional differences in the intensity of transmitted light, where darker graylevels represent longitudinal collagen orientation and brighter graylevels represent oblique-to-transverse collagen orientation (3). Graylevel values were quantified in one 50X image (approx. 2mm²) taken in each pericortical (P), mid-cortical (M), and endocortical (E) subregion in each of the cranial, caudal, medial, and lateral cortical regions of the 50% and 65% sections. Because of thin cortices of the 35% sections, 2 images were analyzed in each region at this location. Using a commercially available image analysis system (Image 1, Universal Imaging Corporation, West Chester, PN) and a video camera and monitor, the images were digitized and stored into memory for analysis. Image graylevels were converted into integer values ranging from 0, 1, 2, ... 255. From the graylevel histogram of each image, a weighted mean graylevel (WMGL) was calculated according to described methods (2). At each section location statistical differences between regions were identified using Fisher's LSD one-way ANOVA test.

RESULTS As shown in the Table, the compression (caudal) cortex is consistently brighter (33%) than in the opposing tension (cranial) cortex at each section location (p < 0.0001). In contrast, differences between the opposing medial and lateral cortices (along the theoretical neutral axis) are invariably small (5.4%) and are not statistically significant at any location (p > 0.3).

DISCUSSION By showing that the collagen fiber organization in the caudal (compression) cortex markedly differs from all other cortical regions examined, and the absence of biomechanically significant differences between the medial and lateral cortices, lends credence to the hypothesis that these ultrastructural anisotropies might adjust regional elastic moduli to optimize the cortices for the cranial-caudal distribution of the predominant strain-mode-related loading regime. These data, however, neither support nor reject the hypothesis that uniform safety factors to failure are the ultimate objective of the osteon-mediated remodeling processes that produced the ultrastructural differences in the equine radius. In the horse third metacarpal, however, it has recently been shown that regional ultrastructural, microstructural (e.g., osteon population density), and mineral content differences do not account for the large non-uniformities in this bone's strain milieu and do not result in uniform safety factors between the "compression" cortical regions (4,6). Based on these data and the histological-mechanical data of Riggs et al. (7), we hypothesize that the regional differences in matrix organization in the horse radius, horse third metacarpal, and other limb bones loaded habitually in bending, may function synergistically with bone curvature, cortical thickness, and geometry to promote loading predictability (6). In turn, the spatial and temporal distribution of the "signal" that mediates bone adaptation and maintenance may be transduced to the cellular level through functional matrix-mediated strains that are linked to, and ensured by, loading predictability (6). If the tension and compression strains that are promoted by the regional differences in collagen organization in the equine radius and other limb bones prove to be important components of a strain history to which the cells are tuned, this would not be wholly unexpected since: 1) locomotion generates bone matrix strains, and greater than 70% of these longitudinal strains are secondary to bending deformations (1), and 2) tension and compression are associated with positive and negative bioelectric potentials, respectively, that are known to promote osteogenesis (5).

Section	Region	Graylevel	(Std)	GL %diff	p value
65%	Cranial	101.19	(25.49)		
	Caudal	153.30	(29.49)	34.0%	p <0.0001
	Medial	96.23	(17.48)		
	Lateral	101.67	(37.64)	5.4%	p > 0.4
50%	Cranial	87.25	(24.69)		
	Caudal	126.30	(34.71)	30.9%	p <0.0001
	Medial	80.10	(16.88)		
	Lateral	72.99	(13.21)	8.9%	p = 0.31
35%	Cranial	100.01	(15.77)		
	Caudal	151.80	(16.27)	34.1%	p <0.0001
	Medial	117.72	(23.33)		
	Lateral	115.57	(27.97)	1.8%	p > 0.4

References 1) Beaupre et al. 1990 J. Orthop. Res. 8:651-661; 2) Boyce et al. 1990 Scan. Microsc. 4:591-603; 3) Boyde and Riggs 1990 Bone 11:35-39; 4) Gross et al 1992 J. Biomech. 25:1081-1087; 5) Martin and Burr 1989 Structure, Function, and Adaptation in Compact Bone, Raven Press; 6) Nelson et al. 1995 Orthop. Res. Soc. Abstract; 7) Riggs et al. 1993 Anat. Embry. 187:231-248; 8) Schneider et al. 1982 Am. J. Vet Res. 43(9):1541-50

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