Abstract 110: Orthopaedic Research Society; 2014.

Trabecular Bone has the Capacity for Hemiosteonal Collagen/Lamellar "Morphotype" Adaptation: Implications for Advancing Understanding of the Emergence of Skeletal Fragility in the Elderly

John G. Skedros, MD[1], Chad S. Mears[2], Richard T. Epperson[2], Roy D. Bloebaum, PhD[2].
[1]University of Utah, Salt Lake City, UT, USA, [2]U. of Utah, Salt Lake City, UT, USA.

Disclosures:
J.G. Skedros: None. **C.S. Mears:** None. **R.T. Epperson:** None. **R.D. Bloebaum:** None.

Introduction: There is substantial interest in determining how trabecular (cancellous) bone adapts to mechanical loads. In orthopaedics this interest primarily reflects the clinical importance of determining why trabecular bone (as a bulk structure and also at the material level) becomes fragile with age and osteoporosis [1,2,3]. In anthropology there is also keen interest in determining how trabecular bone organization might reflect load history, species affiliations, and phylogenetic relationships [2,4-6]. Most studies have focused on the adaptability of trabecular bone in the context of *structural* (architectural) variations, which include: (1) bone volume fraction, (2) degree of anisotropy and trabecular alignment, (3) trabecular number and thickness, (4) structure model index (i.e., rods, plates, or honey-comb morphologies), (5) connectivity, and (6) trabecular spacing. The reasons for this focus are clear: the majority of variance in conventional mechanical tests of bulk specimens can be explained by variations in a few of these trabecular architectural characteristics. However, there is evidence that trabecular bone can also exhibit phenotypic plasticity on its *material* organization, which could have important influences in less studied, but clinically important, mechanical properties including toughness and fatigue resistance at the bulk trabecular and local trabecular (e.g., individual trabeculum) levels. These material variations include: (1) bone tissue mineral density, (2) bone tissue mineral density distribution (BMDD), (3) hemiosteonal packet prevalence, (4) lamellation differences in hemiosteonal packets, and (5) nanoscale mineral grain patterns [7-10]. One characteristic that has not been studied is the possibility that trabecular bone can exhibit mechanically adaptive variations in the collagen/lamellar organization of the hemiosteons that form the "packets". It is well known that cortical (compact) bone has the capacity to adapt in terms of the collagen/lamellar organization of secondary osteons (i.e., Haversian systems). These are called secondary osteon "morphotypes", which are important toughening mechanisms in cortical bone, especially for the nonuniform strain distributions (i.e., tension vs. compression strains) that typically occur in limb bone diaphyses [11-13]. We hypothesized that trabecular bone has the capacity for similar adaptations between regions that are habitually subject to tension vs. compression strains.

Methods: To determine the potential for the adaptive plasticity of trabecular bone, it is important to use a simply loaded model. The deer calcaneus was used for this reason and also because it is known to have significant differences in hemiosteonal packet prevalence, BMDD, and/or tissue mineralization between trabecular bone from the dorsal "compression region" vs. plantar "tension region" [9]. The calcanei from ten adult animals were obtained from the sample used in our prior studies [2,9]. Transverse segments from the middle-third of each calcaneal shaft were embedded in polymethyl methacrylate. These segments were then sectioned and milled to 50 microns and mounted on slides. The bone was fully mineralized and was not stained. Confocal microscopy was used to ensure that there was no, or minimal, overlap of secondary hemiosteonal packets. Then, using the light microscope, images were obtained of the dorsal "compression region" cortical and trabecular bone, and from the plantar "tension region" cortical and trabecular bone (Fig.1). Images were obtained at 10X using circularly polarized light (CPL), and the predominant collagen fiber orientation (CFO) was quantified in terms of weighted mean gray levels (WMGL) as described previously (where brighter gray levels are expressed as higher WMGLs, and reflect "compression-adapted" CFO) [11]. The heterogeneity of CFO (CFO-het) was expressed as the full width at one-half of the maximum of the gray level profile, which is similar to methods used to measure BMDD [7,9].

Results: Data from cortical bone showed expected CFO differences ($p=0.02$): more oblique-to-transverse "compression-adapted" CFO in the dorsal vs. more longitudinal "tension-adapted" CFO in the plantar cortex. Supporting the hypothesis, CFO was also more oblique-to-transverse in the dorsal trabecular and more longitudinal in the plantar trabecular bone (dorsal WMGL 119.6 ± 17.6; plantar 98.3 ± 20.2; $p<0.001$) (Fig. 2). CFO-het was also greatest in the dorsal "compression" trabecular bone ($p<0.01$). Qualitative observations showed that this increased heterogeneity could be seen at the level of individual hemiosteonal packets (i.e., variations in brightness were primarily explained by differences between, not within, packets). This is similar to what is seen in regions that are highly populated with different secondary osteon morphotypes in cortical bone [9].

Discussion: Similar to secondary osteons in cortical bone, hemiosteons in trabecular bone have the capacity to adapt in terms of their collagen/lamellar organization. Consequently, these hemiosteonal "morphotypes" are homologous to the secondary osteon "morphotypes" that have been described in various species, including humans and chimpanzees [9,11-13]. Similar trabecular-level adaptation might be present in bones that are habitually subject to nonuniform strain distributions, including the human femoral neck. It would be important to determine if this occurs and if these putative differences then become deficient with aging, perhaps contributing to the degradation of tissue mechanical properties (i.e., bone quality) in ways that are not detected when using measures of areal or volumetric bone mineral density (BMD; e.g., from DEXA scans). Studies are also warranted during aging of trabecular bone in vertebral bodies and in other areas that are prone to fracture in the elderly.

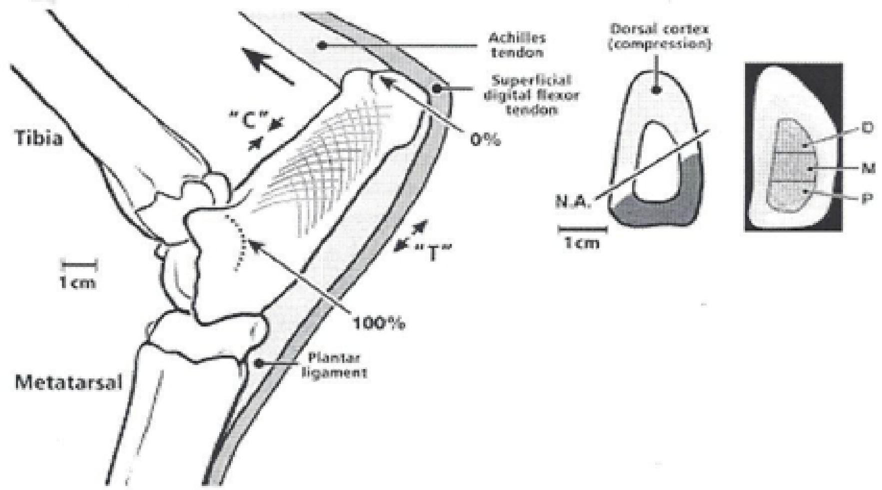
Resolving these issues can be clinically relevant when considering that initial failure of the proximal femur from a sideways fall is associated with failure of just a tiny proportion of the bone tissue (typically 1-6%) [3]. While the initiation of failure in this tiny region is attributed to the lack of structural redundancy [3], the possibility of deficiency in material competence should also be considered.

Significance: There is increasing evidence that material variations help adapt trabecular bone for nonuniform strain distributions that are experienced in many bones.

Acknowledgments: This research is supported by medical funds from the Department of Veterans Affairs, USA.

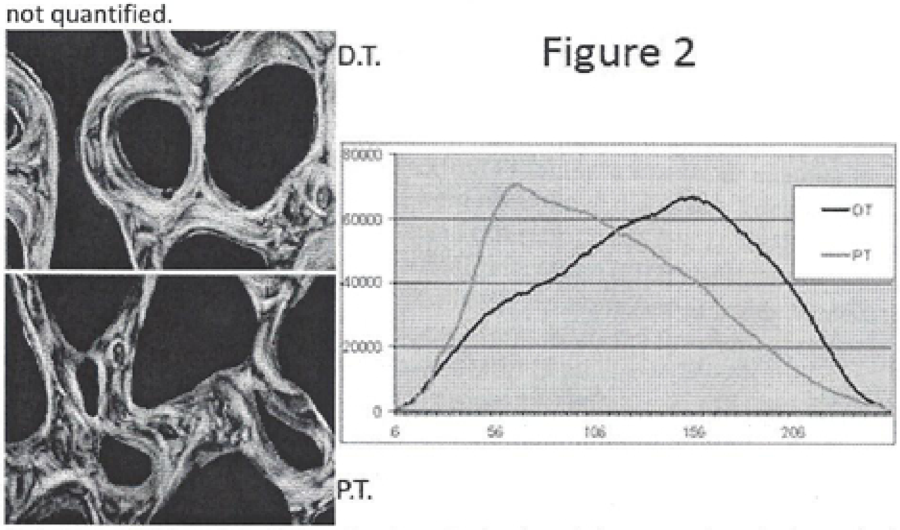
References: **1.** Keaveny et al. (2001) Annu Rev Biomed Eng 3:307-; **2.** Sinclair et al. (2013) J Human Evol 64:366-; **3.** Nawathe et al. (2013) J Bone Min Res, July Epub; **4.** Fajardo et al. (2007) Anat Rec 290:422-; **5.** Kaptoge et al. (2007) Bone 40:506-; **6.** Shaw and Ryan (2012) Am J Phys Anthro 147:187-; **7.** Roschger et al. (2008) Bone 42:456-; **8.** Ciarelli et al. (2009) Bone 45:903-; **9.** Skedros et al. (2012) J Anat 220:242-; **10.** Milovanovic et al. (2013) Microsc Microanal 14:1-; **11.** Skedros et al. (2009) Bone 44:392-; **12.** Skedros et al. (2013) J Struct Bio 181:95-; **13.** Skedros et al. (2011) J Anat, 218:480-.

Figure 1



At left is a lateral view of a deer ankle region showing the calcaneus and the habitual tension (T) and compression (C) loads. At right is a cross-section showing the dorsal (D) and plantar (P) trabecular bone and the intervening middle (M) region that was not quantified.

Figure 2



At left are CPL images of dorsal trabecular (DT) and plantar trabecular bone (PT) from the same calcaneus. The image width is ~2.36 mm. At right are the gray level profiles obtained from these images; toward the left is increased longitudinal collagen and toward the right is increased oblique-to-transverse collagen orientation.