

# Advancing Understanding of Femoral Neck Histomorphology and its Relationship to Load History: Use of Bat and Pigeon Humeri as Models for Adaptation for Habitual Torsion

John G. Skedros<sup>1</sup>, Madison S. Doutré<sup>1</sup>, Eric M. Brown<sup>1</sup>, Chad S. Mears<sup>1</sup>, and Sharon M. Swartz<sup>2</sup>  
<sup>1</sup>University of Utah, Salt Lake City, UT and <sup>2</sup>Brown University, Providence, RI

Disclosures: None of the authors have disclosures

**INTRODUCTION:** It remains uncertain if the habitual load condition in the FN is bending (e.g., as argued by Wolff and accepted as conventional wisdom today in orthopaedic surgery training) or torsion (e.g., as argued by others) (reviewed in [1]). Or perhaps FN loading is sufficiently complex that neither of these specific load histories are appropriate simplifications. For example, it may be that the lateral gluteal muscles produce net compression across the entire FN [2] and this condition changes to comparatively deleterious bending with advanced aging [3]. It has been argued that if this is the case then age-related change in FN load history would be characterized by the emergence of prevalent/predominant tension in the superior cortex [4]. To examine that possibility, this more recent study [4] examined patterns of predominant collagen fiber orientation (CFO) because it is the strongest indirect method for detecting a bending vs. torsion load history [5,6]. However, the CFO data reported in [4] did not support the hypothesis of age-related increased bending (i.e., the emergence of tension in the superior cortex) in the FN of elderly individuals. But reduced differences in CFO between the superior and inferior FN cortices were found, supporting the probability that the strain environments of the superior and inferior cortices become more similar with advanced age [4]. This is consistent with the idea that underloading of the superior FN becomes prevalent with age [3]. The fact that material changes are occurring in addition to structural changes (e.g., enlargement of FN diameter) is important because it leads to the proposal that enhancing only the natural age-related subperiosteal bone apposition that occurs in the FN [3] would not be sufficient to curb fracture risk. The aim of the present study is to consider the histology (especially CFO patterns) of the human FN in a novel context to advance understanding of how bone adapts to torsion vs. bending when the cortex is very thin. Here we examine the histomorphology and cross-sectional structural characteristics of highly torsionally loaded bones from bats and birds. In this context the histological type of bone known as “laminar bone” is considered. Laminar bone is common in birds and can adapt via the modification of the orientations/patterns of primary vascular canals, where highly laminar bone is characterized by high proportions of vascular canals that course circularly, following the contour of the bone’s cortex. Increased “laminarity” correlates with increased oblique-to-transverse CFO, but which is the most proximate adaptation is unclear [7]. In a study of 168 limb bones from 22 species of birds, de Margerie et al. (2005) [8] concluded that the occurrence of laminar bone in long bones with larger diameters reflects optimization for torsion. Bat forelimb limb bones (e.g., humerus and radius) also experience substantial torsion [9,10] but do not have laminar bone [11]. This difference is compelling because it suggests that CFO might be a ‘universally adaptable characteristic’ that can be modified regardless of vascular orientation. Being from a flying mammal that has the ability to form secondary bone rather than adjust laminarity, the bat humerus may provide insights into how FN histomorphology might adapt for habitual torsional vs. bending loads. We hypothesize that the CFO patterns and cross-sectional morphology of the pigeon humeri (strain data from [12]) and bat humeri will resemble those reported previously by our laboratory in the human FN [4] but not in the bat third metacarpal where unidirectional bending is more prevalent [10] as is seen in the proximal human femoral shaft [6].

**METHODS:** Mid-shaft segments were cut transversely from adult: 14 pigeon humeri (*C. livia domestica*), 9 bat humeri and 9 bat third metacarpal (*P. poliocephalus* from [9]). The segments were embedded in polymethyl methacrylate and a 1mm thick section was mounted onto glass slides and ultramilled to 100±5 µm [4,5]. 50x images were obtained in cranial, caudal, ventral, and dorsal cortices. Regional differences in predominant collagen fiber orientation (CFO) were expressed as differences in weighted mean gray-level (WMGL) in circularly polarized light (reported as “CFO-WMGL”) from: (1) the entire cortex, and (2) the cortex excluding the highly birefringent (“bright”) endosteal bone. Fractional area of secondary bone (FASB; i.e., percentage of secondary osteons (Haversian systems)) was also determined and the thickness of each of the four cortices. The entire bone transverse sections were also used to obtain cross-sectional circularity, cortical thinness (K values; closer to 1.0 = thinner cortex), second moments of area, I<sub>max</sub>, I<sub>min</sub>, and J (I<sub>max</sub>+I<sub>min</sub>) [8,11]. Statistical significance (p<0.05) was determined using ANOVA tests and Pearson correlations.

**RESULTS (Table and Fig. 1):** Consistent with expectations for adaptation for habitual torsional loading, no significant regional CFO-WMGL differences (with or without the inclusion of endosteal bone) were found in bat and pigeon humeri. These torsionally loaded bones also had generally and significantly higher CFO-WMGL than the bat third metacarpal (B3M) (where bending is habitual). Although the B3M showed a dorsal vs. caudal CFO-WMGL difference (p<0.01), this was not in the dorsal-ventral direction of bending. Cortical thickness (CT) showed no significant regional differences and did not correlate with FASB or CFO-WMGL in any of the bones. Cortical thinness (K) showed highest values (thinnest walls) in the humeri (pigeon > bat) and lowest in the B3M. FASB was substantially higher in the B3M than the bat humerus; no secondary osteons were seen in the pigeon humeri (entire bone sections examined). The osteons in the bat bones were also in the mid-to-deeper portions of the cortex (i.e., likely deposited in earlier development).

**DISCUSSION:** The bat and pigeon humeri showed the absence of regional variations in CFO-WMGL, which is the ‘expected adaptation’ in habitual torsion [5,6]. This finding in laminar (birds) and non-laminar (bats) bones is an important advance for comparative histomorphological studies because it suggests that CFO-WMGL is the proximate, and perhaps universal, means for adapting cortical bone at the tissue level (perhaps secondary osteons are not needed for this adaptation). In this context, it may be possible to draw board comparisons in histomorphological data between species that otherwise seem highly dissimilar and regardless of their capacity to form secondary osteons. In vivo strain data from pigeon and bat humeri show that their neutral axes (NA) shift broadly throughout the different stages of flight (up to ~40°) [9,12]. It has been argued natural NA shifts in the human FN also contribute to the low regional variations in CFO-WMGL and FASB seen in the FN cortex [4]. However, the relatively low K values (i.e., thin cortices) of these humeri coupled with their lack of regional variations in CFO-WMGL, and the lack of obvious mechanically adaptive secondary osteon distributions (in the bat humeri), places them in the “zone” where the cortex is so thin that material adaptation subsidiary to the role that structural adaptations have in accommodating habitual torsion or bending (Fig. 2, below middle). In vivo strain data showing that the B3M is loaded in dorsal-ventral bending [10] led us to hypothesize that dorsal-ventral differences in CFO-WMGL would be detected and would correlate with this direction of habitual bending (as seen in along the medial-lateral proximal human femoral shaft and in other bones or bone regions where uni-directional bending also predominates [4-6]). However, in the B3M there is substantial reversal of strain mode during the wingbeat cycle, which imparts high compression and high tension to the dorsal and ventral cortices at different times [10]. This likely explains the lack of expected compression- vs. tension-specific dorsal-ventral CFO-WMGL, FASB, and/or cortical thickness differences along this axis. The higher FASB in the B3M when compared to the bat humerus might reflect the generally increased strain magnitudes and microdamage repair toward the distal end of the wing [10]. In their histomorphological study of fluorochrome-labeled limb bones of bats of the same species examined by us, Bennett and Forward [13] also showed that the osteonal remodeling was “... predominantly quiescent [in adults], suggested that remodeling had occurred in an early stage of life, possibly when the wings were being exercised in preparation for anticipated flight.”

**SIGNIFICANCE:** Bat and pigeon humeri exhibit histological and structural characteristics that resemble some findings in the human femoral neck. Use of data from these bones contributes to the development of a large body of data from various species that help when interpreting load history in bone regions where in vivo strain data are not available.

**REFERENCES:** [1] Skedros and Baucom 2007 J. Theoretical Biol 244; [2] Lovejoy 1988 Sci. Am. 289; [3] Mayhew et al. 2005 Lancet 366; [4] Keenan et al. 2014 ORS abstract; [5] Skedros et al. 2009 Bone 44; [6] Skedros et al. 2013 J. Struct. Biol. 181; [7] Skedros and Hunt 2004 J. Anat 205; [8] de Margerie et al. 2005 Anat Record 282; [9] Swartz et al. 1992 Nature 359; [10] Swartz and Middleton, 2008 Cells Tiss Organs 187:59-; [11] Lee and Simons 2015 PeerJ March e823; [12] Biewener and Dial 1995 J. Morphology 225; [13] Bennett and Forward 2010 Austral Zoologist 35.

