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# OSTEON PHENOTYPIC MORPHOTYPES: A NEW CHARACTERISTIC FOR INTERPRETING BONE QUALITY IN CORTICAL BONE

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**INTRODUCTION:** Characteristics of bone organization that may change with age and thereby decrease bone "quality" (i.e., tissue mechanical properties), leading to fragility fractures, include collagen molecular crosslinks, predominant collagen fiber orientation (CFO), viability of bone cell populations, and bone density. An additional related characteristic that has recently received attention because of its importance in influencing fatigue behavior and osteon pullout is regional variations in the proportions of secondary osteon morphotypes [1,2]. In circularly polarized light (CPL) these are seen as variations in the degree of collagen organization/orientation, ranging from "hoop" osteons with a peripheral ring of highly oblique-to-transverse collagen to "distributed" osteons with highly oblique-to-transverse collagen distributed across the osteon wall (Fig. 1). Martin and co-workers [1] have developed a "hoop" score that assigns numerical values to six osteon morphotypes. We have recognized that modifications of this scoring scheme can significantly enhance its utility for inferring adaptation. The present study evaluates how strongly hoop scores correlate with non-uniform strain environments that are typically experienced by appendicular bones. We hypothesized that our modified hoop score will strongly correlate with the prevalent/predominant strain modes (tension, compression, and shear (neutral axis)) in bones that receive habitual bending (e.g., sheep, deer, and horse calcanei, sheep and horse radii, and horse third metacarpals (MC3s)) and weakly correlate in bones where torsion/shear is comparatively prevalent (sheep tibiae). Additionally, we hypothesized that the modified hoop score will correlate strongly with the predominant CFO across the entire microscopic field.

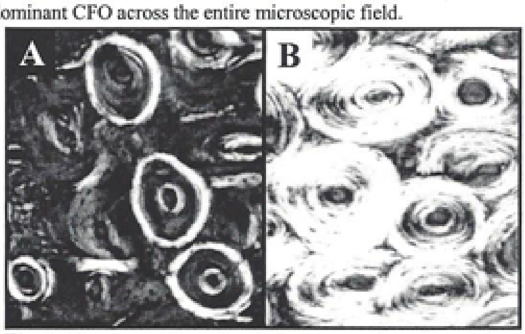


**Fig. 1** CPL images illustrating different osteonal morphotypes in an equine MC3: (A) "Hoop" osteons in a habitual tension region, and (B) "Distributed" osteons in a habitual compression region. Images are of the same unstained bone, with the same magnification and illumination.

**METHODS:** Seven bones of each type were obtained from skeletally mature animals, including calcanei, radii, and MC3s from Standardbred horses (4-8 years old) that had no history of racing, calcanei from wild male mule deer, and radii and calcanei from 2-year-old domesticated sheep. Mid-diaphyseal sections were embedded in PMMA in an undecalcified state, and ultramilled to 100±5 microns. Milled sections were analyzed for CFO using CPL [3]. Regional differences in CFO were quantified in terms of differences in the transmitted light intensity, where darker gray levels represented relatively more longitudinal CFO and brighter gray levels represent more oblique-to-transverse CFO. The anterior, posterior, medial, and lateral cortices of all bones were analyzed. The habitual compressed cortex is anterior in the calcanei and posterior in the radii. The MC3 and sheep tibia are highly torqued, hence do not have the habitually prevalent/predominant tension/compression regions seen in the other bones. **Osteon selection criteria** included: 1) presence of a scalloped cement line, 2) absence of a Volkmann's canal connection, and 3) refilling complete or nearly so [1]. Blinded investigators classified these complete secondary osteons (>10,000) into one of six categories, based on birefringence pattern, with each osteon type receiving "Martin's" numerical value: Dark=0, Distributed=1, Weak Incomplete=2, Weak=3, Incomplete=4, and Hoop=5 ("weak" or "incomplete" refer to a weak or incompletely present hoop). The modified hoop score used in the present study is: Dark=0, Weak Incomplete=1, Weak=2, Incomplete=3, Hoop=4, and Distributed=5. This modification juxtaposes morphotypes associated with oblique-to-transverse CFO, which is more biomechanically relevant when inferring compression vs. tension adaptation. Hoop scores were then calculated for each image by determining the number of each osteon type, multiplying each type by its respective assigned value, summing the products, and then dividing by the total number of quantified osteons [1].

**RESULTS:** Results are summarized in figs. 2 (means & SE) and 3, where SC=sheep calcaneus, DC=deer calcaneus, HC=horse calcaneus, HR=horse radius, SR=sheep radius, MC3=horse third metacarpal, ST=sheep tibia. Significant anterior/posterior differences were found in nearly all bones ($p<0.0001$), except for the MC3s, and sheep tibiae and radii. However, the medial/lateral comparison in the MC3s was statistically significant ($p<0.001$), which in this bone may more closely correspond to regions habitually loaded in compression (medial) vs. tension (lateral). Correlations between the hoop scores of Martin et al. and CFO data from all regions in each bone type demonstrated moderate-to-weak *negative* r values. In contrast, typically high *positive* r values were found when the same comparisons were made between the modified hoop scores and the CFO data.

**Fig. 2 Modified Hoop Scores in Each Bone**

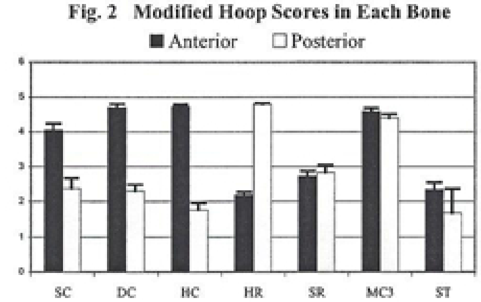


**Fig. 3 CFO vs. Hoop Score (r values) [*p<0.05]**

| Bone | Martin et al. | Modified Score |
|---|---|---|
| Sheep Calcaneus | -0.543* | 0.806* |
| Deer Calcaneus | -0.396* | 0.757* |
| Horse Calcaneus | -0.525* | 0.810* |
| Horse Radius | -0.319* | 0.764* |
| Sheep Radius | 0.056 | 0.343* |
| Horse MC3 | -0.518* | 0.673* |
| Sheep Tibia | -0.216 | -0.061 |

**DISCUSSION:** The modified hoop score typically correlates strongly with the CFO of a strain distribution of habitual bending. In this context, increased "distributed" osteons (i.e., with highly oblique-to-transverse CFO) account for the high hoop scores in the habitually compressed cortices of nearly all bones. Sheep radii are an exception; in these bones the increased oblique-to-transverse CFO in their caudal cortex was strongly influenced by the oblique-to-transverse collagen in the primary bone. There are in vivo strain data suggesting that the neutral axis in equine MC3 courses in a more anterior-posterior direction (hence loading the medial and lateral cortices in compression and tension, respectively) than in a medial-lateral direction. The sheep tibiae showed expected results—regional variations in osteon morphotypes are not expected since more diffusely prevalent shear would be accommodated by relative uniformity of CFO and/or osteon morphotypes. The modified hoop score may prove to be useful for further investigating how distributions of osteon morphotypes, and their potential age-related variations, affect bone tissue mechanical properties.

**REFERENCES:** **1)** Martin et al. 1996. Bone 19:165; **2)** Hiller et al. 2003. J. Orthop. Res. 21:481; **3)** Skedros et al. 1996 Anat. Rec. 246:47.