Antler has Inherent Collagen Fiber Orientation Heterogeneity (CFO-Het): Implications for Investigating Bisphosphonate- and Age-Related Degradation in Human 'Bone Quality'

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Introduction: Antler, one of the toughest natural materials known, is receiving increased attention in orthopaedics because of its potential for advancing our understanding of toughening mechanisms in human bone. The increased incidence of fragility fractures in the elderly is strongly related to degradation of bone toughness. DEXA scans do not correlate strongly with fracture risk because they do not account for 'bone quality' (e.g. tissue/material changes that influence toughness) [1,2]. We quantified material characteristics that are potential microstructural toughening mehanisms in deer antler, which might also be at work in an analogous fashion in human bone and might degrade with age and/or bisphosphonate use. We predict that antler will exhibit evidence of inherent CFO-Het, which may be important in providing it with high toughness.

Methods: 11 Rocky Mountain mule deer antler segments (transverse) were embedded in methacrylate and ultramilled to 75μm. The sections were not stained and were fully calcified. Primary osteonal structures (n = 528; secondary osteons are rare) were cropped from circularly polarized light (CPL) images. Predominant CFO for each osteon was based on gray-level pixels. A gray-level profile for each osteon wall was created. CFO-Het is expressed as the sum of the full width at the half-maximum for each peak of the gray-level profile. Osteon morphotype scores (MTSs) were assigned to each primary osteon (Fig. 1): (1) relatively dark birefringence (BF) (longitudinal CFO) across the entire osteon wall (OW) (2) relatively bright BF (transverse-to-oblique CFO) within the inner half (near the vascular canal) of the OW with relatively dark BF within the peripheral half of the OW, (3) relatively dark BF within the inner half of the OW with relatively bright BF within the peripheral half of the OW, (4) bright BF within both the inner one-third of the OW and the peripheral one-third of the OW with relatively dark BF within the middle one-third of the OW, and (5) bright BF patterns of CFO traversing the majority of the OW. Correlations were used to determine possible associations between MTSs and CFO-Het. Osteon size, shape and elongation data were also obtained for each osteon.

Results: MTS and CFO-Het are not correlated: all MTSs ($r = -0.05$, $p=0.2$), and MTSs 1-4 ($r = -0.03$, $p=0.5$). Predominant CFO was also not correlated (r values <0.1) with osteon size, shape, or elongation. The mean CFO-Het for individual osteons did not differ significantly from the mean CFO-Het for entire images. 70.7% of all osteons were scored as either MTS of 3 or 4, indicating that CFO is highly oblique-to-transverse near the osteonal interfaces. Figure 2 shows CPL images that are representative of the sample of antlers.

Discussion: Lack of correlations between CFO-Het and osteon MTSs, and between predominant CFO and osteon cross-section size, shape and elongation, suggests that the antler tissue has inherent CFO-Het. Hence, CFO-Het and predominant CFO are independent of osteon MTSs and their 2D morphologies and 3D orientations (the latter is inferred from 2D elongation data). Osteonal morphotypes reveal histomorphological patterns that are not shown by CFO-Het and predominant CFO data. This is revealed by MTS data showing 70.7% of the osteons have oblique-to-transverse CFO near their outer interfaces. This likely enhances toughness where microdamage is best accommodated/arrested, similar to the suggested function of the analogous "hooped" secondary osteons in human bones [3-5]. Hypermineralization at these interfaces (previously reported) is also similar to cement lines of human secondary osteons [6]. We have speculated that regional variations in CFO-Het, predominant CFO, and/or osteon MTSs are important toughening mechanisms in human bones [3]. One exemplitive case is the proximal femoral diaphysis where habitual bending requires mechanically adaptive variations in predominant CFO (produced by different osteon morphotypes) and possibly CFO-Het between the medial (“compression”) and lateral (“tension”) cortices. In contrast to antler where there is inherent CFO-Het (i.e., no regional variations in all CFO-related data), mechanically advantageous regional variations in predominant CFO and/or CFO-Het is accomplished through osteon remodeling (not possible in antler) by the production of different osteon morphotypes. In human bone, toughness degrades when these regional variations are not maintained by this phenotypic plasticity of normal osteonal remodeling. Drug-induced insufficiency in this process may be an early stage in the development of atypical fractures that can occur with bisphosphonate treatment (and possibly contribute to age-related skeletal fragility) [5]. Additional studies are needed to determine more specifically how perturbations of normal bone remodeling coupled with the failure to maintain the regional strain-mode-adapted histomorphology increases the propensity for femoral fractures in these patients. We suggest that much can be learned from comparing antler with human histomorphological adaption, especially in terms of the degradation that occurs with senescence and bisphosphonate use.

Significance: Relationships between CFO-Het and osteon morphotypes is a largely unexplored avenue of research in human bone tissue-mechanical properties. Insights from antler regarding how these relationships can work as toughening mechanisms could help to understand more specifically how and why 'bone quality' degrades in some skeletal locations in humans during aging and bisphosphonate treatment.

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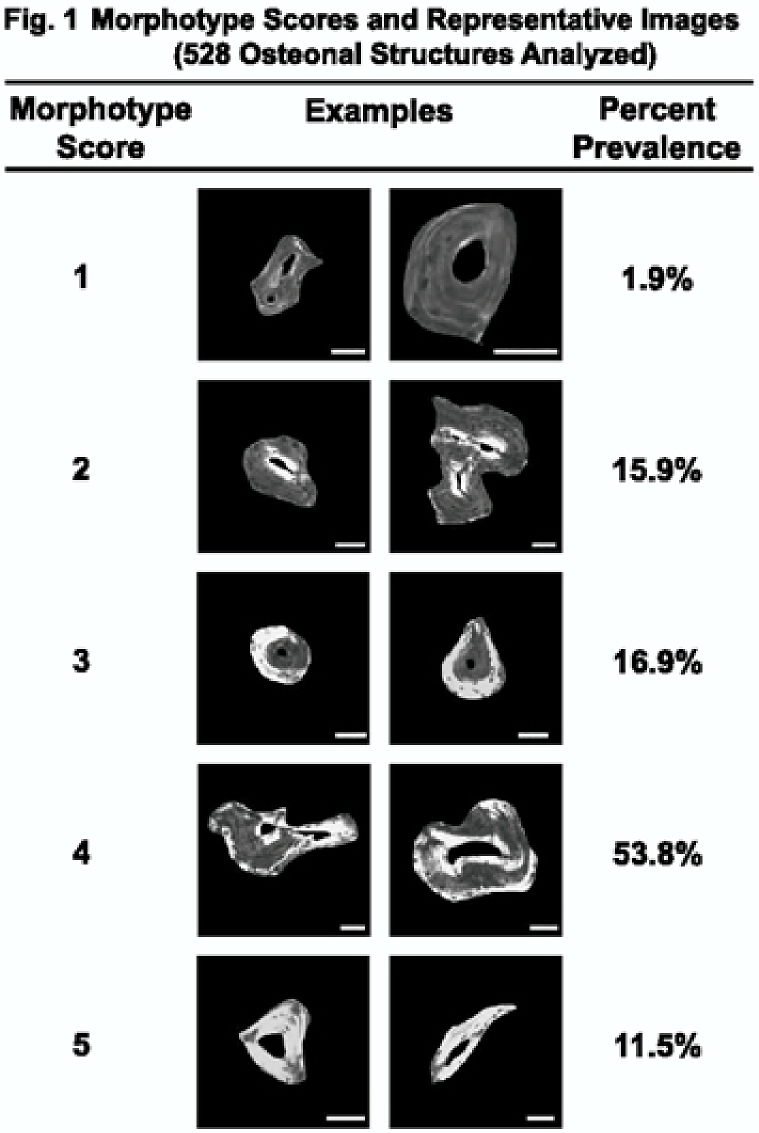
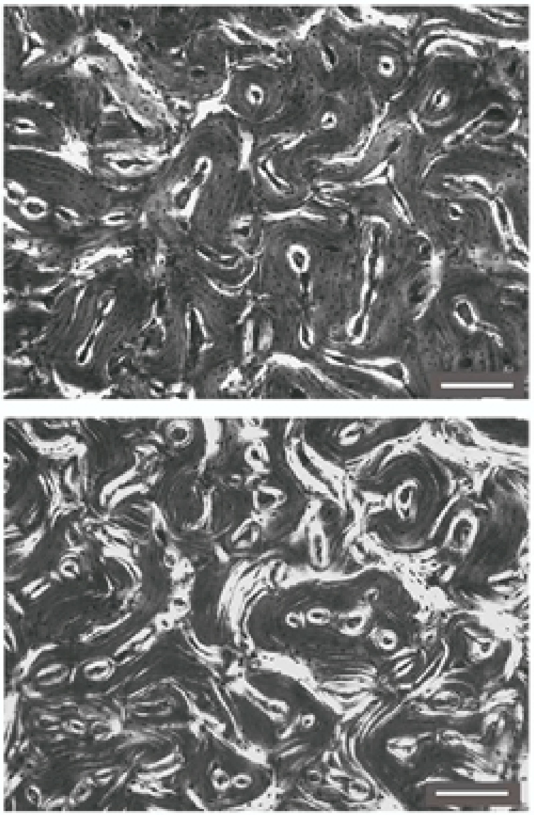
References: [1] Dawson-Hughes et al. 2008 Osteop. Int. 19:449-458; [2] McCreadie and Goldstein 2000 J. Bone Min. Res. 15:2305-2308; [3] Skedros et al. 2011 J. Anat. 218:480-499; [4] Hiller et al. 2003 J. Orthop. Res. 21:481-488; [5] Skedros et al. 2012 ORS abstract 37:1512; [6] Skedros et al. 2005 Anat. Record 286:781-803.



Examples of the 5 osteon morphotypes (antler).

Fig. 2 Representative Antler Images (Circular Polarized Light)



Two CPL images of antler.

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