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THE RELATIVE INFLUENCE OF COLLAGEN CROSSLINKS ON THE MECHANICAL PROPERTIES OF EQUINE CORTICAL BONE

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INTRODUCTION: Decreases in bone mineral density fall significantly short of explaining age-related increases in fragility fractures. Age-related alterations of other histocompositional parameters may contribute significantly to the deterioration of bone "quality" (i.e., tissue mechanical properties). For example, the integrity of the collagen network in bone has been implicated in age-related decreases in toughness [1]. Intermolecular collagen crosslinks that may be involved in this context include the enzymatic cross-links hydroxylysyl-pyridinoline (HP) and lysyl-pyridinoline (LP), and non-enzymatic crosslinks measured by pentosidine (PE) concentration. Investigation of collagen crosslinks and other non-traditionally examined histocompositional parameters may help to clarify their relative contributions in accommodating the non-uniform loading environment experienced by most bones [2]. For example, variations in predominant collagen fiber orientation (CFO) strongly influence energy absorption of cortical bone in the physiologic context of strain-mode-specific (**SMS**) testing (e.g., compression testing of bone from regions habitually loaded in compression) [3,4]. We examined the relative influences of collagen crosslinks and other histocompositional characteristics on the mechanical properties of cortical bone in SMS compression testing of equine third metacarpals (MC3s), which have served as models for understanding how bone tissue adapts to avoid stress fractures associated with a harsh loading environment [5].

METHODS: *Italicized regions = "strain-mode-specific"(SMS) testing* Cubic (5mm) specimens for compression testing (n: dorsal-lateral, **D-L** = 20; *palmar-medial, **P-M** = 20*; lateral, **Lat** = 10; *dorsal-medial, **D-M** = 10*) were machined from 10 skeletally mature horse ~2-9 year-old **[check]** MC3s at mid-diaphysis [6]. Specimens were tested unrestrained to failure in axial compression at 0.001 sec^{-1} [6]. Each specimen was examined for: elastic modulus, yield stress, ultimate stress, pre-yield "elastic" energy absorption, post-yield "plastic" energy absorption, and total (elastic + plastic) energy absorption. Specimen fragments were evaluated for %ash content, osteocyte lacuna population density (Ot.Lc.N/B.Ar; no./mm^2 bone), percent of osteonal (secondary) bone, secondary osteon population density (N.On/T.Ar), predominant CFO (using circularly polarized light), and concentration of collagen crosslinks (HP, LP, PE) [1,2,7]. Data were evaluated using Pearson's correlations (r), and simple linear (r^2) and stepwise multivariable multiple regression analyses (R^2 for cumulative variance).

RESULTS: HP ranged from 21.2 ± 5.0 (lateral) to 28.1 ± 7.2 (habitually compressed dorsal-medial cortex) ($p<0.05$). LP ranged from 4.31 ± 0.9 (lateral cortex) to 5.0 ± 1.5 (both dorsal-medial and palmar-medial cortices) ($p=0.17$). PE ranged from 0.8 ± 0.7 (dorsal-medial cortex) to 2.0 ± 2.4 (palmar-medial cortex) ($p<0.05$). Calculation of the HP/LP ratio ranged from 5.0 ± 1.2 (lateral cortex) to 5.9 ±1.8 (dorsal-medial cortex) ($p=0.15$).

All compression-tested specimens (non-SMS testing): Collagen crosslinks (HP, LP, PE, HP/LP) typically explained << 5% of variance for each mechanical parameter. CFO was the most important explanatory variable in pre-, post-, and total energy absorption (8% r = 0.287, $p<0.05$; 15% r = 0.390, $p<0.01$; 22% r = 0.472, $p<0.001$, respectively). Porosity explained the greatest variance in elastic modulus and yield stress (11%, r = -0.337, $p<0.01$; 7%, r = -0.259, $p=0.05$, respectively), and %ash explained the greatest variance in ultimate stress (23%, r = 0.478, $p<0.001$).

SMS compression specimens: HP/LP ratio explained the most variance in post-yield energy absorption, explaining ~12% of variance (r = -0.34, $p=0.076$). %Ash explained the most variance in both elastic modulus and ultimate stress (20%, r = 0.45, $p<0.05$; 26% r = 0.51 $p<0.01$, respectively). Ot.Lc.N/B.Ar explained the most variance in pre-yield energy absorption and yield stress (18%, r = -0.42, $p<0.05$; 25%, r = -0.50, $p<0.01$, respectively). CFO explained the most variance in total energy (32%, r = 0.57, $p<0.01$).

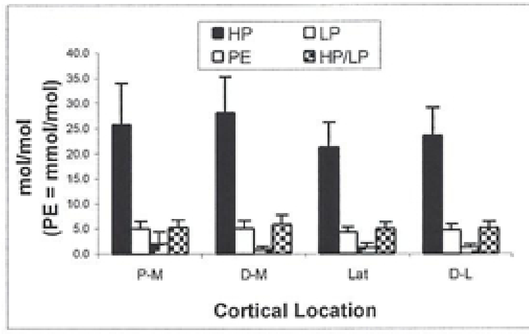

Fig. 1. Collagen cross-links in the four cortical locations.

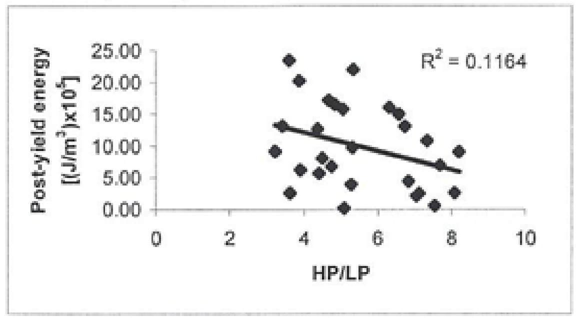

Fig. 2. Relationship between HP/LP and post-yield energy in SMS compression.

DISCUSSION: Results of SMS compression tests reveal positive relationships between *pre-yield* energy absorption and HP, LP, and HP/LP. Correlations were negative between PE and *pre-yield* energy absorption. This finding is consistent with results of previous studies showing that elderly humans having low levels of enzymatic crosslinks and high levels of non-enzymatic crosslinks tend to have more brittle bone, and thus may be at elevated fracture risk [8]. In contrast, results of ***non***-SMS testing demonstrated little or no correlation between the crosslinks and mechanical parameters — clearly demonstrating the value of the more physiologic context of SMS testing. In SMS testing, the HP/LP ratio also emerged as having an important ***negative*** influence in post-yield energy absorption, suggesting that the HP moiety of enzymatic crosslinks hastens failure if the specimen is loaded into the post-yield range. However, the borderline statistical significance (p = 0.076) and low correlation (r = -0.341) of HP/LP with post-yield energy absorption might suggest insufficient statistical power. But this possibility is minimized by the fact that one or more of the other histocompositional characteristics showed much stronger, and statistically significant, correlation with each of the SMS mechanical parameters (e.g., CFO and total energy absorption: r = 0.565, p = 0.002). This fact suggests that collagen crosslinks truly have an important role in determining post-yield behavior. Mechanical testing regimes that are different from those used in our study will probably be needed to better elucidate the relative roles of collagen crosslinks in affecting bone quality. These might include tests that: 1) measure damage accumulation or fatigue behavior that is more representative of the complexities of physiologic loading [9,10], and 2) distinguish between initiation and propagation fracture toughness [11].

REFERENCES: **1)** Wang et al. 2002. Bone 31:1-7; **2)** Wang et al. 2002. Calcif Tiss Int 71:186-192; **3)** Skedros et al. 2000. 24th Am. Soc Biomech 173-174; **4)** Skedros et al. 2001. Trans. 47th ORS:519; **5)** Skedros et al. 2003. J Exp Biol 206:2431-9; **6)** Riggs et al. 1993. Anat Embryol 187:239-248; **7)** Skedros et al. 1996. Anat Rec 246:47-63; **8)** Keaveny et al. 2003 J Bone Min Res 18:S307; **9)** Joo et al. 2004 Trans 50th ORS:515; **10)** Hiller et al. 2003 J Orthop Res 21:481-8; **11)** Vashishth et al. 1997. J Biomech 30:763-9