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THE ROLE OF OSTEOCYTE LACUNA POPULATION DENSITY ON THE MECHANICAL PROPERTIES OF CORTICAL BONE

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INTRODUCTION: It is known that osteocyte lacuna population density (OLPD) declines with age, and is associated with an exponential increase in microdamage [1]. This population decline may contribute significantly to the age-related deterioration of mechanical properties of bone, and to skeletal fragility associated with osteoporosis. Recent *analytical* studies have suggested an important role for osteocyte and lacunar densities in mechanical behavior of bone, including stiffness [2] and strength [3]. If true, these assertions challenge conventional wisdom that osteocyte lacunae are inconsequential in influencing the mechanical behavior of cortical bone [4]. Although up to 42% of lacunae may not contain viable osteocytes [5], both osteocytes *and* lacunae can potentially influence bone remodeling and mechanical properties. For example, osteocyte lacunae may become stress-risers for microdamage formation [6], especially with age or excessive exercise, and osteocytes may be the mechanosensors of microdamage [7], influencing their repair. Thus modifying their concentrations may enhance a bone's fatigue life, hinder stress and fragility fractures, and/or modify other aspects of mechanical behavior. Despite the broad application of such claims, relatively few studies have examined these possibilities.

Investigation of OLPD and other non-traditionally examined histocompositional parameters may help to clarify their relative contributions to the mechanical properties of bone from various cortical regions. For example, it has been shown that variations in predominant collagen fiber orientation (CFO) strongly influence energy absorption of cortical bone in the physiologic context of strain-mode-specific (**S-M-S**) testing (e.g., compression testing of bone from regions habitually loaded in compression; tension testing of bone from regions habitually loaded in tension) [8,9]. In this study, we examined the relative influences of OLPD and other ultrastructural and microstructural characteristics on the mechanical properties of equine and cervine cortical bone in S-M-S testing.

METHODS: *Italicized regions = "strain-mode-specific"(S-M-S) testing*
Equine Specimens: Cubic specimens for compression testing (n: cranio-lateral = 20, *caudo-medial = 20*, lateral = 10, *cranio-medial = 10*) and dumb-bell-shaped specimens for tension testing (n: *cranio-lateral = 20*, caudo-medial = 20) were machined from 10 mature standardbred horse third metacarpals (MCIII) at mid diaphysis [10].
Cervine Specimens: Cylindrical compression specimens (n: *cranial = 8*, caudal = 8) and dumb-bell tension specimens (n: cranial = 9, *caudal = 9*) were machined from 17 skeletally mature male Rocky Mountain mule deer calcanei at mid diaphysis [11].
All bones: Specimens were tested unrestrained to failure in axial compression (strain rate: MCIII, 0.001 sec^{-1}; Calcanei, 0.003 sec^{-1}) or tension (strain rate: MCIII, 0.01 sec^{-1}; Calcanei, 0.003 sec^{-1}) [10,12]. Each specimen was examined for: elastic modulus (EM), yield stress (YS), ultimate stress (US), elastic energy (YNRG; energy absorbed to yield stress), plastic energy (PNRG; energy absorbed from yield stress to ultimate stress), and total energy (TNRG; total energy absorbed). Specimen fragments were evaluated for %ash content (550ºC), OLPD (no./mm^2 bone), percent of osteonal (secondary) bone, secondary osteon population density (OPD), and predominant CFO (using circularly polarized light) [13]. Data were evaluated using Pearson's correlations (r), and multiple regression analyses (R^2 for cumulative variance).

RESULTS:
Equine MCIII: OLPD ranged from 611±69 (habitually tensed cranio-lateral cortex) to 620±76 (habitually compressed caudo-medial & cranio-medial cortices) (p = 0.6).
All compression-tested specimens: OLPD typically explained << 5% of variance for each mechanical parameter.
S-M-S compression specimens: OLPD was the most important explanatory variable for YS and US (r = -0.494, p < 0.01 for both), and was 2nd most important in EM (r = -0.396; p < 0.04) and YNRG (r = -0.419; p < 0.03). The addition of OLPD to multiple regression analyses increased cumulative %variance explained in all S-M-S compression analyses by an average of 28.8% (11.6% absolute).
All tension-tested specimens: There were no instances where OLPD was greater than the fourth-most important explanatory variable.
S-M-S tension specimens: OLPD was an important explanatory variable in EM (2nd position; r = -0.770; p < 0.02), YS (2nd position, r = -728; p < 0.03), and US (3rd position; r = -0.683; p < 0.05).
Cervine calcaneus: OLPD ranged from 485±89 (habitually tensed caudal cortex) to 583±72 (habitually compressed cranial cortex) (p < 0.01).
Compression-tested cranial cortex (S-M-S): OLPD typically explained << 5% of variance for each mechanical parameter.
Compression-tested caudal cortex: OLPD was the most important explanatory variable in YNRG (r = -0.911; p < 0.03) and TNRG (r = -0.855; p < 0.06). In other mechanical parameters, OLPD typically explained << 5% of variance.
Tension-tested cranial cortex: Aside from a moderately correlated 2nd position influence on YS (r = 0.685; p = 0.09), OLPD typically explained << 8% of variance for each mechanical parameter.
Tension-tested caudal cortex (S-M-S): OLPD typically explained << 3% of variance for each mechanical parameter.

DISCUSSION: In S-M-S tests of equine MCIII bone, OLPD emerged as important in influencing EM, YS, and US. However, similar relationships were not found in S-M-S testing of the cervine calcanei. These inconsistent quantitative experimental data draw into question the conclusions of analytical studies that have suggested that OLPD is important in the mechanical behavior of cortical bone. Furthermore, the relatively *uniform* OLPD in the MCIII was influential in explaining variance in several S-M-S mechanical parameters while the comparatively *non-uniform* OLPD in the calcanei explained only a minor proportion of variance.

The negative correlations of OLPD with EM, YS, and US in S-M-S tests of the MCIII imply that lacunae may be stress risers during in vitro testing, making the specimen more likely to fracture. The possibility that these MCIII data are anomalous is supported by the lack of similar behavior in the S-M-S tested calcaneus specimens. This may be due in part to the different strain rates used for these bones, or alternately, the influence of other histocompositional parameters in the calcaneus that may preclude behavior observed in the MCIII. This may include markedly non-uniform CFO and collagen cross-linking between cranial and caudal cortices of the calcanei [14,15].

Previous studies have shown that the relatively uniform cell densities in the equine MCIII (which has a highly non-uniform strain milieu) may reflect the probability that nutritional constraints are vastly more important than any mechanically relevant adaptation [16]. However, OLPD may be correlated with other histocompositional parameters (such as OPD or %ash) that are more proximate in influencing mechanical properties.

The percentage of viable osteocytes could not be determined in this study. Further investigations are necessary to determine the role that the relative percentage of viable osteocytes and the corresponding density of the cellular network may play in influencing bone mechanical properties, especially in the context of influencing the propagation, arrest, and repair of microdamage. Fatigue and fracture toughness testing may reveal important mechanical roles for OLPD that are not evident in this study.

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