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THE INFLUENCE OF COLLAGEN FIBER ORIENTATION ON MECHANICAL PROPERTIES OF CORTICAL BONE OF AN ARTIODACTYL CALCANEUS: IMPLICATIONS FOR BROAD APPLICATIONS IN BONE ADAPTATION

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INTRODUCTION: Degradation of bone *material* properties contributes to skeletal fragility associated with aging and osteoporosis. McCalden et al. [1] documented significant age-related changes in ultimate stress, ultimate strain, and energy absorption in femoral diaphyseal cortical bone. However, relative influences in calcium content, % osteon bone, and porosity explained only 60% of variance in energy absorption. They suggested that other histocompositional characteristics, which are not typically evaluated in such studies, might be influential in the degradation of this important mechanical property. Such variables may include variations in predominant collagen fiber orientation (CFO), collagen cross-linking, and osteocyte lacuna population density (OLPD).

Evaluation of these histocompositional parameters may provide a more complete description of variance in mechanical properties. For example, a previous study that investigated the relative influence of CFO on mechanical properties of cortical bone from the equine third metacarpal (MCIII) showed that variations in CFO strongly influence energy absorption in the physiologic context of strain-mode-specific testing (**S-M-S**; e.g., compression testing of bone from regions habitually loaded in compression; tension testing of bone from regions habitually loaded in tension) [2]. Evaluation of mechanical behavior in S-M-S testing is relevant since many limb bones receive habitual (physiologic) bending during typical loading conditions [3]. For example, such loading is considered prevalent in the human proximal femur and other human long bones.

This study examined the relative influences of CFO and other material characteristics on mechanical properties of cortical bone from an artiodactyl (deer) calcaneus, which is subject to a relatively simple loading regime (compared to the equine MCIII) [4,5]. This model has proved useful for evaluating relative influences of various histocompositional characteristics on the mechanical behavior of cortical bone [6]. Based on the high correlations found in the MCIII, similar findings in the calcanei would support the hypothesis that CFO has an important role in energy absorption. Furthermore, such findings would support the use of the calcaneus model for investigating fundamental issues in bone biomechanics and adaptation.

METHODS: 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. Specimens were tested unrestrained to failure in axial compression or tension (strain rate: 0.003 sec^{-1}) [7]. 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 (550°C), porosity, osteocyte lacuna population density (OLPD; no./mm^2 bone), fractional area of (secondary) osteonal bone (FASB), secondary osteon population density (OPD), and osteonal cross-sectional shape ("shape") and area (OA) [8,9]. Predominant CFO was evaluated using circularly polarized light and corresponding weighted mean graylevels, where darker graylevels represent more longitudinal collagen and brighter graylevels represent more oblique-to-transverse collagen [8]. The data were evaluated using Pearson's correlations (r), and multiple regression analyses (R^2 for cumulative variance).

RESULTS:

S-M-S caudal calcaneus (tension testing): CFO was *the most important explanatory variable in every mechanical parameter*, and explained between 28.5-47.7% of total variance (Table 1). R-values for these correlations exceeded |±0.350|, but none were significant at $p < 0.05$. TNRG, YS, and US showed trends with CFO ($0.05 < p < 0.09$).

S-M-S cranial calcaneus (compression testing): CFO was the *second most important explanatory variable* in YNRG, PNRG, EM, and US. YNRG, PNRG, TNRG and EM showed r-values that exceeded |±0.350|, but none of these correlations were significant at $p < 0.05$. OPD was the most important explanatory variable in energy absorption; porosity and osteon shape were most important in EM, YS, and US.

Caudal calcaneus in compression (Not S-M-S): CFO was the second most important explanatory variable in YNRG and TNRG. With the exception of EM, all parameters showed r-values that exceeded |±0.350|, but none were significant at $p < 0.05$. %Ash and OLPD were the most important explanatory variables in energy absorption data.

Cranial calcaneus in tension (Not S-M-S): CFO was the third most important explanatory variable in YNRG and TNRG. With the exception of EM, all parameters showed r-values that exceeded |±0.350|, but none were significant at $p < 0.05$. %Ash and osteon shape were the most important explanatory variables in energy absorption data.

	YNRG		PNRG		TNRG		EM		YS		US	
S-M-S cranial calcaneus in compression	OPD	87.5	OPD	69.8	OPD	53.3	Porosity	19.1	Shape	47.9	Shape	67.8
	CFO	89.0	CFO	81.5	Porosity	77.2	CFO	26.4	Porosity	49.8	CFO	85.6
	Shape	90.6	%Ash	99.8	%Ash	82.4	[illegible]	35.7	OPD	51.2	Porosity	89.4
	Total	90.6	Total	99.8	Total	82.4	Total	39.9	Total	51.8	Total	90.8
S-M-S caudal calcaneus in tension	CFO	28.5	CFO	43.8	CFO	47.7	CFO	35.9	CFO	45.6	CFO	46.6
	Porosity	53.9	Porosity	77.8	Porosity	76.1	FASB	63.1	OPD	66.6	Porosity	67.6
	OLPD	63.4	FASB	89.4	FASB	82.5	Shape	71.8	OA	86.3	FASB	72.5
	Total	94.3	Total	90.3	Total	82.9	Total	83.1	Total	91.0	Total	89.1

Table 1: Results of multiple regression analyses for S-M-S testing. The top 3 explaining variables and cumulative % variance are listed.

DISCUSSION: Predominant collagen fiber orientation (CFO) was influential in explaining energy absorption in both compression and tension strain-mode-specific (S-M-S) testing. These results are consistent with the findings of recent studies between "tension" and "compression" cortices of equine MCIIIs [2,10] and radii [11].

In a similar study, Riggs et al. [11] examined the relative importance of %ash, density, porosity, volume fraction of secondary bone, and CFO on the mechanical properties of equine radii. In tensile testing they showed that CFO was the most important explanatory variable in EM, US, and strain-to-failure. In the present study, *all* of the mechanical properties were most strongly influenced by CFO in the caudal ("tension") cortex in S-M-S tension testing. In contrast, in the cranial ("compression") cortex, porosity, OPD, and osteon cross-sectional shape were most important in S-M-S compression loading. Such regional adaptations, although conspicuously evident in the calcaneus, may also be present (but less obvious) in most long bones, since habitual bending during controlled ambulation is common [3]. Similar S-M-S CFO adaptations have been detected in various bone types including adult equine MCIIIs, ovine (e.g., sheep) radii, and human proximal femora [8,12,13].

Bone material heterogeneity can influence mechanical properties, especially in the context of fatigue resistance, toughness, and energy absorption. Variations in the material organization between the cranial and caudal cortices probably reflect significant differences in mechanical properties of bone in tension and compression [14]. Similar hypotheses are being investigated in the human femoral neck, which reputedly is a tension/compression (superior/inferior) environment. Calcanei of sheep and deer are being advanced as comparative models for examining histologic adaptation of cortical bone in the human femoral neck in the context of normal loading, intramedullary prosthetic loading, and the material changes associated with age-related fragility fractures.

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