

# Volume effects on yield strength of equine cortical bone

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## A B S T R A C T

Volume effects are a fundamental determinant of structural failure. A material exhibits a volume effect if its failure properties are dependent on the specimen volume. Many brittle ceramics exhibit volume effects due to loading a structure in the presence of “critical” flaws. The number of flaws, their locations, and the effect of stress field within the stressed volume play a role in determining the structure’s failure properties. Since real materials are imperfect, structures composed of large volumes of material have higher probabilities of containing a flaw than do small volumes. Consequently, large material volumes tend to fail at lower stresses compared to smaller volumes when tested under similar conditions. Volume effects documented in brittle ceramic and composite structures have been proposed to affect the mechanical properties of bone. We hypothesized that for cortical bone material, (1) small volumes have greater yield strengths than large volumes and (2) that compared to microstructural features, specimen volume was able to account for comparable amounts of variability in yield strength. In this investigation, waisted rectangular, equine third metacarpal diaphyseal specimens ( $n = 24$ ) with nominal cross sections of  $3 \times 4$  mm and gage lengths of either 10.5, 21, or 42 mm, were tested monotonically in tension to determine the effect of specimen volume on their yield strength. Yield strength was greatest in the smallest volume group compared to the largest volume group. Within each group of specimens the logarithm of yield strength was positively correlated with the cumulative failure probability, indicating that the data follow the two-parameter Weibull distribution. Additionally, log yield strength was negatively correlated with log volume, supporting the hypothesis that small stressed volumes of cortical bone possess greater yield strength than similarly tested large stressed volumes.

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## 1. Introduction

Bone is a dynamic tissue that accommodates changes in

its physiological and mechanical environments. Bones are subject to static and dynamic loads and incur various forms of damage that can contribute to monotonic or fatigue failure.

As bone is an imperfect material, flaws within material volumes can contribute to failure. Knowledge of bone failure mechanisms is essential for understanding the structure and function of skeletal tissue.

Cortical bone microstructure has been considered analogous to that of a fiber ceramic matrix composite with the osteons acting as the fibers within an interstitial matrix (Currey, 1964; Hogan, 1992; Buckwalter et al., 1995). Bone exhibits nonlinear stress–strain behavior under various loading conditions (Fondrk et al., 1988). This nonlinearity has been attributed to various damage mechanisms such as microcrack formation and extension, and progressive fiber failure (Zioupou et al., 1994; Reilly and Currey, 1999; Bigley et al., 2006). Material yield marks the onset of irrecoverable damage involving interactions of damage mechanisms with microstructural components. The complexity of these microstructural interactions extends beyond a single measure of material strength or toughness. Additional information is needed to more completely characterize the failure behavior of bone tissue.

### 1.1. Weibull statistical theory

A “critical” defect is defined as a flaw within a material that acts as a stress concentrator and crack initiation site, or aids in crack propagation. Such defects are by definition responsible for immediate or progressive failure of the material (Wisnom, 1999). Failure can be caused by a single critical defect or several small defects acting together to create a critical flaw. Due to the statistical nature of the occurrence and size of such defects in ceramic materials, many exhibit a volume effect (Wisnom, 1999; Rentzsch, 2003). If failure is initiated by a critical defect, and such defects occur randomly within a material, it follows that for a given stress, large volumes of a material will have higher failure probabilities than small volumes because they have a higher probability of possessing a critical defect (Hertzberg, 1996). The Weibull statistical approach enables failure characterization when defects can be assumed to be randomly distributed throughout the material (Weibull, 1951). Weibull theory can then be used to characterize the variability in strength associated with the size of a structure or test specimen (Wisnom, 1999; Cattell and Kibble, 2001). Although the Weibull distribution has a large range of applicability, to our knowledge, ductile materials such as metals are not normally modeled using the Weibull distribution.

In a two-parameter Weibull strength model, the failure probability,  $P$ , of a volume,  $V$ , of a material subject to a uniform stress field is given by:

$$P = 1 - \exp \left[ V \left( \frac{\sigma_y}{\sigma_0} \right)^{m_s} \right] \quad (1)$$

where  $\sigma_y$  is the yield strength,  $\sigma_0$  is a scale parameter representing the characteristic yield strength of a unit volume, and  $m_s$  is a shape parameter, or Weibull strength modulus (Weibull, 1951; Hertzberg, 1996; Wisnom, 1999; Cattell and Kibble, 2001). The strength modulus describes the failure probability distribution and strength variability among test specimens of the same volume tested under similar conditions. High values of  $m_s$  correspond to low variability in yield strength (Rentzsch, 2003).

### 1.2. Volume effects in bone

Weibull theory has been useful for studies of whole bone strength and failure characterization. Sadananda (1991) performed four-point bending tests on the coracoids, clavicles, and ribs of chickens. He described whole bone fracture for the different bones and reported that the coracoids had a distinct Weibull distribution when compared to the clavicles and ribs. The distribution distinction was attributed to microstructural differences in the load bearing components. This investigation demonstrated the versatility of the Weibull distribution in its ability to describe the mechanical effects of intrinsic microstructural differences. Furthermore, it was proposed that probabilistic analysis may be useful for the estimation of whole bone fracture risk.

Pithioux et al. (2004) applied the Weibull distribution to compact bone failure. Bone was three to four times more brittle under dynamic loading than under quasi-static loading. The Weibull strength modulus was lower for quasi-static ( $m_s = 5.77$ ) than dynamic ( $m_s = 7.31$ ) bovine cortical bone tests demonstrating that bone is more brittle under a dynamic load than under a quasi-static load. Weibull theory has also been used to model the role of volume effects in fatigue of cortical bone. Small volumes of cortical bone exhibit greater fatigue strengths and longer fatigue lives than large volumes (Taylor, 1998; Bigley et al., 2007).

We sought to more directly test for volume effects on cortical bone strength by testing specimens of different volumes. We hypothesized that the monotonic yield strength of compact, cortical bone specimens varies with volume in accordance with the Weibull theory. Specifically, smaller volumes will have greater yield strength when compared to larger stressed volumes of bone. Additionally, we hypothesized that the observed volume effect explains the variability of yield strength of equine cortical bone not accounted for by microstructural variables. Equine third metacarpal bone was studied because fatigue related injuries are of particular interest in Thoroughbred racehorses (Nunamaker et al., 1990).

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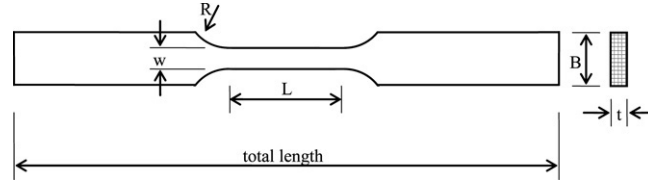
## 2. Methods

### 2.1. Experimental preparation

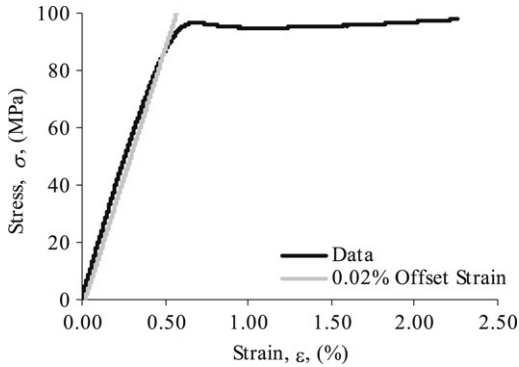
Twenty four third metacarpal (cannon) bone specimens from twelve necropsied Thoroughbred racehorses (3 females (F), 3 males (M), 6 castrated males (G); aged 3–7 years) were studied. Bone specimens were stored in sealed containers at  $-20^\circ\text{C}$  and hydrated with saline at room temperature during machining or testing.

Beams nominally  $140 \times 15 \times 6$  mm, were cut with a bone saw (Hobart Corp, Troy, OH) from the dorsal region of the mid-diaphysis of the left and right cannon bones. The beam’s long axis was aligned with the bone’s anatomical long axis. The width (15 mm) and thickness (6 mm) corresponded to the circumferential and radial anatomic directions, respectively.

Using a computer numerical control (CNC) mill (Prolight 1000, Light Machines Corporation, Manchester, NH) specimens were wet machined into rectangular beams to provide



**Fig. 1 – Diagram of tensile test specimen. All specimens had nominal dimensions of: Total length = 100 mm, R = 4.875 mm, w = 4 mm, B = 10 mm, and t = 3 mm. The gage length, L, was variable at L = 10.5, 21, or 42 mm.**



**Fig. 2 – Typical stress–strain curve with definitions of yield strength based on the 0.02% offset method.**

three different volume groups. The gage lengths of the rectangular waisted tensile test specimens had nominal volumes of 126, 252, or 504 mm<sup>3</sup> (Fig. 1). The machined specimens were then lightly polished with 800-grit carbide paper to remove surface artifacts from the machining process.

The specimens were tested in random order. Each specimen was thawed and placed in a beaker of calcium buffered saline, at 37 °C, where it was thermally equilibrated for 30 min, and then monotonically loaded while constantly irrigated with calcium buffered saline (Gustafson et al., 1996). Testing was performed using an MTS 810 servohydraulic testing machine (MTS Corporation, Eden Prairie, MN) running Testware SX software in accordance with the guidelines specified in the American Society for Testing and Materials Standard E8M-01.

All loading was conducted in displacement control at a strain rate of 0.005 s<sup>-1</sup> based on previously reported *in vivo* strain rates for the Thoroughbred racehorses (Nunamaker et al., 1990). Each specimen underwent ten preconditioning cycles (–0.1% to 0.1% strain) using a 2 Hz sinusoidal waveform. Strain was measured by a calibrated extensometer (suitable for the specimen's gage length: MTS models 632.26E–30, 632.26B–30, 632.12B–30) attached with elastic bands to the waisted region. Gage length extenders were used to ensure that strain was measured over the same relative proportion (73%) of the gage region for each volume group. Load was measured using a 2446 N (550 lb.) capacity load cell (MTS model 661.18C–02). The initial elastic modulus was obtained from linear regression between 0.8 MPa and 65% of the maximum stress of the stress–strain curve (Fig. 2). Yield stress and strain were determined using a 0.02% offset strain criterion.

## 2.2. Analysis of variance

A mixed model repeated measures analysis of variance (ANOVA) was performed on the yield strength,  $\sigma_y$ , using horse as a random repeated subject, age as a continuous variable, sex (F, M, G) and volume category ( $V_1$ ,  $V_2$ ,  $V_3$ ) as fixed three level factors, and elastic modulus as a covariate. Tukey pairwise comparisons were used to determine *post hoc* differences. Significant differences were reported when  $p < 0.05$  (SAS v9.1.3, Cary, NC).

## 2.3. Weibull analyses

The Weibull strength modulus,  $m_s$ , and characteristic yield strength,  $\sigma_0$ , were determined for the three different volume groups based on their respective failure probabilities. Within each group, yield strength was ranked from the least to greatest and the respective failure probabilities were calculated using the Bernard median rank (Wisnom, 1999; Cattell and Kibble, 2001). Median rank is an estimator of the true failure probability,  $P_i$ , for the  $i$ th ranked yield strength,  $\sigma_i$ , and is given by

$$P_i = \frac{i - 0.3}{n + 0.4} \quad (2)$$

where  $i$  is the rank of the specimen's yield strength, and  $n$  is the group sample size ( $n = 8$  for each volume group) (Cattell and Kibble, 2001). For each volume group, Eq. (1) can be rewritten in the linear form by taking the natural logarithm twice,

$$\ln \ln \left( \frac{1}{1 - P_i} \right) = m_s \ln(\sigma_i) - m_s \ln(\sigma_0) \quad (3)$$

where  $P_i$  is the failure probability,  $m_s$  is the Weibull strength modulus, and  $\sigma_0$  is the characteristic yield strength. Regression of the failure probability against the yield strength allows each volume's strength modulus and characteristic yield strength to be calculated (SAS v9.1.3, Cary, NC).

## 2.4. Combined volume effects analysis

For tensile specimens, the stress is nominally uniform over the entire gage length so Eq. (1) is applicable. Assuming equal material failure probabilities per unit volume, the volume effect on the yield strength ratio for specimens of volumes,  $V_A$  and  $V_B$  is

$$\frac{\sigma_A}{\sigma_B} = \left( \frac{V_B}{V_A} \right)^{1/m_V} \quad (4)$$

where  $\sigma_A$  and  $\sigma_B$  are the respective yield strengths, and  $m_V$  is the Weibull volume modulus (Wisnom, 1999; Cattell and Kibble, 2001). For geometrically similar specimens with

uniform stress distributions the volume effect on yield strength can be determined from a “log-log” plot of Eq. (4), where the slope is the negative reciprocal of the Weibull volume modulus,  $m_V$  (Wisnom, 1999; Cattell and Kibble, 2001). The distinction between strength modulus,  $m_s$ , and volume modulus,  $m_V$ , is in the manner of determination. The strength modulus is determined within individual volume groups while the volume modulus is determined across different volume groups. For a material that can be described by the Weibull distribution, the two modulus values should be similar because they are derived from the same distribution (Wisnom, 1999; Cattell and Kibble, 2001).

## 2.5. Histomorphometry

Transverse sections of the specimens were cut just proximal and distal to the fracture surface of each fragment using a low speed diamond saw (Isomet Buehler, Lake Bluff, IL). These sections ( $100 \pm 10$  micrometers thick) were subsequently mounted onto glass slides using Eukitt mounting media (Calibrated Instruments, Hawthorne, NY) and underwent histomorphometric analysis.

The histomorphometric analysis was conducted over the entire cross section (nominally  $3 \times 4$  mm) on both the proximal and distal section from each specimen. Images of the cross section were acquired using an Olympus BH2 microscope and CCD camera with an objective magnification of 10X. A  $3 \times 3$  grid, totaling nine images, spanned the entire cross section. This analysis area was defined as  $B.Ar = 2.6 \times 3.2 \text{ mm} = 8.32 \text{ mm}^2$ .

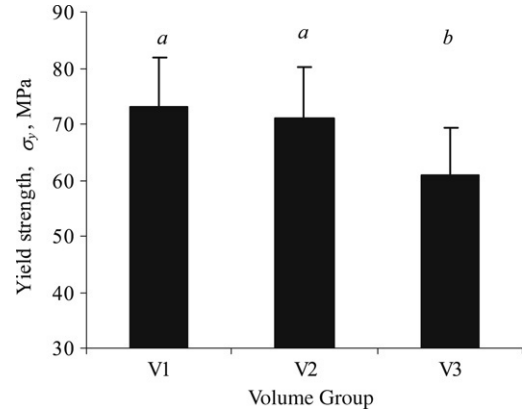
The equine specimens were highly remodeled, leaving very few primary osteons. Secondary osteons were identified and counted by the presence of a prominent cement line. Additionally, secondary osteons were counted as complete if they possessed three radii covering greater than 80% of the osteonal area. The number of osteons in each analysis area was counted and divided by the analysis area, B.Ar, to obtain the osteon density (On.Dn,  $\#/mm^2$ ).

$$On.Dn = \frac{N.On}{B.Ar}. \quad (5)$$

Two perpendicular cement line diameters were measured and averaged for six randomly selected osteons in each of the nine fields, resulting in a total of 54 osteon diameters per section. The osteon diameter (On.Dm, mm) for each specimen was defined as the average of the cement line diameters ( $54 \text{ diameters/section} * 2 \text{ sections/specimen} = 108 \text{ diameters}$ ) for each specimen.

Haversian canal diameters (H.Dm, mm) were measured, in the same manner as cement line diameter, for each of the six randomly selected osteons. Porous cavities (resorption cavities and Volkmann’s canals) were counted (N.Po) and measured using two perpendicular diameters (Po.Dm, mm) across each of the nine fields. Total porosity (PO, %) was defined as the combined Haversian canal area and porous cavity area divided by the total analysis area (B.Ar).

$$PO = \frac{\frac{\pi}{4} [N.Po (Po.Dm^2) + N.On (H.Dm^2)]}{B.Ar}. \quad (6)$$



**Fig. 3 – Least squares mean yield strength for each volume group adjusted for age, sex, and horse effects (mean  $\pm$  standard deviation). Different letters reflect significant differences ( $p < 0.05$ ).**

Univariate regression analyses (SAS v9.1.3, Cary, NC) were performed to determine whether histomorphometric parameters correlated with specimen volume or the measured mechanical properties, including elastic modulus, yield strength, and fatigue life. Additionally, a combination of forward and backward step analysis procedures were used to determine the best fitting multiple regression model, with the dependent variable being yield strength, and independent variables of specimen volume, elastic modulus, and the histomorphometric parameters. Statistical significance was reported for  $p < 0.05$ .

## 3. Results

### 3.1. Analysis of variance

The mixed model repeated measures analysis of variance resulted in significant effects of elastic modulus ( $p = 0.007$ ), and volume category ( $p = 0.002$ ) on yield strength,  $\sigma_y$ , while age ( $p = 0.613$ ), and sex ( $p = 0.699$ ) had nonsignificant effects. Subsequent *post hoc* comparisons showed that the largest volume,  $V_3$ , had a significantly lower mean yield strength,  $\sigma_y$ , than both the smallest volume,  $V_1$  ( $p = 0.001$ ) and the intermediate volume,  $V_2$  ( $p = 0.004$ ). Statistically significant differences did not exist between volume groups  $V_1$  and  $V_2$  ( $p = 0.435$ ). Yield strength and volume data are summarized in Table 1; while Fig. 3 presents yield strength across the groups,  $V_1$ ,  $V_2$ , and  $V_3$ .

### 3.2. Individual Weibull analyses

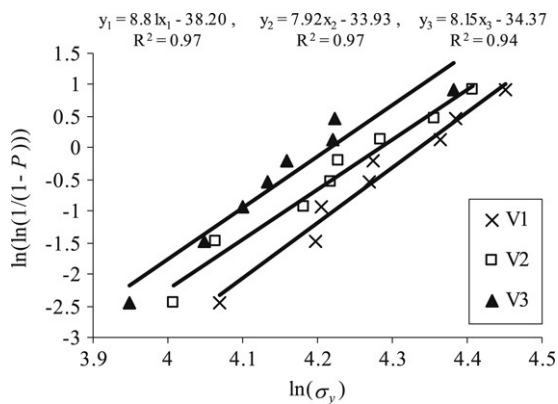
All three volume groups,  $V_1$ ,  $V_2$ , and  $V_3$ , had significant regressions of the transformed failure probability ( $P$ ) (Eq. (3)) against  $\ln(\sigma_y)$  ( $p < 0.001$ , Fig. 4), demonstrating that the two-parameter Weibull model is applicable for the analysis. Individual Weibull strength moduli,  $m_s$ , and characteristic yield strength,  $\sigma_0$ , were calculated for each group (Eq. (3), Table 1). The Weibull strength moduli were not different, (ANOVA,  $p = 0.701$ ), between the volume groups, as expected because the slopes are related to the specimens’ material failure properties.

**Table 1 Summary of physical, mechanical, and histomorphometric parameters for the 24 specimens (mean  $\pm$  standard deviation)**

Parameter	Specimen volume group		
	$V_1$	$V_2$	$V_3$
Age (years)	4.6 $\pm$ 1.2	4.3 $\pm$ 1.3	3.9 $\pm$ 0.8
Volume, $V$ , (mm <sup>3</sup> )	123.9 $\pm$ 1.8	238.2 $\pm$ 11.8	492.0 $\pm$ 14.2
Elastic modulus, $E$ , (MPa)	14 536 $\pm$ 1962	13 528 $\pm$ 2495	16 312 $\pm$ 2211
Yield strength, $\sigma_y$ , (MPa)	72.47 $\pm$ 8.73	68.49 $\pm$ 9.19	64.04 $\pm$ 8.43
Weibull strength modulus, $m_s$	8.81	7.92	8.15
95% confidence interval for $m_s$	(7.19, 10.43)	(6.55, 9.29)	(6.02, 10.28)
Characteristic yield strength, $\sigma_0$ , (MPa)	76.40	72.54	67.84
Osteon diameter, On.Dm, ( $\mu$ m)	167 $\pm$ 20	157 $\pm$ 15	159 $\pm$ 10
Porosity, PO, (%)	4.4 $\pm$ 2.1	4.1 $\pm$ 2.4	3.4 $\pm$ 1.0
Osteon density, On.Dn, (#/mm <sup>2</sup> )	24.5 $\pm$ 7.6	24.5 $\pm$ 6.5	23.0 $\pm$ 3.8

**Table 2 Regression model for predicting  $\log(\sigma_y)$  from  $\log(V)$  and  $\log(E)$  ( $R^2 = 0.45$ ,  $p = 0.002$ )**

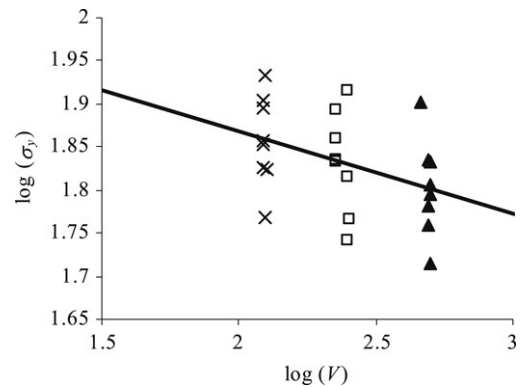
Variable	Parameter estimate	Standard error	$p$ value
Model	-	0.045	0.002
Intercept	0.267	0.562	0.639
$\log(V)$	-0.135	0.040	0.003
$\log(E)$	0.453	0.140	0.004



**Fig. 4 – Weibull probability plot for each volume group. Slopes are the Weibull strength modulus,  $m_s$ . Intercepts on  $\ln(\sigma_y)$  axis are used to determine  $\sigma_0$ .**

### 3.3. Combined volume effects analysis

Transformed yield strength,  $\log(\sigma_y)$ , was significantly dependent on  $\log(V)$  (Fig. 5, linear regression for all specimens,  $p = 0.046$ ,  $R^2 = 0.17$ ). The correlation was negative, demonstrating that yield strength decreases with increasing volume. Additionally, multiple linear regression was performed on the transformed yield strength with respect to the transformations of both volumes,  $\log(V)$ , and elastic modulus,  $\log(E)$ . The multiple regression was also statistically significant (Table 2, multiple linear regression,  $p = 0.002$ ,  $R^2 = 0.45$ ) with significant coefficients of  $-0.135$  for  $\log(V)$  and  $0.453$  for  $\log(E)$ . Accounting for the effect of elastic modulus, the Weibull volume modulus,  $m_V = 7.40$ , fell within the 95% confidence intervals of all three strength moduli ( $m_s$ ) obtained from the individual analyses (Table 1) (Cattell and Kibble, 2001).



**Fig. 5 – Volume effects on yield strength ( $p = 0.046$ ,  $R^2 = 0.17$ ).**

### 3.4. Histomorphometry

The summarized histomorphometric parameters for the fatigue data set are presented in Table 1. As expected, no significant correlations were detected between specimen volume measures and the histomorphometric parameters. Regression analyses between the histomorphometric parameters and the mechanical properties of elastic modulus,  $E$ , and yield strength,  $\sigma_y$ , exhibited four significant results (Table 3). Porosity was negatively correlated with elastic modulus ( $p = 0.022$ ,  $R^2 = 0.22$ , Fig. 6), but had no significant correlation with yield strength ( $p = 0.094$ ,  $R^2 = 0.12$ ). Osteon diameter was positively correlated with yield strength ( $p = 0.002$ ,  $R^2 = 0.37$ , Fig. 7) but had no significant correlation with elastic modulus ( $p = 0.236$ ,  $R^2 = 0.06$ ). Elastic modulus was positively correlated with yield strength ( $p < 0.050$ ,  $R^2 = 0.16$ , Fig. 8), and had no significant correlation with volume ( $p = 0.073$ ,  $R^2 = 0.14$ ). There was no significant correlation between osteon density and either elastic modulus ( $p = 0.415$ ,  $R^2 = 0.03$ ) or yield strength ( $p = 0.305$ ,  $R^2 = 0.05$ ).

Multiple linear regression demonstrated that yield strength was significantly predicted by osteon diameter ( $p = 0.019$ ), elastic modulus ( $p = 0.004$ ), specimen volume ( $p = 0.003$ ). Osteon density and porosity did not meet the model selection criteria. Regression model coefficients, and partial  $R^2$  values, for predicting yield strength from histomorphometric parameters, elastic modulus, and specimen volume are given in Table 4.

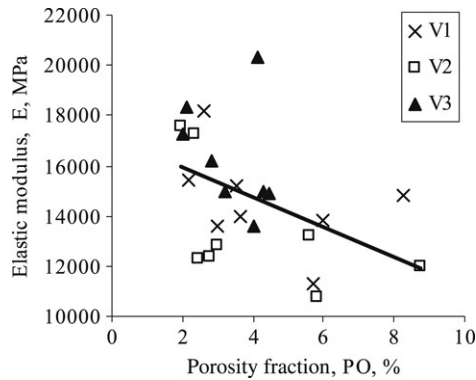
**Table 3 Summary of regression analyses between specimen volume and histomorphometric parameters for the 24 specimens**

Regression parameters	Volume		Elastic modulus		Yield strength	
	<i>p</i>	<i>R</i> <sup>2</sup>	<i>p</i>	<i>R</i> <sup>2</sup>	<i>p</i>	<i>R</i> <sup>2</sup>
Osteon size, On.Dm	0.325	0.04	0.236	0.06	<b>0.002</b>	<b>0.37</b>
Porosity fraction, PO	0.336	0.04	<b>0.022</b>	<b>0.22</b>	0.094	0.12
Osteon density, On.Dn	0.626	0.01	0.415	0.03	0.305	0.05
Volume, V	–	–	0.073	0.14	<b>0.048</b>	<b>0.17</b>
Elastic modulus, E	–	–	–	–	<b>&lt;0.050</b>	<b>0.16</b>

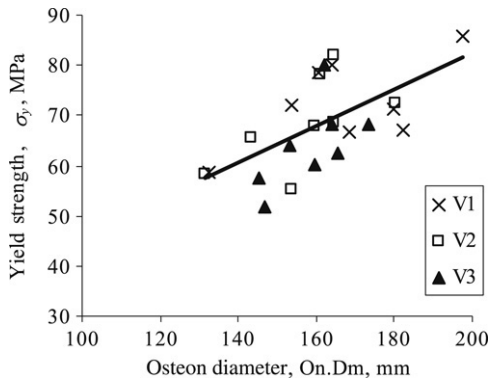
Bold values indicate statistically significant (*p* < 0.05) correlations between the histomorphometric parameter and the mechanical property.

**Table 4 Multiple regression model for predicting yield strength from histomorphometric parameters, specimen volume, and elastic modulus (*R*<sup>2</sup> = 0.64, *p* < 0.001)**

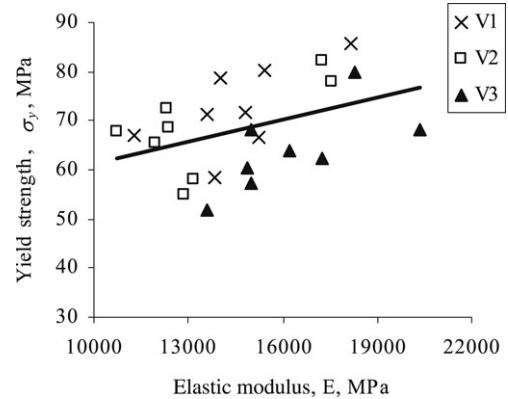
Variable	Parameter estimate	Standard error	<i>p</i> value	Partial <i>R</i> <sup>2</sup>
Intercept	13.2901	13.9898	0.354	–
Osteon diameter, On.Dm	0.2219	0.0872	0.019	0.37
Elastic modulus, E	0.0019	0.0006	0.004	0.19
Specimen volume, V	–0.0298	0.0089	0.003	0.08



**Fig. 6 – Linear regression results of elastic modulus vs. porosity for the 24 specimens. Regression parameters are slope: –601.7, intercept: 17, 164 MPa, *p* = 0.022, *R*<sup>2</sup> = 0.22.**



**Fig. 7 – Linear regression results of yield strength vs. osteon diameter for the 24 specimens. Regression parameters are slope: 0.361, intercept: 10.2 MPa, *p* = 0.002, *R*<sup>2</sup> = 0.37.**



**Fig. 8 – Linear regression results of yield strength vs. elastic modulus for the 24 specimens. Regression parameters are slope: 0.002, intercept: 45.9 MPa, *p* < 0.050, *R*<sup>2</sup> = 0.16.**

#### 4. Discussion

Stress fractures are an important clinical consequence of excessive fatigue damage in bone. Thoroughbred racehorses are liable to suffer these bone fatigue injuries during their training regime (Nunamaker et al., 1990). These failures can occur when the material is repetitively loaded to, or below, the respective yield point. Detailed knowledge of yield point behavior may contribute to a better understanding of the failure mechanisms of bone. Although not completely understood, variations in the microstructure seem to play a significant role in these mechanical processes and those concerned with fracture (Schaffler et al., 1987, 1995; Nalla et al., 2003; Currey, 2004; Wang and Puram, 2004). High yield strength and low post-yield deformation have been correlated with high elastic modulus and mineral content, respectively (Currey, 2004). The higher these values, the more the bone tissue behaves like a brittle ceramic.

Initiation of microscopic mechanical damage in the form of microcracks and diffuse tissue damage has been observed between 0.4% and 0.5% tensile strain (Schaffler et al., 1995; Boyce et al., 1998; Reilly and Currey, 1999). It has been suggested that the interactions of such damage causes bone fatigue strength to exhibit a volume effect (Taylor, 1998). In the present investigation, uniaxial monotonic tests were conducted on waisted rectangular specimens from equine cortical bone. The hypothesis that small volumes of cortical bone exhibit greater yield strengths than similarly tested

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large volumes was supported by three different statistical analyses.

Mixed model repeated measures analysis of variance supported the hypothesis that, when variations in elastic modulus are considered, a larger specimen volume resulted in lower yield strength for similarly tested specimens, consistent with the presence of a volume effect. Post hoc Tukey comparisons demonstrated statistically significant differences between both the largest and smallest, and the largest and mid-sized volume groups.

Regressions of failure probability on yield strength for each volume group are consistent with a two-parameter Weibull distribution. Additionally, the similarity in Weibull moduli across the individual volume groups suggests that the bone material in all three groups yielded in a similar manner (Wisnom, 1999; Cattell and Kibble, 2001). If different flaw populations were contributing to yield in each of the different volume groups, or different test parameters (e.g. material types, strain rate, or loading mode) were being used, the individual volume groups would be expected to exhibit different Weibull moduli, but they did not.

The regression between the logarithm of yield strength and the logarithm of volume confirmed a significant volume effect on the yield strength. The Weibull modulus, or slope, derived from tests involving similar stress states, characterizes the variation in yield strength of a particular material independent of its volume. When variability in elastic modulus was accounted for via multiple regression, the similarity of the Weibull volume modulus ( $m_V = 7.40$ ) to the individual moduli from the independent Weibull analyses ( $m_{s1} = 8.81$ ,  $m_{s2} = 7.92$ ,  $m_{s3} = 8.15$ ) supports the hypothesis that the latter moduli are independent of volume, which in turn implies a definitive volume effect on yield strength for this material (Wisnom, 1999; Cattell and Kibble, 2001).

Microstructural features have been demonstrated to affect the mechanical properties of cortical bone (Gibson et al., 1995; Martin et al., 1996; Fyhrie and Vashishth, 2000; Gibeling et al., 2001; Skedros et al., 2003; Bigley et al., 2006). The mechanical performance of cortical tissue is thought to be optimized by adaptations in the various microstructural features (Gibson et al., 2006). The current histomorphometric analysis represents relevant microstructural features. In this study we hypothesized that the observed volume effect explains the variability in yield strength of equine cortical bone not accounted for by microstructural variables. This hypothesis was confirmed using univariate and multiple regression analyses between the microstructural features and the mechanical properties.

Several points must be considered when interpreting these results. The specimens came from a sample of twelve horses. There may have been differences from horse to horse not accounted for by elastic modulus, age, gender or other differences with any certainty. The application of the Weibull theory to bone specimens has limitations. For a material having inhomogeneities of dimensions approaching the size of the test specimen a characteristic volume must be present, however in its power law form (Eq. (1)), no characteristic material volume is identifiable (Bazant and Zdenek, 2005). For this reason the Weibull theory is only applicable when these microstructures are sufficiently small compared to the

test specimen size (Choi and Goldstein, 1992; Taylor et al., 1999; Bazant and Zdenek, 2005). Additionally, Weibull theory describes brittle material failure in which a microscopic crack grows to macroscopic size. It does not account for local stress redistributions, or energy dissipation mechanisms associated with crack propagation in quasi-brittle materials (Bazant and Zdenek, 2005). The incorporation of microstructural energy release, stress redistribution, and load sharing mechanisms requires more advanced size effect analyses (Bazant and Zdenek, 2005). The Weibull theory is empirically based and is capable of modeling failure for a number of materials. To that end, its wide applicability should be used with caution when analyzing evolutionary data. Otherwise, however, the present data are consistent with the Weibull theory and support the existence of volume effects in cortical bone for specimens substantially larger than osteons and other material inhomogeneities.

Over the course of daily activities, bones routinely accumulate damage due to fatigue loading. This damage has been associated with the activation of remodeling responses that are apparently the only means for its removal (Mori and Burr, 1993; Martin, 2002). Targeted remodeling responses address the damage problem without increasing the overall bone volume, thereby avoiding the metabolic costs of added bone mass (Martin, 2003; Daly et al., 2004). The present results demonstrate that small volumes of bone have greater yield strengths than large volumes, establishing the additional benefit of a light, metabolically less expensive skeleton.

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## 5. Conclusions

1. The individual yield strength data for each volume tested follow the two-parameter Weibull distribution. Each distribution was characterized by a similar Weibull modulus.
2. Log yield strength was negatively correlated with log volume, supporting the hypothesis that small stressed volumes of cortical bone possess greater yield strength than similarly tested large stressed volumes.
3. The observed volume effect explained the variability in yield strength of equine cortical bone not accounted for by microstructural variables. For this reason it becomes difficult to compare strengths between specimens of different volumes without considering the effect of stressed volume.

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