Mechanical and morphological properties of trabecular bone samples obtained from third metacarpal bones of cadavers of horses with a bone fragility syndrome and horses unaffected by that syndrome

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Objective—To determine morphological and mechanical properties of trabecular bone of horses with a bone fragility syndrome (BFS; including silicate-associated osteoporosis).

Sample—Cylindrical trabecular bone samples from the distal aspects of cadaveric third metacarpal bones of 39 horses (19 horses with a BFS [BFS bone samples] and 20 horses without a BFS [control bone samples]).

Procedures—Bone samples were imaged via micro-CT for determination of bone volume fraction; apparent and mean mineralized bone densities; and trabecular number, thickness, and separation. Bone samples were compressed to failure for determination of apparent elastic modulus and stresses, strains, and strain energy densities for yield, ultimate, and failure loads. Effects of BFS and age of horses on variables were determined.

Results—BFS bone samples had 25% lower bone volume fraction, 28% lower apparent density, 18% lower trabecular number and thickness, and 16% greater trabecular separation versus control bone samples. The BFS bone samples had 22% lower apparent modulus and 32% to 33% lower stresses, 10% to 18% lower strains, and 41% to 52% lower strain energy densities at yield, ultimate, and failure loads, compared with control bone samples. Differences between groups of bone samples were not detected for mean mineral density and trabecular anisotropy.

Conclusions and Clinical Relevance—Results suggested that horses with a BFS had osteopenia and compromised trabecular bone function, consistent with bone deformation and pathological fractures that develop in affected horses. Effects of this BFS may be systemic, and bones other than those that are clinically affected had changes in morphological and mechanical properties. (*Am J Vet Res* 2012;73:1742–1751)

Horses that have a BFS (including SAO [ie, osteoporosis associated with pulmonary silicosis]) may have deformities in multiple bones (eg, scapulae and pelvic bones), osteoarthrosis of cervical vertebrae, non-displaced rib fractures, or complete displaced fractures of bones (eg, pelvic bones).[1–3] Clinically affected horses most commonly develop nonspecific lameness, deformities (ie, bowing) of scapulae, and, in severely affected horses, lordosis.[3] Treatment is primarily palliative, and the most severely affected horses die or are euthanized for humane reasons because of catastrophic fractures. The etiology of this BFS is unknown; however, most affected horses have concurrent pulmonary silicosis associated with a history of exposure to soil containing silicon dioxide, which is cytotoxic.[3]

Bone tissue from horses with severe SAO is qualitatively characterized by a mosaic of reversal lines and large osteoclasts with high numbers of nuclei.[2] To the

Abbreviations

AD	Apparent density
BFS	Bone fragility syndrome
SAO	Silicate-associated osteoporosis
SED	Strain energy density

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authors' knowledge, the morphological and functional effects of such characteristics on the biological and mechanical properties of bone are unknown. The purpose of the study reported here was to determine the effects of a BFS on morphological and mechanical variables of trabecular bone tissue samples collected from bones (ie, third metacarpal bones) that do not typically develop clinically detectable abnormalities in affected horses. We hypothesized that the morphological and mechanical properties of trabecular bone samples collected from third metacarpal bones of cadavers of horses with a BFS would be different than those of trabecular bone samples obtained from cadavers of horses without a BFS and that affected horses would have weaker trabecular bone versus unaffected horses. In addition, we hypothesized that differences in bone tissue strength would be related to differences in the quantity and amount of bone mineralization. Findings of the study reported here were expected to enhance understanding of the pathogenesis of BFS.

Materials and Methods

Samples—Bone samples of cadavers of 19 horses (median age, 15 years; age range, 4 to 29 years; 9 sexually intact females, 5 sexually intact males, and 5 castrated males; various breeds) with a BFS diagnosed via bone scintigraphy or necropsy[1,2] were included in the study. Sixteen of these horses had SAO as determined on the basis of histopathologic evidence of granulomatous fibrosing bronchiolitis with particulates in macrophages.[2] All horses with this BFS had a skeletal deformity or lameness and were euthanized or died as a result of the condition. Bone samples of cadavers of 21 horses (median age, 14 years; age range, 2 to 28 years; 6 sexually intact females, 4 sexually intact males, and 11 castrated males; various breeds including 4 Thoroughbreds) without a BFS were included in the study; these horses had been necropsied by personnel of the California Animal Health and Food Safety Laboratory System and the Veterinary Medical Teaching Hospital, University of California-Davis. Horses without a BFS had been euthanized or died as a result of colic (n = 4 horses), renal disease (1), neoplasia (3), athletic repetitive overuse injury (carpal fracture, tibial fracture, metacarpophalangeal luxation, or unknown musculoskeletal injury; 4), undiagnosed illness (3), or unknown causes (6). Clinical records of horses without a BFS were reviewed, and information regarding previous athletic use or workload that could affect mechanical or morphological properties of bone was recorded. Four of the horses without a BFS (the 4 Thoroughbreds) had been in race training prior to euthanasia. Six of the horses without a BFS had a history of a mild workload (pleasure or trail riding) or paddock confinement. Previous athletic use or workload was unknown for 11 of the horses that did not have a BFS.

Because clinically detectable bone deformities typically develop in the axial portion of the skeleton and proximal aspects of the appendicular portion of the skeleton in horses with a BFS, bones in these regions of severely affected horses are likely to have pathological fractures and bone remodeling associated with fracture repair. Therefore, trabecular bone samples were obtained from third metacarpal bones of horses because such bones were likely to have changes in morphological and mechanical properties attributable to the primary effects of this BFS, rather than effects of fractures or bone remodeling. One trabecular third metacarpal bone tissue sample was obtained from the left forelimb of each of 18 horses with a BFS and all 21 of the horses without a BFS; 1 trabecular bone sample was obtained from the right forelimb of one of the horses with a BFS. Because 1 bone sample was obtained from the right forelimb of 1 horse with a BFS, interlimb variability of variables was assessed via evaluation of samples from contralateral (right) forelimbs of 8 horses without a BFS.

Bone core samples were obtained from third metacarpal bones of horses with a BFS (including horses with SAO; BFS bone core samples) and horses without a BFS (control bone core samples). A 25-mm-thick transverse section of the distal aspect of each metacarpal bone was prepared with a band saw[a] and jig; the

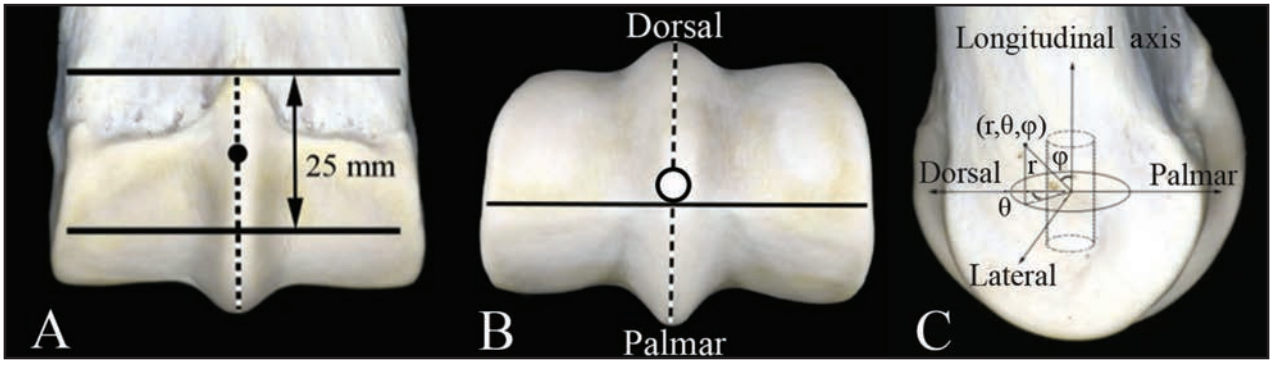

Figure 1—Illustrations depicting anatomic landmarks for collection of samples of third metacarpal bones of cadavers of horses with or without a BFS. A—View of the palmar aspect of a third metacarpal bone. Transverse sections (25 mm thick) of bone were obtained by use of a band saw. Proximal and distal limits of the transverse sections are indicated by the solid horizontal lines. The center (black dot) of each bone section was located 70% of the distance from the most distal point of the midsagittal ridge to the proximopalmar margin of the midsagittal ridge (dashed line). B—View of the distal articular surface of a third metacarpal bone. Bone core samples (6 mm in diameter) were collected from transverse sections of bone at a location (circle) dorsal to the transverse ridge (solid line) along the line of the midsagittal ridge (dashed line). C—View of the lateral aspect of a metacarpal bone. The location of the core bone sample (cylinder) within the third metacarpal bone and a 3-D coordinate system are indicated. The direction of the preferred trabecular orientation vector (r) is indicated by the midsagittal offset angle (**θ** [positive in the lateral direction from the dorsal aspect and negative in the medial direction from the dorsal aspect]) and the longitudinal offset angle (**ϕ**).

location and orientation of transverse sections of bone were standardized with respect to anatomic landmarks (**Figure 1**). For each third metacarpal bone, the apex of the midsagittal ridge of the distal aspect of the bone was marked with indelible ink (from the proximodorsal margin to the proximopalmar margin). The transverse ridge was marked with indelible ink (from the medial border to the lateral border). Because third metacarpal bones were of various sizes, the center of each transverse section was located 70% of the distance from the most distal aspect of the midsagittal ridge to the proximopalmar margin of the midsagittal ridge; this method was intended to standardize the site from which transverse sections of bone were collected.

A longitudinally oriented cylinder of bone (bone core) was obtained from each transverse section of bone. These bone core samples were obtained with a 6-mm-diameter diamond-tipped coring bit[b] mounted on a drill press[c] operating at 760 revolutions/min. During drilling, bone samples were continuously irrigated with saline (0.9% NaCl) solution and drilling was performed for brief, intermittent periods to ensure bone core samples remained cool. Each bone core sample was obtained from a location in the transverse bone section that was dorsal to the transverse ridge of the third metacarpal bone (Figure 1); this was intended to avoid obtaining bone core samples from regions predisposed to osteochondrosis (ie, the palmar aspect of the condyles of third metacarpal bones).

To identify the orientation of each bone core sample relative to third metacarpal bones (Figure 1), the distolateral surface of each sample was marked with indelible ink prior to collection. The distal and proximal aspects of each bone core sample were removed with a rotating saw that had 2 parallel blades spaced 15.0 mm apart[d] to achieve a length-to-diameter ratio of 2.5.[4] By use of a micrometer, 3 length and diameter measurements were determined for each bone core sample, and the mean of each of these variables was calculated prior to testing. Bone core samples were wrapped in paper moistened with calcium-buffered saline (0.9% NaCl) solution[5] and stored in sealed plastic bags at –20°C until testing; this was intended to maintain hydration, prevent mineral dissolution, and preserve mechanical properties of samples.

High-detail radiographic images of each bone core sample were obtained for evaluation of sample integrity. Radiography (35 kVp; 3 mA; duration, 2 minutes; focal film distance, 46 inches) was performed with a cabinet x-ray unit[e] and mammography film.[f] Radiography revealed a defect in 1 control bone core sample; that sample was not tested. Results were reported for 19 BFS and 20 control bone core samples.

Morphologic analysis—Bone core samples were thawed, and the central portion of each sample was imaged (beginning 4.5 mm distal to the proximal aspect of the sample and extending distally for 5.568 mm) with a high-resolution micro-CT scanner[g] (70 kVp; 114 μA; mean of 3 images with a 300-millisecond integration time). Micro-CT images of each bone core sample comprised 6-μm voxels. Serial tomograms were created by use of raw data via a cone beam–filtered projection algorithm.[6] To exclude data for debris that had been deposited on the periphery of bone core samples during collection, data for a portion of the sample (diameter, 3.744 mm; area, 0.1101 cm^2) were used for calculation of morphological variables (**Figure 2**).

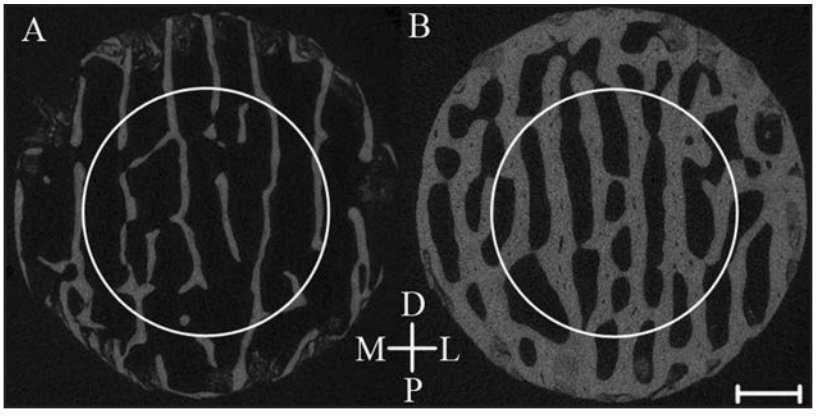

Figure 2—Representative cross-sectional micro-CT images of a bone core sample obtained from a distal third metacarpal bone of a cadaver of horse with a BFS (A) and a horse without a BFS (B). White circles indicate the regions that were used for calculation of morphological variables. The cross indicates anatomic orientation (dorsal [D], palmar [P], medial [M], and lateral [L]) of bone samples. Bar = 1 mm.

Table 1—Mean ± SD values of morphological variables of bone core samples obtained from third metacarpal bones of cadavers of 19 horses with a BFS and 20 horses without a BFS (control samples).

Variable	BFS	Control	*P* value
Bone volume fraction	0.27 ± 0.06	0.36 ± 0.12	< 0.001
AD (mg of hydroxyapatite/mL)	269 ± 65	372 ± 119	< 0.001
Mean mineralized bone density (mg of hydroxyapatite/mL)	923 ± 24	923 ± 24	0.500
Trabecular number	1.8 ± 0.3	2.2 ± 0.6	< 0.001
Trabecular thickness (mm)	0.14 ± 0.02	0.17 ± 0.03	< 0.001
Trabecular separation (mm)	0.59 ± 0.11	0.51 ± 0.11	0.030
Connectivity density	12.8 ± 5.5	12.5 ± 5.5	0.595
Degree of anisotropy	2.37 ± 0.33	2.39 ± 0.29	0.072
Preferred orientation vector (mm)	0.76 ± 0.15	0.72 ± 0.11	0.831
θ (°)	5.0 ± 30.5	5.3 ± 20.9	0.345
φ (°)	8.6 ± 7.7	8.0 ± 4.3	0.554

P values are results for effects of BFS in a complete ANOVA model (which included BFS status, age, and the interaction of age × BFS status). θ = Midsagittal offset angle (relative to the dorsal midline aspect of bone samples). φ = Longitudinal offset angle (relative to the longitudinal aspect of bone samples).

Gray values in micro-CT images were converted to mineral density values (mg of hydroxyapatite/mL) by means of a multistandard hydroxyapatite calibration phantom. Proper calibration of the micro-CT machine had been confirmed prior to the start of the study. Values of morphological variables were determined with software.[g] The AD was calculated as the total mineral content of the volume of interest of a bone core sample divided by the total volume of that sample (Figure 2). A threshold value of 650 mg of hydroxyapatite/mL was used to distinguish mineralized tissue (ie, bone; ≥ 650 mg of hydroxyapatite/mL) from nonmineralized tissue (< 650 mg of hydroxyapatite/mL) in the bone core samples. Bone volume fraction was calculated as the number of voxels identified as mineralized bone (bone volume) divided by the total volume of a bone core sample. Mean density of mineralized bone was calculated as the mean density of the voxels identified as mineralized bone. For each bone core sample, the number of trabeculae, mean trabecular thickness, and mean trabecular separation were calculated via direct morphometric analysis.[7] Connectivity density was calculated by dividing the connectivity value by the total bone core sample volume, where the connectivity value is the maximum number of trabeculae that could be broken before the sample was separated into 2 parts.[8] Anisotropy of each bone core sample was evaluated via the mean intercept length method.[9] Briefly, the value of the longest vector was divided by the value of the shortest vector. Isotropic bone has a degree of anisotropy equal to 1. Bone is anisotropic when the degree of anisotropy is > 1. The preferred trabecular orientation is the direction of the longest vector. Coordinate values pertaining to the longest vector were used to calculate the offset of the preferred orientation relative to the longitudinal axis and the dorsal aspect of the sagittal plane of the third metacarpal bone (Figure 1). Resulting vectors were plotted in a 3-D space. To facilitate statistical analysis of longitudinal offsets, vectors within the distal 4 quadrants (dorsodistolateral, dorsodistomedial, palmarodistolateral, and palmarodistomedial) were plotted as negative vectors in the proximal 4 quadrants (dorsoproximolateral, dorsoproximomedial, palmaroproximolateral, and palmaroproximomedial). Data for the palmar quadrants (palmaroproximolateral and palmaroproximomedial) were transferred to the opposite (ie, dorsal) quadrants (dorsoproximolateral and dorsoproximomedial) for statistical evaluation of the mediolateral offset with respect to the dorsal aspect of the sagittal plane.

Mechanical testing—Prior to mechanical testing, bone core samples were thawed to room temperature (approx 25°C). Bone core sample orientation (distolateral aspect oriented up or down and north, south, east or west) within the test platens and the order in which samples were tested were determined with a software-based randomization procedure.[h] Milled aluminum end caps were bonded[i] to the proximal and distal ends of the bone core samples to mitigate artifacts associated with loading of samples attributable to abnormally unconstrained trabeculae at the ends of samples.[10] Each bone core sample was compressed in a single load to failure at an apparent strain rate of 0.01 seconds^{-1} via a servohydraulic materials testing system.[j] Load[k] and actuator displacement data were sampled at a rate of

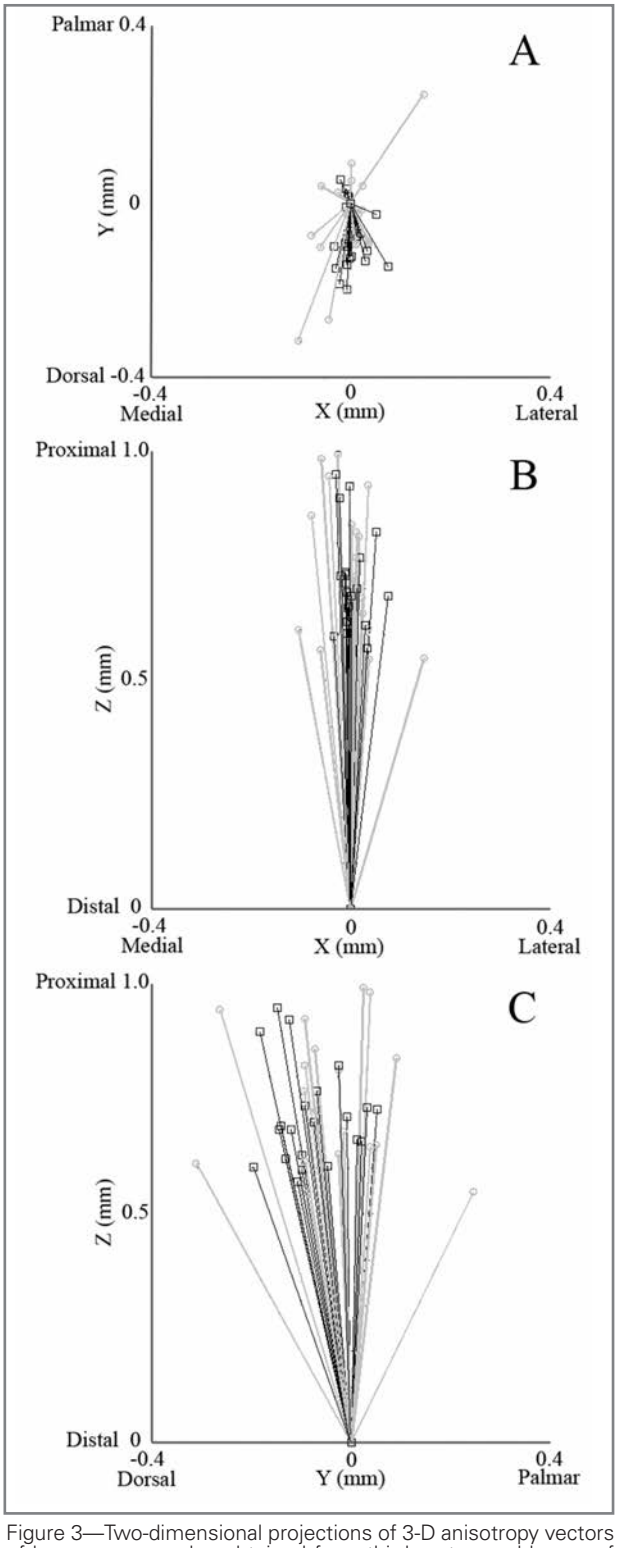

Figure 3—Two-dimensional projections of 3-D anisotropy vectors of bone core samples obtained from third metacarpal bones of cadavers of 19 horses with a BFS (gray lines and circles) and 20 horses without a BFS (black lines and squares) in X- and Y- (A), X- and Z- (B), and Y- and Z- (C) axes. Numbers on axes are the distance of anisotropy vectors (in millimeters) from the middle plane of bone core samples (0 mm) in palmar or dorsal, proximal or distal, and medial or lateral directions.

100 Hz during mechanical testing and were smoothed with a 5-point moving mean.

Several mechanical properties were calculated with a custom software program.[l] Apparent stress and strain were calculated via normalization of load and displacement data to cross-sectional area and original length of bone core samples, respectively. Compressive modulus; yield, ultimate, and failure stresses; yield, ultimate, and failure strains; and yield, ultimate, and failure SEDs were determined via evaluation of stress-strain curves for each bone core sample tested. Ultimate stress and strain were determined at the point of maximum stress. The modulus was defined as the slope of the stress-strain curve from 35% to 65% of the maximum stress. The yield point was defined as the point at which the stress-strain curve diverged from a linear line by a 0.2% strain offset.[11] Failure point (after ultimate load) was defined as the point at which load was 85% of the maximum load. All stress-strain curves had a toe region that was considered an artifact.[12] Therefore, origins of coordinates of data were moved to the strain-axis intercept of the linear regression line used to calculate modulus. Strain energy density was calculated as the area under the stress-strain curve, not including the toe region.

Statistical analysis—Wilcoxon signed rank tests were performed to compare data for bone core samples obtained from the right versus left forelimb and data for the toe regions of stress-strain curves for BFS versus control bone core samples. The effects of BFS status (BFS vs control bone core samples), age (ie, age of horse from which bone sample was obtained; continuous variable), and the interaction of BFS status and age on mechanical and morphological variables were determined via ANOVA.[m] Normality of data was assessed via evaluation of normal probability plots of ANOVA residuals; few mild deviations of data from normality were detected. For each pair of bone core samples obtained from right and left forelimbs of the same horse, only data for one of the bone core samples were included in ANOVA analyses. Values of $P \leq 0.05$ were considered significant for all statistical analyses.

Simple linear regression analyses were performed to determine relationships between mechanical and morphological variables because morphological variables that can be assessed in vivo may be useful for estimating fracture risk. Linear regression relationships for BFS bone core samples were compared with those for control bone core samples via a likelihood ratio test:

$$F = \frac{\frac{RSS_{Total} - (RSS_{BFS} + RSS_{Control})}{\Delta p}}{\frac{RSS_{BFS} + RSS_{Control}}{dfe_{BFS} + dfe_{Control}}}$$

where F is the likelihood ratio, RSS_{Total} is the total residual sum of squares, RSS_{BFS} is the residual sum of squares for BFS bone core samples, $RSS_{Control}$ is the residual sum of squares for control bone core samples, Δp is the difference in the number of estimated parameters between regressions of data for all samples and regressions of data from BFS versus control samples, dfe_{BFS} is the error degrees of freedom (total degrees of freedom – model degrees of freedom) for BFS bone core samples, and $dfe_{Control}$ is the error degrees of freedom for control bone core samples.

Results

Interlimb comparison—Comparison of values of mechanical and morphological variables for control bone core samples obtained from right versus left forelimbs was performed via a Wilcoxon signed rank test. No significant differences were detected between right forelimb and left forelimb control bone core samples for any mechanical or morphological variable.

Morphological properties—Differences were detected between values of morphological variables for BFS bone core samples and those for control bone core samples (**Table 1**). Mean bone volume fraction and AD were significantly ($P < 0.001$) lower (25% and 28% lower, respectively) in BFS bone core samples versus control bone core samples. Mean trabecular number and thickness were significantly ($P < 0.001$) lower (18% lower) in BFS bone core samples versus control bone core samples. Trabecular separation was significantly ($P = 0.030$) higher (16% higher) in BFS bone core samples versus control bone core samples. Connectivity density and mean mineralized bone density

Table 2—Analysis of variance model results for effects of BFS status, age of horse, the interaction of age X BFS status, and a complete model (including BFS status, age, and interaction of age X BFS status) for morphological variables of bone core samples obtained from third metacarpal bones of cadavers of 19 horses with a BFS and 20 horses without a BFS.

	BFS status		Age		Age X BFS status interaction		Complete model	
Variable	R^2	***P* value**	R^2	***P* value**	R^2	***P* value**	R^2	***P* value**
Bone volume fraction*	0.23	0.002	0.31	< 0.001	0.39	0.020	0.61	< 0.001
AD (mg of hydroxyapatite/mL)*	0.23	0.002	0.27	< 0.001	0.36	< 0.001	0.57	< 0.001
Mean mineralized bone density (mg of hydroxyapatite/mL)	0.00	0.937	0.04	0.224	0.05	0.442	0.05	0.557
Trabecular number*	0.15	0.014	0.16	0.012	0.20	0.019	0.43	< 0.001
Trabecular thickness (mm)*	0.22	0.002	0.41	< 0.001	0.50	< 0.001	0.66	< 0.001
Trabecular separation (mm)	0.11	0.035	0.00	0.798	0.05	0.407	0.17	0.085
Connectivity density	0.00	0.889	0.08	0.084	0.08	0.217	0.09	0.348
Degree of anisotropy*	0.00	0.889	0.14	0.018	0.16	0.048	0.24	0.025
Preferred orientation vector	0.02	0.374	0.03	0.259	0.08	0.219	0.08	0.386
θ (°)	0.00	0.972	0.03	0.302	0.05	0.433	0.07	0.463
φ (°)	0.00	0.791	0.03	0.340	0.03	0.637	0.04	0.742

*Variable is significant ($P \leq 0.05$) in the complete ANOVA model.
See Table 1 for remainder of key.

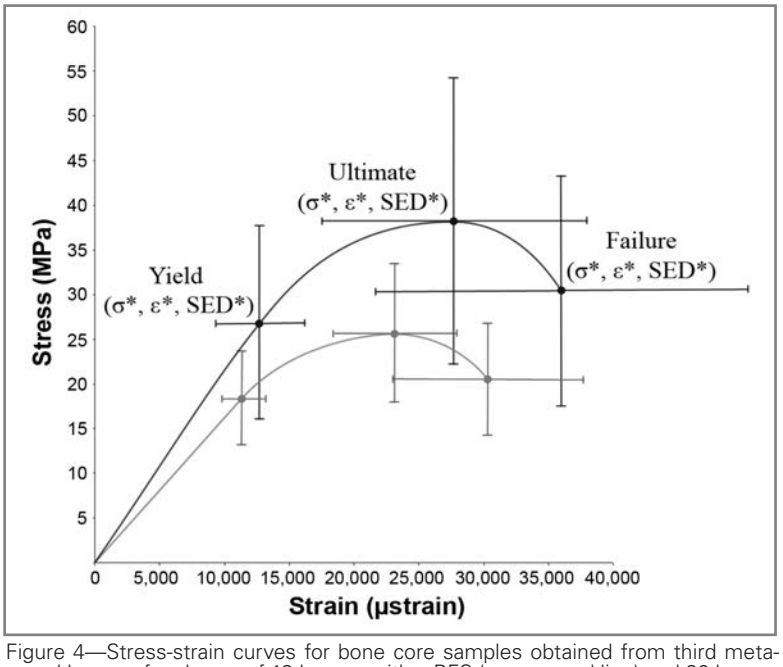

Figure 4—Stress-strain curves for bone core samples obtained from third metacarpal bones of cadavers of 19 horses with a BFS (gray curved line) and 20 horses without a BFS (black curved line). Circles indicate mean values of yield, ultimate, and failure stress (σ), strain (ε), and SED. Vertical and horizontal bars indicate SDs for stress and strain, respectively. *Values of variables for bone core samples obtained from horses with a BFS are significantly ($P \leq 0.05$) different from those for bone core samples obtained from horses without a BFS.

were not significantly different between BFS and control bone core samples (< 3% difference in values).

Mean values of degree of anisotropy were not significantly different between BFS and control bone core samples. Degree of anisotropy vectors were plotted to determine orientation of vectors relative to anatomic structures of third metacarpal bones (**Figure 3**). Vectors were typically oriented along the longitudinal axes of bone core samples with small longitudinal (BFS bone core samples, 8.6°; control bone core samples, 8.0°) and lateral (BFS bone core samples, 5.0°; control bone core samples, 5.2°) offsets. The BFS bone core samples had greater variation in longitudinally and laterally orientated offset angles than did control bone core samples (Table 1).

Significance of contributions of BFS status and age of horses to trabecular morphology of bone core samples was determined via ANOVA models (**Table 2**). Contributions of BFS status alone were significant and accounted for 11% to 23% of the variability in bone volume fraction ($P = 0.002$), trabecular number ($P = 0.014$), trabecular thickness ($P = 0.002$), trabecular separation ($P = 0.035$), and AD ($P = 0.002$). Contributions of age alone were significant and accounted for 14% to 41% of the variability in bone volume fraction ($P < 0.001$), trabecular number ($P = 0.012$), trabecular thickness ($P < 0.001$), AD ($P < 0.001$), and degree of anisotropy ($P = 0.018$). Contributions of the interaction of age × BFS status were significant and accounted for 16% to 50% of the variability in bone volume fraction ($P = 0.020$), AD ($P < 0.001$), trabecular number ($P = 0.019$), trabecular thickness ($P < 0.001$), and degree of anisotropy ($P = 0.048$). Contributions of BFS status, age, and the interaction of age × BFS status (complete model) were significant and accounted for 24% to 66% of the variability in values of the following variables: bone volume fraction ($P < 0.001$), AD ($P < 0.001$), trabecular number ($P < 0.001$), trabecular thickness ($P < 0.001$), and degree of anisot-

Table 3—Mean ± SD values of mechanical variables of bone core samples obtained from third metacarpal bones of cadavers of 19 horses with a BFS and 20 horses without a BFS (control samples).

Variable	BFS	Control	*P* value
Modulus (GPa)	1.62 ± 0.37	2.09 ± 0.62	0.027
Yield stress (MPa)	18.2 ± 5.3	26.7 ± 11.0	< 0.001
Ultimate stress (MPa)	25.5 ± 7.8	38.4 ± 16.5	< 0.001
Failure stress (MPa)	20.4 ± 6.2	30.6 ± 13.2	< 0.001
Yield strain (μstrain)	11,334 ± 1,665	12,576 ± 3,427	0.026
Ultimate strain (μstrain)	23,091 ± 4,746	28,159 ± 11,320	0.002
Failure strain (μstrain)	30,293 ± 7,379	36,137 ± 15,105	0.003
Yield SED (kJ/m^3)	109 ± 43	184 ± 103	< 0.001
Ultimate SED (kJ/m^3)	396 ± 197	830 ± 675	< 0.001
Failure SED (kJ/m^3)	578 ± 293	1162 ± 928	< 0.001

See Table 1 for key.

Table 4—Analysis of variance model results for effects of BFS status, age of horse, the interaction of age X BFS status, and a complete model (including BFS status, age, and interaction of age X BFS status) for mechanical variables of bone core samples obtained from third metacarpal bones of cadavers of 19 horses with a BFS and 20 horses without a BFS.

	BFS status		Age		Age X BFS status interaction		Complete model	
Variable	R^2	*P* value	R^2	*P* value	R^2	*P* value	R^2	*P* value
Modulus (GPa)*	0.18	0.006	0.19	0.007	0.29	0.003	0.38	< 0.001
Yield stress (MPa)*	0.20	0.005	0.28	0.001	0.35	< 0.001	0.53	< 0.001
Ultimate stress (MPa)*	0.20	0.004	0.30	< 0.001	0.37	< 0.001	0.57	< 0.001
Failure stress (MPa)*	0.20	0.004	0.31	< 0.001	0.37	< 0.001	0.57	< 0.001
Yield strain (μstrain)*	0.05	0.162	0.21	0.004	0.22	0.014	0.32	0.004
Ultimate strain (μstrain)*	0.08	0.079	0.35	< 0.001	0.36	< 0.001	0.52	< 0.001
Failure strain (μstrain)*	0.06	0.136	0.41	< 0.001	0.41	< 0.001	0.55	< 0.001
Yield SED (kJ/m^3)*	0.19	0.006	0.27	0.001	0.32	0.001	0.55	< 0.001
Ultimate SED (kJ/m^3)*	0.16	0.011	0.31	< 0.001	0.34	< 0.001	0.58	< 0.001
Failure SED (kJ/m^3)*	0.16	0.013	0.35	< 0.001	0.38	< 0.001	0.62	< 0.001

See Table 2 for key.

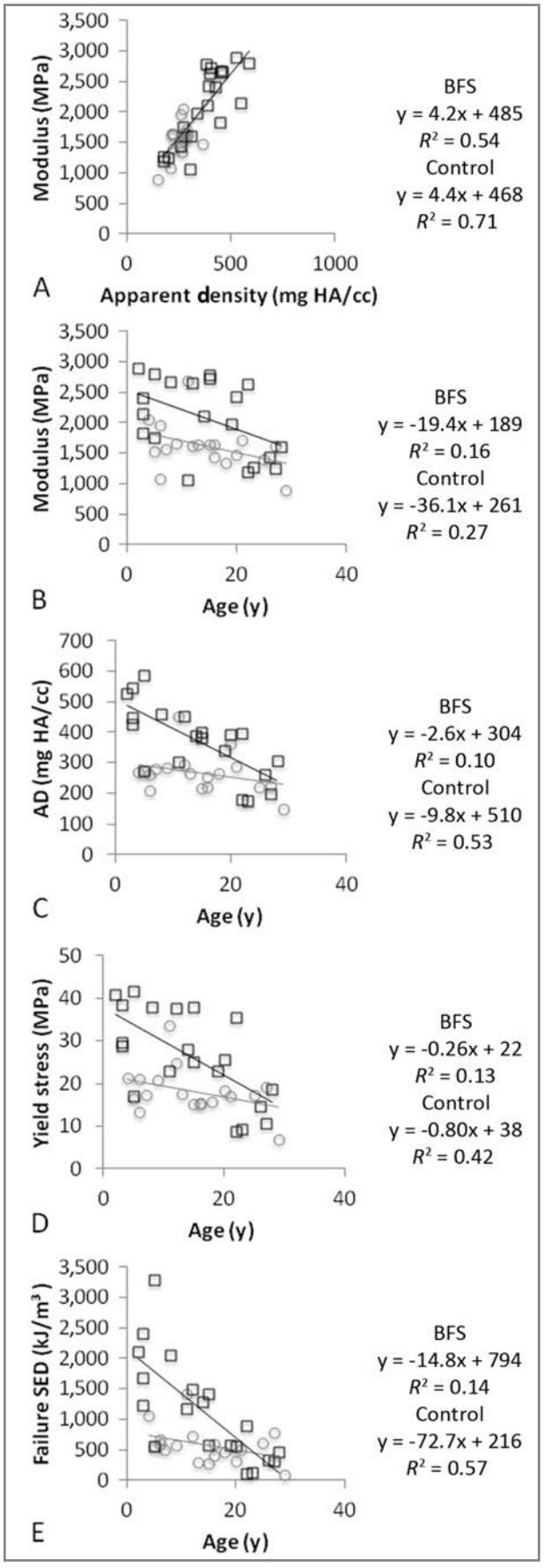

Figure 5—Linear regression analysis results for modulus versus AD (A), modulus versus age (B), AD versus age (C), yield stress versus age (D), and failure SED versus age (E) for bone core samples obtained from third metacarpal bones of cadavers of 19 horses with a BFS (gray circles [values for horses] and gray lines [regression lines]) and 20 horses without a BFS (control samples; black circles [values for horses] and black lines [regression lines]). Equations and R^2 values for BFS and control bone core samples are indicated.

ropy (P = 0.025). Significant effects of age, BFS status, or the interaction of age × BFS status were not detected for mean mineralized bone density, connectivity density, or preferred orientation vector.

Mechanical properties—Stress-strain curves were plotted for BFS and control bone core samples (**Figure 4**). Results of statistical analyses for which stress-strain curve toe region data were included were similar to results of analyses for which such data were excluded. No significant differences were detected between BFS and control bone core samples regarding stresses and strains within the toe regions of stress-strain curves. Data in the toe regions of stress-strain curves were likely influenced by artifacts during initial loading of bone samples; therefore, all values in the toe region were excluded via movement of the strain origin[12] for subsequent analyses.

Significant differences between BFS and control bone core samples were detected for all mechanical variables (**Table 3**). The mean modulus in BFS bone core samples was approximately 22% lower than it was in control bone core samples. Mean stresses at yield, ultimate, and failure loads in BFS bone core samples were 32% to 33% lower than they were in control bone core samples. Mean strains at yield, ultimate, and failure loads in BFS bone core samples were 10% to 18% lower than they were in control bone core samples. Mean SEDs at yield, ultimate, and failure loads in BFS bone core samples were 41% to 52% lower than they were in control bone core samples.

Several multivariable ANOVA models were evaluated to determine the significance of contributions of BFS status and age to values of bone core sample mechanical variables (**Table 4**). Contributions of BFS status alone were significant and accounted for 16% to 20% of the variability in modulus (P = 0.006); yield (P = 0.005), ultimate (P = 0.004), and failure (P = 0.004) stresses; and yield (P = 0.006), ultimate (P = 0.011), and failure (P = 0.013) SEDs. Contributions of age alone were significant ($P \leq 0.007$ for all variables) and accounted for 19% to 41% of the variability in values of all measured mechanical variables. Contributions of the interaction of age × BFS status were significant ($P \leq 0.014$ for all variables) and accounted for 22% to 41% of the variability in values of all measured mechanical variables. Contributions of BFS status, age, and the interaction of age × BFS status (complete model) were significant ($P \leq 0.004$ for all variables) and accounted for 32% to 62% of the variability in values of all measured mechanical variables.

Regression analyses—The relationships among age and values of morphological and mechanical variables were compared for BFS and control bone core samples via simple linear regression analyses. Because

Table 5—Comparisons of linear relationships between various independent and dependent variables for bone core samples obtained from third metacarpal bones of cadavers of 19 horses with a BFS versus bone core samples obtained from cadavers of 20 horses without a BFS.

Independent variable	Dependent variable	F statistic	*P* value
AD	Modulus	0.048	0.827
Age	Modulus	5.364	0.026
Age	AD	11.952	0.001
Age	Yield stress	9.169	0.005
Age	Yield strain	2.834	0.101
Age	Yield SED	10.489	0.003
Age	Ultimate stress	10.493	0.003
Age	Ultimate strain	5.890	0.020
Age	Ultimate SED	10.959	0.002
Age	Failure stress	10.500	0.003
Age	Failure strain	5.214	0.028
Age	Failure SED	11.815	0.001

Results are F statistic and associated *P* values. Significant ($P \leq 0.05$) *P* values indicate that the relationship between variables in a row is significantly different between BFS and control bone core samples.

AD was highly correlated ($R^2 = 0.99$; $P < 0.001$) with bone volume fraction (AD = 1,038•bone volume fraction – 6), AD was used as the explanatory morphological variable for regression analyses. The AD was also correlated ($R^2 = 0.73$; $P < 0.001$) with modulus (modulus = 0.0044•AD + 0.44) as well as other mechanical variables, such as yield stress and failure SED ($R^2 = 0.36$ to 0.82; **Figure 5**).

The AD and modulus of bone samples decreased with age of the horse (Figure 5). Because AD and modulus were high for bone samples obtained from young horses without BFS and were low for bone samples obtained from horses of all ages that had BFS, the effect of age on AD and modulus was greater for control bone core samples than it was for BFS bone core samples. The slope magnitudes of the linear regression lines for age versus modulus and age versus AD were 1.7 and 3.7 times greater, respectively, for control bone core samples versus BFS bone core samples. Slopes and intercepts of regression equations for BFS and control bone core samples were compared via likelihood ratio tests. The BFS and control bone core samples had significantly different relationships between age and modulus, AD, strains at ultimate and failure loads, and stresses and SEDs at yield, ultimate, and failure loads (**Table 5**). However, relationships between modulus and AD for BFS and control bone core samples were not significantly different.

Bone samples obtained from cadavers of racehorses—Four of the 21 horses without a BFS were in race training when they died or were euthanized. The control bone core samples obtained from cadavers of these horses had higher values for mechanical and morphological properties versus control bone core samples obtained from cadavers of other horses without a BFS. This finding may have been attributable to the young adult ages of the 4 horses in race training or to the high-intensity exercise associated with race training. To evaluate potential bias, all statistical analyses were repeated without data for bone samples obtained from the 4 racehorses. Results for these analyses indicated data for BFS and control bone core samples were not significantly different regarding 6 variables (trabecular number and separation, modulus, and strains at yield, ultimate, and failure loads) for which significant differences were detected via initial analyses that included data for bone samples obtained from the 4 racehorses. Results of analyses excluding data for racehorse bone samples indicated mean trabecular number was 8% lower in BFS bone core samples than it was in control bone core samples (vs 18% lower for initial analyses including data for racehorses), mean trabecular separation was 11% greater in BFS bone core samples than it was in control bone core samples (vs 16% for initial analyses), mean modulus was 19% lower in BFS bone core samples than it was in control bone core samples (vs 22% for initial analyses), and mean strains at yield, ultimate, and failure loads were 2% to 4% lower in BFS bone core samples than they were in control bone core samples (vs 10% to 18% for initial analyses).

Discussion

In the present study, we investigated the effect of a BFS on morphologic and mechanical properties of trabecular bone samples of third metacarpal bones via micro-CT and compressive mechanical testing. Horses with a BFS had osteopenic bone, as indicated by 25% lower bone volume fraction and 28% lower AD in BFS bone core samples versus control bone core samples. Trabecular geometry of bone core samples obtained from cadavers of horses with a BFS was consistent with osteopenia, and those bone samples had fewer and thinner trabeculae with greater separation than did bone samples obtained from cadavers of horses without a BFS. The reduced bone volume fraction in BFS bone core samples resulted in a corresponding reduction in compressive material properties. The BFS bone core samples had 22% lower modulus; 32% to 52% lower stress and SED at yield, ultimate, and failure loads; and 10% to 18% lower strain at yield, ultimate, and failure loads, compared with control bone core samples. Bone sample architecture (degree of anisotropy and connectivity density) and mineralization (mean mineralized bone density) did not seem to be affected by BFS status.

Results of the present study indicated osteopenia developed in third metacarpal bones, which do not typically have clinically detectable signs (eg, bone deformation and fracture) of BFS in affected horses. Clinically, signs of BFSs (including SAO) are detected in bones of the axial portion and proximal aspects of the appendicular portions of the skeleton in affected horses.[1–3] Abnormal radiographic patterns have been reported[2] to develop in all bones of those portions of the skeletons of affected horses, except the carpal and third metatarsal bones; bone deformation and radiographic abnormalities are most commonly observed in the scapula, vertebral column, ribs, and pelvic bones of such horses.[1–3] Scintigraphic abnormalities are typically detected in the scapula, ribs, cervical vertebrae, and pelvic bones of affected horses.[1] Nondisplaced fractures are commonly detected in the ribs, whereas displaced fractures are commonly detected in the pelvic bones and scapulae of horses with a BFS. Despite the lack of reports describing clinically detectable abnormalities

in the distal aspects of limbs of horses with a BFS, osteopenia was detected in samples of third metacarpal bones obtained from cadavers of BFS-affected horses in the study reported here.

The finding of the present study that mechanical properties of BFS bone core samples were worse than those of control bone core samples was primarily attributed to reduced bone quantity, rather than reduced bone quality. The BFS bone core samples had decreased bone quantity, as indicated by low AD and bone volume fraction values. However, mineralization was not different between the 2 groups of bone core samples, as indicated by the finding that mean mineralized bone density was not significantly different between BFS and control bone core samples. The relationship between AD and apparent modulus in bone core samples of the present study was similar to that reported for bone samples in other studies,[13,14] and this relationship was not different between BFS and control bone core samples. This finding indicated trabecular bone samples of each group were similar with regard to these 2 mechanical variables.

Findings of the present study were consistent with histopathologic results of other studies in which bone samples from horses with SAO were examined. Rib samples obtained from horses with SAO have high numbers of enlarged osteoclasts, fragmented trabeculae, and a mosaic of cement lines,[2] which is consistent with high bone turnover and active bone remodeling. An increase in activation frequency of bone remodeling units in bone typically causes decreases in bone tissue volume and mean tissue mineralization, which are attributable to a formation deficit that normally occurs during refilling and reduced tissue age, respectively.[15] Although results of the present study indicated that horses with a BFS had less trabecular bone than did horses without a BFS, a decrease in the amount of bone mineralization was not detected for BFS bone core samples via micro-CT. The finding that the amount of mineralization was not different between the 2 groups of bone samples may be attributable to inaccuracy of the micro-CT method or to severity of the BFS in affected horses; BFSs may develop in multiple stages, with periods of high bone turnover followed by periods characterized by recovery of bone mineralization. For example, horses with a BFS from which bone samples were obtained in the present study may have previously had high bone turnover, but may not have had high bone turnover at the time of death or euthanasia and bone sample collection. Therefore, histologic indicators of high bone turnover may be detected in bone samples that have an amount of mineralization typical of a healthy horse.

Findings of the present study regarding mechanical properties of BFS bone core samples were consistent with the fact that BFS-affected horses are predisposed to fractures. To the authors' knowledge, it is unknown why bones in the proximal aspects of limbs of severely affected horses develop deformation and complete fracture, whereas other bones in such horses do not develop these abnormalities. This clinical finding could be unique to bones with a flat bone architecture (ie, scapula and pelvis). This clinical finding could also be attributable to the ratio of the volume of trabecular bone tissue to the volume of compact, cortical bone tissue in bones of horses. The trabecular-to-cortical bone ratio is generally greater in long bones in the proximal aspects of limbs versus long bones in the distal aspects of limbs of horses. Although samples of compact, cortical bone obtained from cadavers of horses with a moderate to severe BFS have an increased number and size of resorption cavities,[2] trabecular bone may be preferentially or more severely affected than cortical bone in such horses. If this theory were true, the mechanical integrity of bones in the proximal aspects of limbs of horses with a BFS would be more severely compromised than would bones in the distal aspects of limbs of such horses.

Results of the present study indicated morphological and mechanical properties of trabecular bone samples obtained from horses with a BFS were comparable to those in bone samples obtained from old horses without a BFS. Bone samples obtained from old horses without a BFS had worse mechanical and morphological properties than did bone samples obtained from young horses without a BFS. The effects of BFS on bone samples seemed to be similar to the effects of aging of horses; bone samples obtained from horses of all ages that had a BFS had mechanical and morphological properties comparable to those of bone samples obtained from old horses without a BFS.

Detection of osteopenia of the distal aspects of metacarpal bones may be useful for identification or staging of BFSs in horses. Currently, the most useful and reliable diagnostic finding for diagnosis of a BFS is scintigraphic detection of increased radiopharmaceutical uptake in multiple sites in multiple bones (most commonly scapulae, ribs, cervical vertebrae, and pelvic bones).[1] Unfortunately, this imaging modality is expensive to perform and may not be an option for many horse owners. Ultrasonographic evidence of thickening of scapular spines is supportive for a diagnosis of BFS, but this finding has only been detected in horses with a moderate to severe BFS.[16] To the authors' knowledge, no reliable and economical imaging modality exists for the detection of BFSs in mildly affected horses. Because third metacarpal bones are located in the distal aspects of limbs, they are more accessible for routine diagnostic imaging (eg, radiography) than are bones in proximal aspects of limbs.

In the present study, the complete ANOVA model (including age, BFS status, and the interaction of age × BFS status) accounted for 61% of the variability in bone volume fraction. Because of the high correlation of AD with bone volume fraction and modulus, clinicians may be able to assess BFS status, progression of a BFS, and risk of fracture for a horse if age and bone volume fractions of distal metacarpal condyles are known. Accordingly, future research should be conducted to develop imaging techniques that can be used to quantify bone volume fraction in vivo, particularly in third metacarpal bones, for identification of horses with a BFS.

Bias in the present study may have been related to characteristics of the horses that were included. Bone samples in this study were obtained from cadavers of horses that were euthanized or died because of severe clinical signs of a BFS. Consequently, results of this study reflect findings for horses with a severe BFS.

Moreover, horses with a BFS often have lameness in ≥ 1 limb; affected horses may respond by reducing locomotion. Such disuse of limbs may have contributed to osteopenia and other changes in morphological variables of bone samples in this study.

Potential bias in data of control bone core samples could be related to high mechanical and morphological values for bone samples obtained from racehorses. Age and workload of these horses could have contributed to high values of the variables. The 4 racehorses were younger than all horses with a BFS. When data for the racehorses were removed from analysis, the negative relationship between AD and age remained. Exclusion of data for samples obtained from racehorses decreased sample size, and comparisons for some of the variables became nonsignificant. However, comparisons for the most important variables maintained significance, including bone volume fraction, AD, mean mineralized bone density, stresses, and SEDs. Thus, exclusion of data for racehorses did not seem to affect the primary findings of the present study.

Results of the present study indicated that horses with a BFS (including horses with SAO) had trabecular bone osteopenia. The worse compressive material properties of bone samples obtained from cadavers of those horses were associated with reduced quantity of bone, versus bone samples obtained from cadavers of unaffected horses. Differences in mineralization between bone samples obtained from cadavers of horses with a BFS and those obtained from cadavers of horses without a BFS were not detected. Osteopenia and changes in mechanical properties of bone likely contribute to high risk for pathological bone fracture in horses with a severe BFS.

a. Butcher Boy Bandsaw, American Meat Equipment LLC, Selmer, Tenn.
b. Model 102065, Starlite Industries Inc, Rosemont, Pa.
c. Model J-2530, JET, La Vergne, Tenn.
d. IsoMet low speed saw, Buehler, Evanston, Ill.
e. Faxitron Series 43805 X-ray System, Hewlett Packard Corp, Buffalo Grove, Ill.
f. Kodak Oncology Film, Carestream Health Inc, Rochester, NY.
g. µCT 35, Scanco Medical AG, Basserdorf, Switzerland.
h. RAND function, Microsoft Office Excel, Microsoft Corp, Redmond, Wash.
i. All Purpose Instant Krazy Glue, Krazy Glue, Columbus, Ohio.
j. Model 809 and Testware SX software, MTS Systems Corp, Minneapolis, Minn.
k. Model 662.20C-04, MTS Systems Corp, Minneapolis, Minn.
l. MATLAB, version 2011a, MathWorks, Natick, Mass.
m. Proc GLM, SAS, version 9.2, SAS Institute Inc, Cary, NC.

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