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Estimating the Compressive Strength of the Porcine Cervical Spine

An Examination of the Utility of DXA

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Study Design. The failure strength of porcine spinal units was correlated with vertebral size and bone mineralization. The accuracy of the resulting predictive equations was tested with an independent sample of spinal units.

Objectives. To determine if dual energy x-ray absorptiometry (DXA)-obtained measures of bone mineralization can be used to accurately predict the compressive tolerance of porcine spinal units.

Summary of Background Data. Porcine spinal units are often used in place of cadaveric tissues, and normalization is used to improve the transferability of model results. In compressive testing, normalization can be performed to the estimated compressive strength. Bone mineralization measures have been shown to be positively correlated with compressive tolerance and have been used to predict the tolerance of human spinal units. However, the accuracy of these predictive equations has not been assessed with an independent sample.

Methods. Twenty porcine cervical spinal units were scanned (DXA) to obtain measures of bone mineral content (BMC) and bone mineral density (BMD). The units were compressed to failure, and the failure loads were correlated with the measured bone mineralization and endplate area of the spinal unit. The regression equations were used to predict the compressive tolerance of an independent sample of spinal units.

Results. BMC ($P = 0.078$) and BMD ($P = 0.2834$) were not significantly correlated to compressive strength. Endplate area was the most highly correlated variable, with an r^2 of 0.5329. The use of a predictive equation including BMC on the second independent sample resulted in errors of estimation of 1.4 ± 1.2 kN, corresponding to 13% of the average compressive strength. In comparison, the use of an equation employing endplate area alone resulted in estimation errors of 11%.

Conclusions. Measures of BMC/BMD did not enhance predictions of compressive strength and will not reduce errors in compressive load normalization in a porcine model. The poor correlations found between BMC and compressive strength may be due to the non–load-bearing anterior processes of the porcine cervical spine.

Key words: compression, accuracy, DXA, porcine, animal model, normalization. **Spine 2005;30:E492–E498**

Understanding the mechanical behavior of the human spine is the focus of research taking place in many laboratories throughout the world. Much of this research is examining the behavior of the spine in isolated tissues or segments to allow detailed analysis of tissue response to a controlled loading scenario. The goal of spinal research is to take the laboratory results and apply them to humans to reduce spinal injury or improve function. To facilitate this transfer of knowledge, many researchers have used cadaveric spinal samples.[1–8] However, cadaveric tissue testing can be limited by the cost and availability of the tissues.[9] Additionally, cadaveric tissues lack homogeneity[10] and can therefore exhibit a large variability of properties and responses. This large variability exists as age, activity, degeneration, and ethnic background can affect the physical and mechanical properties of the spinal tissues.[11–17]

To reduce the cost and variability associated with cadaveric testing, researchers have used animal models. The porcine cervical spine has been shown to be similar to the human lumbar spine in terms of both anatomy and function.[18,19] The similarities between the human and porcine spines have led to the use of the porcine model in a variety of spinal research.[20–31] To allow animal model results to be interpreted in terms of human load exposure and tolerance, normalization of tissue loads can be used. In terms of spinal compression, loads can be normalized as a percentage of the maximum compressive tolerance of the spinal unit. To allow normalization of compressive loads in this manner, an estimate of the ultimate compressive tolerance of a spinal unit is necessary and must possess two characteristics. First, the estimate cannot be destructive as it will then prevent any further testing of the segment. Second, the estimate must be as accurate as possible. Errors in the estimation of the compressive tolerance will affect the load normalization and thus lead to errors when relating animal model results to human load exposures.

Dual energy x-ray absorptiometry (DXA) is a nondestructive method to measure bone mineral content (BMC), which has been shown to be correlated to vertebral strength, with reported correlation coefficients (r) ranging from 0.47[32] to 0.92.[33] Additionally, the inclu-

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sion of endplate or cross-sectional area with measures of bone mineralization in regression analyses has been shown to improve the correlation between bone mineral properties and failure load in human spinal tissues,[34–36] increasing the correlation coefficient as much as 0.23 in the work of Biggemann *et al*.[37] Despite the strong correlations between bone mineralization and failure loads, to the authors' knowledge, no previous studies have tested the accuracy of the regression equations on a second independent specimen sample.

Given the need for accurate and nondestructive methods to estimate the compressive tolerance of porcine spinal units, an *in vitro* study was conducted to assess the relationships between bone mineral measures, endplate area, and compressive tolerance in porcine cervical spinal units. Additionally, the accuracies of the resulting predictive regression equations were tested with an independent tissue sample.

■ Materials and Methods

Twenty frozen porcine cervical spines were obtained from a local abattoir (10 spines on two separate occasions, denoted sample 1 and sample 2). Specimens were kept frozen during storage and scanning. DXA scans were performed with a fan beam system (QDR 4500/W, Hologic Inc., Bedford, MA) using a high resolution spine scan sequence provided by the manufacturer. On each day of testing, a quality control calibration of the DXA system was performed using a spine phantom. A coefficient of variation lower than 1% was considered acceptable based on manufacturer specifications. Specimens were placed on their left side (lateral scan), with the specimen aligned to ensure the scan path of the system followed the longitudinal axis of the cervical spine. The image was sectioned using custom software (Hologic Inc.). BMC and bone mineral density (BMD) were determined for each vertebra in the cervical spine. Each measured variable was separately averaged for the two vertebrae of each functional spinal unit (FSU) to obtain one representative value.

Before dissection, the spines were allowed to thaw overnight. Each specimen was dissected to obtain two osteoligamentous FSUs (C3C4 and C5C6). The exposed intervertebral discs were assessed according to the classifications of Galante[39] to ensure the use of only nondegenerated specimens (Grade 1). After disc assessment, the endplates were measured in the anterior-posterior and medial-lateral directions to allow calculation of endplate area. As in the work of Callaghan and McGill,[27] endplate area was calculated using the equation for surface area of an ellipse ($\pi/4 * A * B$), where A is the anterior-posterior length (perigee) and B is the medial-lateral width (apogee) of the vertebral endplate. The average area of the two exposed endplates was used to represent the FSU.

The upper and lower endplates were mounted in aluminum cups through placement on nonexothermic dental plaster (Denstone, Miles, South Bend, IN), with the midplane of the intervertebral disc aligned parallel to the surface of the cups. The superior vertebral cup was mounted to the actuator of a servohydraulic materials testing machine (8872, Instron Canada, Toronto, Ontario, Canada). The lower cup was placed on a bearing covered surface to allow unconstrained translation and axial rotation of the inferior vertebrae (Figure 1).

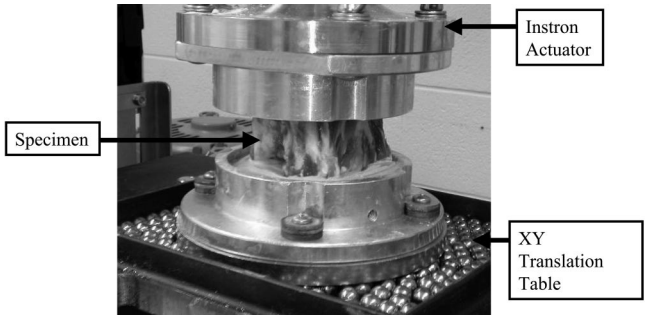

Figure 1. Illustrative diagram of testing fixtures with a specimen mounted.

Before failure testing, specimens were preloaded with 300 N for 15 minutes.[19,27,40] After application of the preload, specimens were loaded to failure at a rate of 3,000 N/s. This load rate was chosen to mimic the compressive load rate obtained using a rigid link model (GOBER, University of Waterloo, Waterloo, Ontario, Canada) during the performance of a dynamic, two-handed symmetrical floor-to-waist height lift. Failure (ultimate strength) was defined using three definitions previously described by Gunning *et al*.[40] Ultimate strength 1 was defined as a 3.125% drop in the force feedback within a 10 millisecond window, with the maximum force sustained before failure taken as the compressive strength of the specimen. This failure definition was sensitive enough to identify the sharp drop in compressive resistance at failure, given the delay in the force feedback loop. Ultimate strength 2 was based on the work of Brinckmann *et al*[36] and was taken as a deviation of 5% from a straight line fit to the linear portion of the deformation curve. Additionally, ultimate strength 3 was defined as the point on the load-deformation curve where the slope fell to zero.[41] Force output was sampled at 1,000 Hz. Throughout testing, specimens were surrounded in saline-soaked gauze and plastic wrap to prevent moisture loss.

The first 20 FSU tested (sample 1) were used for development of the regression equations. T-tests were used to test for differences between the two spinal levels used. Findings with $P < 0.05$ were considered statistically significant. The relationships between BMC, BMD, endplate area, and failure loads were assessed using stepwise multiple linear regression analysis with Statistical Analysis Software (version 5.1.2600, SAS Institute Inc., Cary, NC). Stepwise regression was chosen to allow identification of independent contributors to the correlation. An additional 20 FSU (sample 2) were scanned and compressed to failure to test the accuracy of the predictive equation. Accuracy was measured as the absolute error between the predicted strength and measured failure loads. Comparison was made to two other methods of compressive strength prediction:

1. Using the combined mean of all 40 FSU as an estimate of failure strength.
2. Determining the failure strength of a FSU and using that strength as a prediction for the adjacent FSU from the same spine.

A one-way analysis of variance was used to test for significant differences ($P < 0.05$) between all methods of prediction. T-tests were performed between the units used to develop the equation (sample 1) and those used to test its accuracy (sample 2) to ensure that differences in size or bone mineralization did not bias the results.

Table 1. Average Endplate Area, Bone Mineral Content, Bone Mineral Density, and Failure Loads Shown for Both Spinal Levels From Sample 1, Isolated by Level

	N	Endplate Area (mm^2)	Bone Mineral Content (g)	Bone Mineral Density (g/cm^2)	Failure Load (kN)		
					Ultimate Strength 1	Ultimate Strength 2	Ultimate Strength 3
C3C4	10	718 (63)	9.87 (1.66)	0.89 (0.10)	10.39 (1.09)	5.46 (2.34)	9.03 (2.55)
C5C6	10	724 (60)	9.35 (1.67)	0.96 (0.11)	10.54 (1.18)	6.22 (1.82)	7.30 (2.99)
Total	20	721 (60)	9.61 (1.66)	0.93 (0.11)	10.46 (1.11)	5.84 (2.10)	8.23 (2.81)
P		0.6134	0.0901	0.0013	0.5992	0.4730	0.2479

Values are mean (SD). The results of the paired *t* test are given; $P < 0.05$ was taken to be statistically significant.

■ Results

Equation Development

There were no significant differences for endplate area, BMC, or failure loads between the C3C4 and C5C6 levels of sample 1 (Table 1). It should be noted that five specimens exhibited loading curves that did not reach the criteria of ultimate strength definition 3; therefore, only 15 values were available for comparison. BMD was found to be significantly different, but because of its poor performance in the regression analysis, differences were not examined any further. Additionally, regression analysis revealed that all correlations with endplate area and bone mineralization were reduced with the use of ultimate strength definitions 2 and 3. Therefore, no further analysis was performed using these definitions. Comparison of endplate area, BMC, BMD, and failure load (ultimate strength 1) between the two samples revealed no significant differences (Table 2). Stepwise regression analysis found that endplate area (r = 0.73) was the only significant variable in the regression, with BMC (r = 0.28) and BMD (r = 0.18) improving the model correlation only slightly (Table 3). BMC and BMD were not significantly correlated to failure load ($P = 0.078$ and $P = 0.283$, respectively). The relationships of endplate area and BMC with failure load are illustrated in Figures 2 and 3. The resulting equations (with and without BMC) were:

$$\textit{Compressive strength}\ (kN) = 1.34752 + 0.00941 \times \textit{endplate area}\ (mm^2) + 0.24276 \times BMC\ (grams) \quad (1)$$

$$\textit{Compressive strength}\ (kN) = 0.65470 + 0.01361 \times \textit{endplate area}\ (mm^2) \quad (2)$$

Equation Validation

The predictive equation based solely on endplate area (equation 2) resulted in a lower absolute error of estimation when compared with the two-variable equation (equation 1, Figure 4), although the improvement was not significant. Comparison of the estimation methods revealed that the average absolute error of the estimated strength exceeded 1 kN for all methods (Figure 4). Differences were not significant between any of the methods of estimation.

■ Discussion

The results of this study indicate that predictive equations developed on a sample of porcine spinal units resulted in errors greater than or equal to 10% when predicting the compressive strength of a second, independent sample. An equation using both BMC and endplate area resulted in errors of estimation of approximately 13%. The predictive equation using endplate area alone resulted in an error of estimation of 11%. This reduction in error, although not statistically significant, may be valuable in estimating the compressive strength of a porcine cervical spinal unit. The poor performance of the two-factor equation is likely due to the low correlations of BMC and BMD with failure load. These low correlations are in contrast to the strong correlations previously reported in cadaveric specimens.[34,37,42–44] However, previous work relating the BMD and compressive strength of ovine lumbar vertebral bone was also unable

Table 2. Average Endplate Area, Bone Mineral Content, Bone Mineral Density, and Failure Load Shown for Sample 1 and Sample 2

	N	Endplate Area (mm^2)	Bone Mineral Content (g)	Bone Mineral Density (g/cm^2)	Failure Load (kN)
Sample 1	20	721 (60)	9.61 (1.66)	0.93 (0.11)	10.46 (1.11)
Sample 2	20	726 (67)	8.95 (1.78)	0.90 (0.11)	11.10 (1.73)
Total	40	723 (63)	9.29 (1.73)	0.91 (0.11)	10.78 (1.47)
P		0.7993	0.2372	0.3741	0.1750

Values are mean (SD). The results of the *t* test are given; $P < 0.05$ was taken to be statistically significant. Only failure loads based on ultimate strength definition 1 have been included.

Table 3. Results of the Stepwise Regression Analysis

Variable	Partial R^2	Model R^2	*P*
Area	0.5329	0.5329	0.0003
BMC	0.0802	0.6131	0.0777
BMD*	0.0314	0.5644	0.2834

$P < 0.05$ was considered statistically significant.
*A separate model was run to analyze BMC and BMD due to the number of FSU tested.

to find a significant correlation.[45] Deloffre *et al*[45] attributed the lack of correlation to ovine anatomy, with the large vertebral arches contributing to mineralization measures while they were not included in the compressive tests. Previous *in vivo* work has shown that the posterior elements can contribute as much as 44% of the BMC in males and 53% in females when DXA scanning is performed in the anterior-posterior direction.[46] To reduce the errors when obtaining an anterior-posterior scan, a lateral scan was used in this study. In taking a lateral scan, the anterior processes of the porcine cervical spine likely contributed to measures of bone mineralization and affected determinations of BMD while they did not bear any of the compressive load. The lateral scans obtained in this study did not allow for clear delineation of the anterior processes from the vertebral body; therefore, it was not possible to accurately quantify the error this may have introduced into the regression. This is a limitation of DXA as it is a two-dimensional imaging technique that does not allow separation of overlapping structures in the scan plane.

The low correlation between BMC and compressive tolerance may have also been affected by the lack of information concerning the structural organization of the vertebral trabecular bone. Oden *et al*[47] found that including the principal angle of the trabeculae (0° being 100% longitudinal, 90° being completely transverse) in a regression analysis improved the correlation between failure stress and BMD by 13%. Using BMC as a surrogate measure of bone strength ignores the role that distribution of mineral within the structure plays. A technique that accounts for structural organization of the trabecular bone as well as BMC may improve the accuracy of estimating compressive strength; however, such a measure is not possible with DXA. The trabeculae of quadruped vertebrae have been shown to have a longitudinal orientation, similar to that of human vertebrae[48]; however, it was not measured directly in the current study.

Comparison of the error of estimation among the compressive strength predictive methods studied revealed no significant differences. Therefore, the preferred method of estimating failure strength will be dictated by infrastructure, time, and cost. Using a predictive equation based on endplate area is labor intensive while developing the equation for the population of interest. The advantage is low cost and time to perform the estimate once the equation has been developed. Establishing a stable average compressive tolerance magnitude for a desired model or population requires a large sample of specimens and substantial testing time. This method also assumes that the tissue properties do not differ from sample to sample or that a homogeneous population exists, clearly an assumption not well suited for human subjects or even animal models with varying age and size. No statistical differences were found between the endplate area, BMC, BMD, or compressive tolerance of the first and second sample groups used in this study, suggesting that further specimens from the same source would exhibit compressive behavior similar to that reported above. It cannot be assumed that the values obtained in this study would represent the compressive tolerance of porcine specimens of a different age or size or that the results can be scaled to a different FSU model. However, once a population average is obtained, cost and time of prediction would be minimal. Prediction of compressive tolerance based on the strength of the adjacent segment requires less labor than the establishment of a population mean or the development of a regression equation. This

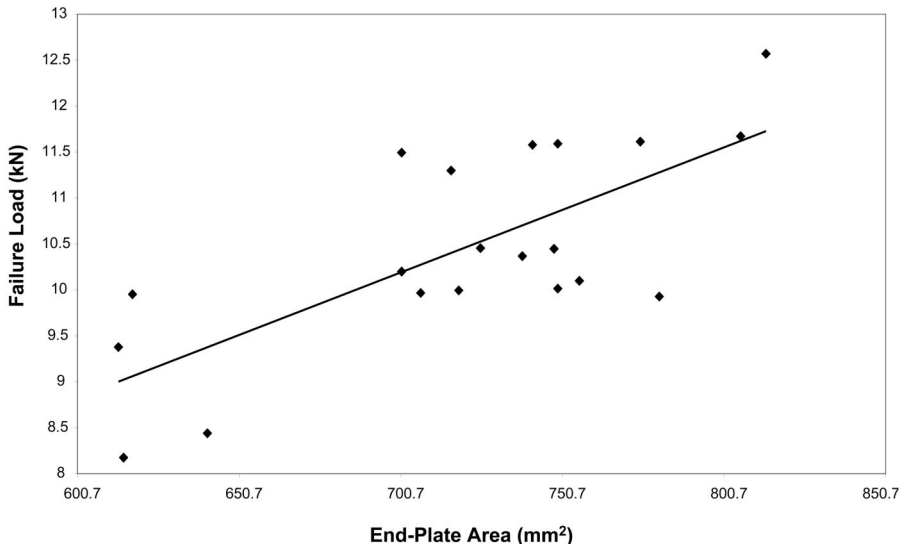

Figure 2. Scatter plot of failure load (kN) obtained during compressive testing *versus* endplate area (mm^2).

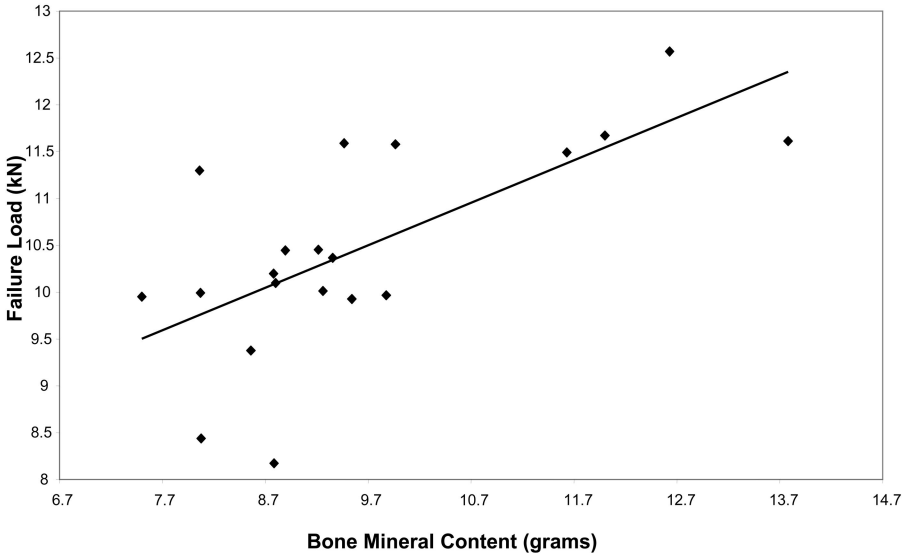

Figure 3. Scatter plot of failure load (kN) obtained during compressive testing *versus* BMC (grams) as obtained from DXA scan.

technique has been used previously to predict human intervertebral joint failure strength in submaximal fatigue testing.[49] The disadvantage of this method is a reduction in the number of specimens available for submaximal testing from each donor.

One limitation of this work was the freezing of spines throughout storage and scanning. Although freezing has not been shown to have any effect on BMC values,[50] it has been shown to increase compressive strength.[27] As all specimens were scanned frozen and thawed overnight before testing, it was expected that the effects of freezing would be constant across specimens. This study was also limited by the application of a purely compressive load. Physiologic loading results in a combination of loads in the sagittal, frontal, and transverse planes. However, in any *in vivo* movement, there will be compressive loading because of the action of the musculature, and isolated loading provides information regarding the tissue changes and injury mechanisms associated with that exposure.

The failure loads obtained in this study are higher than previously reported for the porcine cervical spine. Callaghan and McGill[27] reported an average compressive strength of 7.8 ± 0.89 kN for specimens with an average endplate area of 6.2 ± 0.5 cm^2. Using porcine cervical specimens comprised of three vertebrae, Yingling *et al*[19] reported failure loads of 6.8 ± 1.2 kN for a sample with an average endplate area of 5.8 ± 0.5 cm^2. The lower loads recorded in the previous studies are likely due to the use of smaller specimens as the vertebrae of the specimens used in this study were larger, with an average endplate area of 7.2 ± 0.6 cm^2 and compressive strength of 10.78 ± 1.47 kN.

Errors in the estimation of failure loads in this work are lower than those reported in earlier studies. In 1989,

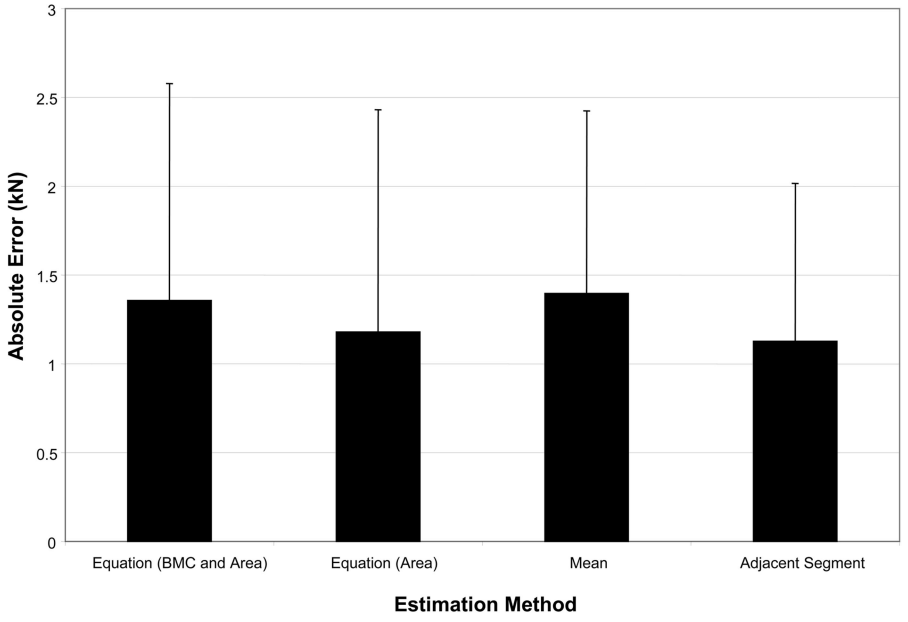

Figure 4. Bar chart showing the average absolute error (N = 20) of estimation in kN (+ 1 standard deviation) for estimation using the predictive equation (both one and two variable models), estimation using the sample mean, and estimation using the adjacent segment. Differences were not significant.

Brinckmann *et al* published two studies[35,36] examining the use of QCT measured bone density and endplate area to predict the compressive strength of cadaveric spinal units. The authors found a standard error of estimation of approximately 1 kN. Similarly, Brinckmann *et al*[35] calculated a standard error of estimation of 0.89 kN from the work of Hansson *et al*,[34] who predicted the compressive strength of human lumbar vertebrae using DPA obtained BMC values. Biggemann *et al*[37] reported a standard error of the estimate of 0.74 kN for cadaveric specimens with a mean compressive strength of 5.3 kN. In the present study, the largest standard error of the mean for the absolute error of estimation for all methods was 0.28 kN. However, standard error measures assess the variability of prediction, not accuracy. To achieve a measure of accuracy, Eriksson *et al*[38] divided the standard error of the estimate by the mean and expressed this measure as a percentage. Applying this equation to the work of Brinckmann *et al*,[36] Hansson *et al*,[34] and Biggemann *et al*[37] reveals accuracy errors of 19%, 23%, and 15%, respectively. The 0.28 kN standard error of the mean for absolute error of estimation obtained in this study results in a 3% accuracy error. These comparisons should be interpreted with caution, as previous work has reported standard errors of estimation based on data from the specimens used to develop the equation; therefore, no true measures of accuracy are possible without testing the developed equations on an independent sample. In contrast, the current study tested a different sample of specimens to establish the accuracy of the predictive equation. Therefore, the estimation errors observed in Figure 4 cannot be directly compared with literature values.

The larger errors in earlier studies[34,36,37] are likely due to the use of human cadaveric specimens. The cadaveric specimens used were not controlled for age and were much older than the porcine model used in this study. The increased variability in age in the cadaver based work may lead to increased variability in the measures of bone mineralization. The porcine specimens used in this study had a range in BMD measures of 0.52 g/cm^2 and 7.7 g in BMC, which appears to be similar to those observed in studies on cadaveric tissues.[34,38,51] However, the cadaveric data may be more scattered about the mean, leading to increased variability. Furthermore, the age and activity controlled animal population employed would not display the thickened or osteoporotic trabeculae observed in some human samples. This may have affected the observed correlations despite the range of mineral values observed. Quantification of the differences in BMC/BMD with the above studies is not possible because of differences in scanning techniques; therefore, the magnitude of the error due to sample selection cannot be assessed.

The findings of this study indicate that an equation for predicting compressive strength that was developed on a tissue sample may result in estimation errors exceeding 10% of the actual compressive tolerance of a specimen. Additionally, it was found that measures of BMC did not reduce the error in predicting the compressive tolerance of a porcine intervertebral joint when compared with the use of a regression equation based on endplate area, a population mean estimate, or the use of the tolerance of an adjacent segment from the same spine. The results of this study indicate that DXA-obtained measures of bone mineralization are not useful in estimating the compressive tolerance in this animal model and would result in poor load magnitude normalization.

■ Key Points

- DXA-obtained bone mineral measures did not improve estimates of the compressive tolerance in porcine cervical spinal units.
- Endplate area ($P = 0.0003$) was significantly correlated ($r = 0.73$) with the compressive strength of the porcine spinal units, whereas bone mineral content ($P = 0.0777$, $r = 0.28$) and bone mineral density ($P = 0.2834$, $r = 0.18$) were not.
- An equation using endplate area resulted in average errors of estimation of 11%. Including bone mineral content increased the error to 13%, although the increase was not significant. This error magnitude should be considered by researchers when normalizing load exposures.
- The poor correlation is likely due to the anterior processes of the porcine cervical spine, which contributed to the amount of measured bone mineral but did not bear any compressive load.

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