

Comparison of bending modulus and yield strength between outer stratum medium and stratum medium zona alba in equine hooves

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Objective—To determine whether the bending modulus and yield strength of the outer stratum medium (SM) differed from those of the SM zona alba (SMZA) and to what degree they differed. In addition, a comparison was made among our values and values reported elsewhere.

Sample Population—10 normal equine feet.

Procedure—A 3-point bending technique was used to determine the bending modulus and yield strength of the outer SM and SMZA. Efforts were made to minimize biological and technical factors that could influence the bending modulus.

Results—Bending modulus of the outer SM was (mean \pm SD) 1876 ± 41.3 MPa, whereas mean value for the SMZA was 98.2 ± 36.8 MPa. Mean yield strength was 19.4 ± 2.6 MPa for the outer SM and 5.6 ± 1.7 MPa for the SMZA. Values for bending modulus and yield strength differed significantly between the outer SM and SMZA. Significant differences were not detected when the outer SM was loaded in bending from the outer or inner surface.

Conclusions and Clinical Relevance—Potentially, the SMZA could serve as a mechanical buffer zone between the rigid hoof wall and bone and laminar tissues. This buffer zone potentially assists the feet of horses in transmitting a load through the tissues and prevents the most susceptible tissues from becoming damaged. More consistency among tissue selection, preparation, and testing protocols must be attained before an accurate 3-dimensional finite-element model of an equine foot can be constructed. (*Am J Vet Res* 2001;62:745–751)

Understanding the biomechanical behavior of the feet of horses is necessary for a more accurate and complete understanding of their biomechanical functions and limitations. Biomechanical behavior reflects how a foot functions mechanically during the acceptance, dissipation, and transmission of load while under quasistatic or dynamic loading conditions. Early investigations by Peters¹ and Lungwitz² focused on the relationship between deformation of hooves and altered conformations of the limbs and feet of horses. Hood^a and Colles³ directed attention more specifically toward deformation at various regions of the stratum medium through the use of transducers and strain gauges applied to the outer surface of a hoof. These studies provided more detailed information regarding

specific regions of microstrain and, therefore, provided a more precise understanding of deformation of the wall in those areas.

Results of those studies revealed that the biomechanical behavior of the feet of horses is complex. Contributing to this complexity is the gross anatomic configuration and component microarchitecture and material properties, all of which play an important role in the overall behavior of equine feet.⁴ It is the combination of these factors that enables the feet of horses to withstand the incredible mechanical forces placed on them during performance conditions. It has been estimated that mechanical forces on each foot range from zero when not bearing weight to 9,000 N during galloping. Therefore, to completely understand how the foot functions as a whole, it is important to understand each of these factors and to combine them in an articulated fashion. Investigators at several laboratories have attempted to apply these data toward a common goal, establishment of 3-dimensional finite-element computerized models of an equine foot to be used in research simulating loading conditions related to athletic performance and disease conditions.⁵⁻⁷

Several investigators^{6,8-10} have applied various techniques to more completely define the modulus, J-integral value, and yield point for the stratum medium. However, there is great disparity among the data, especially with regard to the elastic modulus. The variation in data is striking and must be accounted for before analysis of those results can be applied to future research.

When considering the behavior of the coffin bone and outer stratum medium, it is logical to assume that a mechanical buffer zone exists between the relative stiffness of the bone and wall and the flexibility of the dermis and laminar interface. Such zones of mechanical transition of the stratum medium would decrease the incidence of fractures or ruptures when loads are rapidly applied. The stratum medium zona alba (SMZA) potentially provides this buffer zone and allows loads to be transmitted from the outer stratum medium to the more sensitive laminar interface without inflicting damage to adjacent tissues. Because of the way the equine hoof wall moves when placed under typical loading conditions,^a testing the stiffness (ie, elastic modulus) of the stratum medium and SMZA under bending conditions, versus under axial tension or compression, more closely resembles natural physiologic motion. Therefore, we hypothesized that the bending modulus and yield strength of the outer stratum medium and SMZA would differ significantly and that the outer stratum medium would have higher val-

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ues for both variables, indicating greater intrinsic rigidity and strength than for the SMZA.

Materials and Methods

Sample population—Ten normal feet were collected from euthanized horses (1 foot/horse) during a single visit to an abattoir. Feet were considered to be normal when they did not have grossly visible external lesions (eg, wall cracks, founder rings, or evidence of abscesses) or internal lesions (eg, laminitis) on sagittal sectioning. Whole feet were stored overnight in a controlled environment (3 C and relative humidity of 41%), because testing could not be completed on the same day as collection of samples. Within 12 hours after the feet were collected, tissue samples were prepared. Samples subsequently were subjected to the testing protocol within 4 hours after preparation.

Sample preparation—A 1-cm-wide midsagittal section of tissue was removed from each foot. From this section of tissue, a full-thickness section of stratum medium was obtained. The stratum medium was divided between the outer stratum medium and the SMZA. The zona alba has not been consistently defined among researchers when describing a specific region of an equine foot.^{11-13,b} For our study, we defined the zona alba (ie, SMZA) as the nonpigmented innermost region of the stratum medium extending from the coronary epidermis to the solar surface; it did not include the striated yellow-colored region at the sole that often is referred to as the white line. We identified the boundary of the SMZA as the point at which the color changed from that of the wall to that of the nonpigmented region of the SMZA (Fig 1). The laminar tissue on the inner surface of the SMZA was completely removed. Separation of the stratum medium and removal of laminar tissue was accomplished manually to avoid effects of heat incurred during machine preparation of tissues. Tissue sections obtained from each foot spanned the region from 1 cm distal to the coronary band to 1 cm proximal to the solar margin. Thus, the most distal aspect was proximal to the terminal papillae of the laminar interface. Sections were maintained in paper towelettes that had been moistened but not soaked with physiologic saline (0.9% NaCl) solution, and samples then were placed in plastic bags and sealed to prevent drying of the samples prior to testing.

Base (ie, width) and height of each tissue sample were determined at 3 separate locations along the length of the sample, and mean of the 3 measurements for each variable of each sample was used in calculations for the bending modulus. Samples were taken to the materials testing laboratory

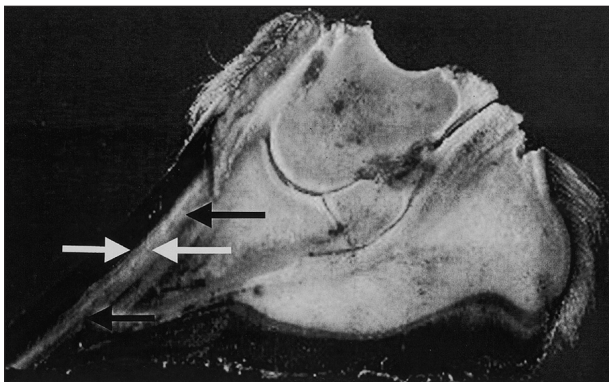


Figure 1—Sagittal section of an equine hoof revealing regions of the stratum medium and stratum medium zona alba (SMZA) that were used in determining the bending modulus. A length of both tissues was obtained from the dorsal surface (black arrows). The region classified as the SMZA is indicated (white arrows); the SMZA was separated from the outer stratum medium prior to testing.

and separately tested with a load, using a testing machine.^c Supports on which the samples were placed for testing were set at a distance of 2.54 cm, which represented a constant value for the sample length during loading. A single upper contact provided a load to the specimen at the midpoint with a ventral displacement rate of 5.1 mm/min to create a condition of relatively slow quasistatic loading conditions, similar to those occurring naturally in a standing horse. The load cell (load capacity of 450 kg) measured the applied load to the tissue sample, whereas a transducer^d measured displacement of the sample over time. The testing load used for these tissues ranged from 0 to 91 kg with a data acquisition sampling rate of 10 Hz.

Initially, each sample was placed with its outer surface facing downward on the supporting bridge, and the bending force (ie, load) was applied in a downward direction contacting the inner surface of the sample. Samples from each foot were tested in an alternating order: outer stratum medium was tested first, and SMZA was tested subsequently. After collection of these data was completed, the outer stratum medium samples were repositioned with the inner surface facing downward on the supports, and samples were tested again with the load applied in a downward direction contacting the outer surface. Digital data acquired with the testing machine included displacement of the sample versus applied force. These data were imported into and plotted by a computer program^e for determination of the slope of the line (Fig 2) that was necessary for calculating the bending modulus of each sample. These calculations were accomplished by use of a computer spreadsheet program.^f Mean regression coefficient for slope of the outer stratum medium samples was 0.997, whereas it was 0.986 for the SMZA. This reflected slightly more variability in the data as a result of increased elasticity of the SMZA.

Analysis of variables—The elastic modulus is defined in basic terms as the ratio between stress and strain, where stress is the load being applied to a material, and strain is the deformation that is detected in response to the applied stress. When measured in bending, it often is termed the bending modulus. We calculated the bending modulus, using the following equation:

$$E = \frac{k(L^3)}{48I}$$

where E is the elastic (bending) modulus, k is the slope of the force-displacement curve, L is the length of sample, and I is the cross-sectional moment of inertia of the sample.

The moment of inertia, used to adjust for the cross-sectional tissue area under bending loading, was defined by the following equation:

$$I = \frac{b \cdot h^3}{12}$$

where b is the base (ie, width) of the sample, and h is the height of the sample. This accounted for discrepancies that may have existed between the overall cross-sectional measurements between samples following sample preparation.

Force-displacement data typically display 2 separate regions along the curve (Fig 2): an elastic and a plastic region, which are separated by the yield point. These terms are commonly applied to engineering materials to distinguish between recoverable deformation (elastic) and permanent deformation (plastic). In such cases, the initial linear portion of the response closely approximates the elastic response, and the nonlinear portion beyond that represents permanent deformation. For biological materials, these phenomena are not always applicable or known, because there can be substantial nonlinear recoverable elastic deformation without material damage and associated permanent deformation. For

our study, the elastic region refers to the linear portion of the response, and the plastic region indicates the nonlinear portion. As indicated previously, the bending modulus is derived from the slope of the initial linear part of the curve.

A 2-tailed paired *t*-test was used to detect significant differences between the bending modulus of the outer stratum medium and the SMZA for each horse when tissues were bent in similar directions. A 2-tailed paired *t*-test also was used to compare the bending modulus of the outer stratum medium when loaded in opposite directions. A value of $P < 0.05$ was used to discriminate significance.

In addition to determining the bending modulus of these tissue samples, calculations were used to ascertain the yield strength for each sample. Yield strength is the stress in the material at the yield point and is calculated by use of the following equation:

$$S_y = \frac{F_y L h}{8I}$$

where S_y is the stress at the yield point, and F_y is the force at the yield point. The yield point is often defined roughly as the point at which the force-displacement curve becomes nonlinear. We determined this point in a systematic way,

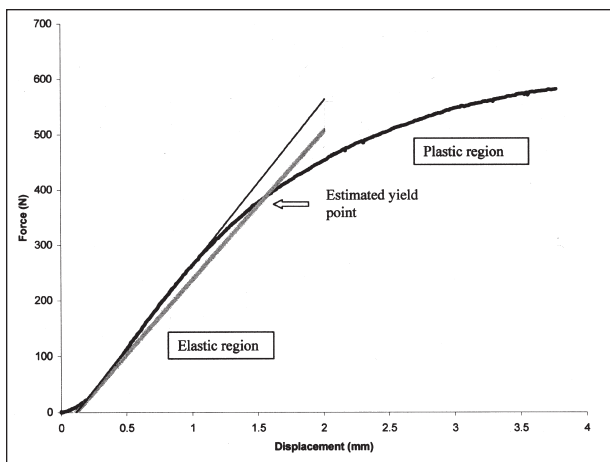


Figure 2—Force-displacement curve (thick black line) of a representative sample of outer stratum medium. The plastic and elastic regions of the curve are indicated, and the linear portion of the curve was used to determine the bending modulus. The original slope (thin black line) was used to determine the bending modulus, whereas the line representing 90% of the original slope (gray line) was used to determine the yield point of each sample.

using a stiffness-reduction technique. The slope of the original linear portion of the force-displacement curve (ie, the stiffness) was reduced by 10%, and another straight line was constructed that had a slope of 90% of the original (Fig 2). The intersection of the reduced stiffness line with the force-displacement curve defined the yield point. A 2-tailed paired *t*-test was used to determine whether the yield strength of the outer stratum medium and SMZA differed significantly ($P < 0.05$) for each horse.

Results

Load-displacement data were collected for all 20 tissue samples for which bending modulus and yield strength were calculated. During initial loading of the outer stratum medium, with the outer surface facing downward, bending modulus ranged from 97.7 to 240.9 MPa (mean \pm SD, 187.6 ± 41.3 MPa). Bending modulus of the SMZA, when loaded in the same direction, ranged from 60.0 to 163.1 MPa (mean, 98.2 ± 36.8 MPa). Subsequent loading of the outer stratum medium in the opposite direction resulted in values for the bending modulus that ranged from 89.2 to 232.6 MPa (mean, 181.5 ± 41.8 MPa). Values obtained for the outer stratum medium, regardless of the direction in which they were loaded, differed significantly ($P = 0.004$) from values for the SMZA. All samples of the outer stratum medium had a higher value for bending modulus than did the corresponding SMZA, except for 1 specimen; the outer stratum medium of that particular specimen had a bending modulus of 97.7 MPa in 1 direction and 89.2 MPa in the opposite direction, whereas the SMZA had a bending modulus of 163.1 MPa.

Values for bending modulus of the outer stratum medium of the same sample, when bent in opposing directions, were analyzed. However, values for each sample did not differ significantly ($P = 0.358$).

Mean yield strength for outer stratum medium was 19.4 ± 2.6 MPa, whereas that for the SMZA was 5.6 ± 1.7 MPa. Similar to the bending modulus, values differed significantly ($P < 0.001$) between the outer stratum medium and SMZA. For all of the samples of the outer stratum medium, values for yield strength were higher than values for the corresponding SMZA.

Values obtained for the elastic modulus vary greatly among reports (Table 1). Values range from extremely low (3.7 MPa) in 1 report⁹ to extremely high

Table 1—Results for the elastic modulus of the stratum medium reported in various studies

Breed	Control for hydration*	Testing protocol	Elastic modulus (MPa)	SD (%)†
Ponies ¹⁹	-/-	C	3.722 ± 0.111 ‡	2.98§
Varied ¹⁴	Fully hydrated	C	240 to 480	NA
Varied ¹³	+/+	C	172 to 500	NA
Varied ¹¹	-/+	T	410 to 14,600	0.486 to 7.8
Varied ²⁰	-/+	T	280 ± 70 to 850 ± 160 ¶	18.9 to 25.0
Varied ⁶	+/+	C, T	460 ± 114 to $1,049 \pm 231$ ¶	22.02 to 24.78
Warmbloods ⁷	+/+	T, B	761.8 ± 295.4 ‡	38.77§
Varied#	+/+	B	187.56 ± 41.31 ‡	22.02§

*The first symbol represents whether the sample was controlled for hydration from the time of sample collection until preparation, whereas the second symbol represents whether the sample was controlled for hydration from the time of sample preparation until testing. †Percentage of mean value for elastic modulus. ‡Mean \pm SD. §Mean. ||Range. ¶Range of mean \pm SD values. #Represents values determined in the study reported here. - = Hydration control was not attempted. + = Samples were controlled for hydration loss. C = Compression. T = Tension. B = Bending. NA = Not available.

(1,049 Mpa) in another report⁵ and are characterized by a wide SD, at times up to 38% of the mean.⁶ Data obtained from the study reported here were within this range. Values for our study were 187.6 ± 41.3 MPa for the outer stratum medium and 98.2 ± 36.8 MPa for the SMZA, with the SD being approximately 22 and 36% of these means, respectively.

Discussion

Biomechanical research of equine feet has been directed toward several aspects. The architectural and material properties of the laminar interface have been studied.¹⁴ This is a tissue of major importance, because, in combination with the dermis, it provides the junction between the coffin bone and stratum medium. Intuitively, these are the most susceptible locations to injury when a foot becomes overloaded.¹⁵ In addition, the stratum medium has received considerable attention as a whole unit as well as regionally with regard to anatomic location (dorsal vs lateral or medial, outer wall vs inner wall).^{5,6,8,16-18}

Given the perceived mechanical role of the hoof wall, a substantial amount of research has been directed toward the stratum medium, including how it behaves under load and its intrinsic material properties. When referring to material properties of tissues, several engineering terms are commonly used for their descriptions. These include elastic or bending modulus, J-integral value or fracture resistance, proportional limit or yield point, and ultimate strength.^{19,20} One of these characteristics (ie, elastic modulus) has been described in detail for the stratum medium. This material property, also called Young's modulus, is defined as the rigidity or intrinsic stiffness of a tissue. The term bending modulus also is commonly used and refers to the same characteristic, with the testing protocol employing a bending of the sample instead of testing the tissue by placing it in tension or compression. Bertram and Gosline^{16,17} and Leach and Zoerb¹⁸ documented that the elastic modulus and J-integral (ie, fracture resistance) of the stratum medium could be substantially affected by altering the relative hydration of the tissue.¹⁶ The optimal relative hydration of the stratum medium, approximately 75%, was necessary to maintain maximal fracture-toughness, and deviations resulted in early appearance of cracks and crack growth.¹⁷ Finally, the yield point (ie, measure of stress at which permanent damage to the tissue began) was higher for the outer wall than the inner wall.⁸

Additional studies have concentrated on determining other factors that potentially impact material properties of the stratum medium. Reilly et al²¹ focused on diversity of tubular density among regions of the stratum medium and contrasts that are evident among breeds of horses. It was hypothesized that variations in tubular density may impact the rigidity in various regions along the stratum medium.

The hypothesis for the study reported here was that a difference existed between the elastic modulus and yield strength of the outer stratum medium and SMZA, with the outer stratum medium having higher values for both material properties. Data from our study support that hypothesis and, in turn, support the

proposed theory that the SMZA is a mechanical buffer zone between the outer stratum medium and laminar interface. When a load is applied to an equine digit, the stratum medium displaces and deforms to a greater magnitude, and at times in a different direction, from the displacement and deformation of the distal phalanx.⁸ This probably results from differences in the direction of the applied loading forces and unique material properties of the stratum medium and distal phalanx. Because of this, the inner wall, laminar interface, and submural dermis must accommodate this variability in movement. It has been documented that material properties of the submural dermis and laminar corium are vastly different from those of the outer stratum medium, indicating a higher elasticity, lower ultimate strength, and lower Poisson ratio than that of the outer stratum medium.^{6,14} The value from our study for elastic modulus of the SMZA (98.2 MPa) was between those of the outer stratum medium (187.6 MPa) and the laminar corium (2.1 MPa).⁷ This supports the concept of a gradient (ie, mechanical buffer zone) between the inner and outer components of the digit.

An accurate determination of the material properties of the various anatomic components of an equine foot is necessary for establishing a computerized 3-dimensional model of the foot for future research. Determination of the elastic modulus of the equine stratum medium has been accomplished by several researchers.^{6,8-10,16-18} Therefore, it may be assumed that this specific mechanical property of the stratum medium would be established and characterized. However, this is not the case (Table 1).

As stated previously, values of the stratum medium and SMZA for 1 of the feet in our study were reversed, compared with values for all other feet. One consideration is human error and that the sample data were inadvertently reversed. On the basis of the numbers, this was an attractive explanation; however, we determined that this was not the case. Therefore, it is more likely that this foot had pathologic changes that were not grossly visible within the stratum medium or SMZA, or both, that became evident during determination of the elastic modulus. We eliminated the data for this foot, recalculated the mean \pm SD elastic modulus, and obtained the following results. For the stratum medium, the mean increased, but SD decreased (194.6 ± 24.3 MPa), whereas for the SMZA, both the mean and SD decreased (88.9 ± 32.4 MPa). Consequently, the percentage of the mean that is represented by the SD decreased for the stratum medium (from 22 to 12%), but it remained essentially the same for the SMZA (36%).

Values for elastic modulus determined from the study reported here add to the dissimilarities reported elsewhere for elastic modulus of the hoof wall, all of which must be accounted for before a finite-element model can be adequately constructed. Some of the variability of the reported data relates to variations in the experimental design and methods used to control for biological and technical factors that impact measurements (Table 1). When selecting hooves for use in an experiment, biological factors such as breed, foot size, and sex of the horse must be considered. Although it is

accepted that the overall structure and components of equine hooves are conserved among breeds of horses, ponies, and donkeys, it is still unknown whether more specific aspects of the stratum medium such as tubular density and hydration gradients are comparable. Therefore, all of these factors may potentially impact the elastic modulus of the stratum medium.

Hinterhofer⁶ and Butler⁹ provided evidence that breed and foot size may affect determination of the elastic modulus. Hinterhofer⁶ used Warmblood horses and calculated a mean elastic modulus of 761.8 ± 295.4 MPa. Butler⁹ used ponies exclusively and determined an elastic modulus of only 3.7 ± 0.1 MPa. We did not control for breed or foot size, and our mean elastic modulus was between values reported in those other studies.^{6,9}

In addition to those 2 studies, Reilly, et al²¹ documented a difference in tubular density when moving from the inner to outer surface through the hoof wall. In another study, Reilly, et al²² also documented a significant difference between horses and ponies in tubule density in 3 of the 4 zones of the stratum medium. This variation in tubular density potentially influences the elastic modulus of the tissue selected; therefore, it is important to select hooves from horses of several breeds when attempting to determine the average elastic modulus for the equine species. By incorporating several breeds in sample selection, it is logical that the influence of hoof size with regard to tubule density and bending modulus should be decreased.

The inner stratum medium is hydrated to a greater degree than is the outer surface,⁸ providing a gradient of relative hydration from the lamellar interface to the outer stratum medium. Because of this hydration gradient and the influence that the relative hydration of the stratum medium will have on material properties, hydration is a biological factor of paramount importance.

Finally, with regard to biological factors, sex of a horse also could influence the determined elastic modulus. Endogenous steroids affect the cornification process of the epidermis.²³ By including stallions, mares, and geldings in the protocol, the influence of sex on the elastic modulus will be minimized.

These biological factors may provide an explanation for the reversed values obtained for tissues from 1 foot in our study. In that foot, the outer stratum medium had a lower bending modulus (97.7 MPa) than did the respective SMZA (163.1 MPa). It is possible that tubular density in that hoof was altered as a result of an unrecognized pre-existing lesion. If the SMZA were undergoing premature cornification, it is plausible that the tubular density and hydration of the SMZA and stratum medium would be altered and reflected as changes in the elastic modulus. We considered performing histologic examinations to confirm this or to reveal other possible pathologic changes; however, it was deemed to be beyond the focus of our study.

In addition to biological factors that must be accounted for, there are technical factors involving collection, preparation, and testing of samples that can potentially affect results. Included in these are environmental influences prior to and at the time of collection of tissues, time elapsing between collection of tissues and preparation, time elapsing between prepara-

tion of tissues and testing, location for selection of samples, and testing protocol used. Studies have revealed that the relative hydration of the stratum medium can have a significant influence on the elastic modulus and J-integral values obtained during testing.¹⁶ It follows that environmental influences such as exposure of a foot to ground moisture prior to tissue collection or application of a topical agent to the surface of a hoof may influence its moisture content. Preliminary studies by our laboratory group have revealed that the greatest changes in relative hydration develop within the first 24 hours after exposure to a new environment.

Because it is established that relative hydration affects the elastic modulus of the stratum medium, it is imperative to determine the relative hydration of tissue samples prior to testing or to have control data available for assessing environmental effects on the tissues at the time of collection. In some studies, the relative hydration of the tested samples has been reported, whereas in others, investigators only tested samples when fully hydrated, a nonphysiologic condition. Analysis of preliminary data collected for the study reported here indicated that, using the protocol described, the relative hydration of the stratum medium from feet collected at the abattoir was $86.4 \pm 2.9\%$.

Time and method of tissue storage are technical factors that play a major role in hydration status and subsequent determination of the elastic modulus. Included in this are the techniques used and the time that elapses between hoof collection and sectioning as well as between sectioning and the actual testing procedure. From a physiologic standpoint, it would be ideal to subject tissues to the testing procedure immediately after a horse has been euthanatized and tissues collected. However, this is not always feasible. It is important to limit the interval from tissue collection to sectioning and subsequent testing. Also, it is important to limit dehydration of samples by use of adequate storage techniques. Review of other studies revealed large disparities with regard to the time that elapsed between tissue collection and sectioning. In 3 studies,^{10,16,18} days elapsed before the tissues were tested. In another study,⁶ only 2 hours elapsed from tissue collection to sectioning, but those authors did not mention the interval from sectioning to application of the testing procedure. Most reports contain comments that the tissues were not frozen and that the tissues were maintained in plastic bags to help prevent dehydration of the samples. The effects of freezing on elastic modulus of the stratum medium are not known at this time.

In the study reported here, feet were collected approximately 12 hours after horses were euthanatized, and each whole foot was stored in a temperature- and humidity-controlled environment to minimize loss of moisture. By maintaining the hooves in this way, exposed surface area of the samples was minimized when compared with storage after sectioning of the stratum medium. In this study, there was a 4-hour time lapse between sectioning of the tissues and application of the testing procedure. During that time, tissues were maintained at room temperature (24 C) in towelettes dampened with physiologic saline solution, which limited desiccation.

Location on each hoof from which samples are collected is another technical factor that must be addressed. Zones of decreasing tubular density throughout the thickness of the stratum medium²² and increasing hydration gradients from outer to inner surface⁶ potentially affect the elastic modulus; therefore, it is imperative to precisely define the location from which samples will be obtained. Although most reports contain adequate descriptions concerning location of tissue samples with regard to dorsal versus lateral, most lack sufficient descriptions in regard to depth or thickness of the obtained stratum medium. Terms such as outer and inner have been used when describing regions of the stratum medium; however, a lack of definition for these terms is apparent. There may be substantial differences in the elastic modulus of sectioned tissues with respect to the thickness of tissues and tubular density in the tested region. Because of the possible influences of tubular density and hydration gradient on the tissue's material properties and the concept that the SMZA potentially provides a buffer zone between the rigid hoof wall and flexible laminar interface, we chose to compare the elastic modulus of the outer stratum medium and SMZA, establishing strict definitions for each.

The testing protocol used for determining the index of the relative elasticity of tissues is a technical factor of major importance in any study. In most studies, investigators applied tension^{5,6,10,16} or compression^{5,8,9,18,g} to the tissues, whereas in the study reported here, force was applied to produce bending, which represents a more physiologic deformation of a hoof when under load. Some studies involved repeated application of the loading force to the same sample without commenting on the potential for the tissue sample to have surpassed its yield point, possibly becoming damaged during earlier testing.⁶ The rate of loading on a tissue can affect the elastic modulus.¹⁰ However, it is unknown at this time what loading rate would most represent physiologic conditions during various gaits.

It is important to be familiar with intrinsic properties of the tissues and whether the tissues are considered to be isotropic or anisotropic. If a tissue sample has differing properties when tested in various directions, it is considered to be anisotropic, whereas if the properties are similar in all directions, it is described as isotropic. Orientation of anisotropic tissue samples during testing will influence the calculated elastic modulus, because values for the elastic modulus and yield strength of these tissues will differ when tested in various directions. Because of its unidirectional tubular configuration, stratum medium is an anisotropic tissue. Although testing samples in various directions would result in differing values for the elastic modulus, their relationship to each other should remain constant for each of the various testing protocols (ie, bending, compression, or tension). Therefore, as stated previously, it is important to bear in mind the direction of sample testing when comparing values reported here with values from other studies.

Studies regarding the elastic modulus of the equine hoof wall must include certain controls to create more uniform data that can be used in the development of a 3-dimensional finite-element model of an

equine foot. To ensure this necessary uniformity in data collection, we recommend the data collected and controls used include a description of the tubular density and relative hydration of the samples being tested, the samples be maintained in a manner that preserves physiologic hydration until the time of testing, and the bending testing protocol be used, which more accurately reflects the physiologic manner in which a foot of a horse deforms under physiologic loading conditions. We have attempted to incorporate these factors and have provided evidence to support the concept that there is a mechanical gradient between the stratum medium and the coffin bone that permits transmission of high loads without damage to the tissues. It should be realized that even when these controls are used in data collection, true variability may exist among breeds of horses. Therefore, it may be difficult to develop a single finite-element model that will accurately reflect the feet of all horses.

^aHood DM, Hogan HA, Grosenbaugh DA, et al. Dorsal hoof wall deformation in static weight bearing, in *Proceedings*. 10th Annu Meet Assoc Equine Sports Med 1991;44.

^bAngerhausen H. *Histologie zur weissen linie am huf des pferdes*. PhD dissertation, Tierärztlichen Hochschule, Hannover, Germany, 1941.

^cTesting machine model 1125, Instron, Canton, Mass.

^dLVDT model E100 ± 0.254 mm, Lucas Control Systems, Hamton, Va.

^eTable Curve 2D, version 2.03, Jandel Scientific, Chicago, Ill.

^fMicrosoft Excel 97, Microsoft Corp, Redmond, Wash.

^gLeach DH. *The structure and function of the equine hoof wall*. PhD dissertation, Department of Veterinary Medicine, Western College of Veterinary Medicine, University of Saskatchewan, SK, Canada, 1980.

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