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Effect of Specimen Size and Aspect Ratio on the Tensile Properties of Porcine Aortic Valve Tissues

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Abstract—The measurement of mechanical properties of biological tissues is subject to artifacts such as natural variability and inconsistency in specimen preparation. As a result, data cannot be easily compared across laboratories. To test the effects of variable specimen dimensions, we systematically modified the size and aspect ratio (AR) of porcine aortic valve tissues and measured their stiffness and extensibility. We found that: (i) as the AR of circumferential specimens increased from 1:1 to 5:1, their stiffness increased by 36% ($p<0.001$) and their extensibility decreased by 21% ($p<0.001$); (ii) as the AR of radial specimens increased from 0.8:1 to 4:1, their stiffness increased by 36% ($p<0.001$) and their extensibility decreased by 34% ($p<0.001$); (iii) as the size of circumferential specimens was reduced from 128 to 32 mm^2 at fixed AR (2:1), their stiffness decreased by 6% ($p=0.05$), and their extensibility increased by 17% ($p<0.001$); and (iv) as the size of radial specimens was reduced from 72 to 32 mm^2 at fixed AR (2:1), their stiffness decreased by 7% ($p=0.03$) and their extensibility increased by 16% ($p=0.005$). Thus, as specimens of constant length became narrower, they became stiffer and less extensible, and as specimens of fixed aspect ratio became smaller, they became less stiff and more extensible. Statistical models of these trends were predictive and can thus be used to integrate materials test data across different laboratories.
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Keywords—Stiffness, Extensibility, Predictive model, Specimen area.

INTRODUCTION

The ability to accurately characterize the mechanical properties of heart valve tissues is important for a number of reasons. First, since the mechanical properties of replacement materials should mimic those of native tissues,[12–14,17] precise measurement of mechanical properties is required. Second, finding differences in mechanical properties between intact and failed bioprosthetic valves may help identify the mechanism of their failure. Third, identifying the subtle changes in tissue mechanics that accompany disease and aging may help researchers develop strategies to treat these conditions in patients. It is, therefore, critical that the mechanical properties of biological tissues, such as heart valves, be measured accurately in any study of their function.

Uniaxial tensile testing is perhaps the most popular means of measuring the mechanical properties of soft tissues. Although it is used to quantify one-dimensional mechanics, it can be adapted to analyze anisotropic materials by testing specimens at different orientations. Multiaxial testing is more appropriate for anisotropic tissues but unidirectional testing is easier to perform and control, and it produces data that can be easily analyzed and interpreted. This viewpoint is supported by the wealth of data in the literature on the one-dimensional mechanical properties of soft biological tissues.[1,2,5,7,9–11,15–18]

These reports, however, are full of inconsistent and contradictory data. For example, the elastic modulus of circumferentially oriented native porcine aortic valve tissues has ranged from 6.7 MPa[11] to 17.97 MPa,[10] and the extensibility has ranged from 12.79%[10] to 50%.[15] Similarly, the modulus of radially oriented tissues has ranged from 1.85 MPa[10] to 4 MPa,[15] and the extensibility has ranged from 33%[10] to 83%.[15] Normal biological variability is believed to account for the majority of the scatter of data within test samples in any given laboratory. Inconsistency in extracting material parameters from highly nonlinear material test data is another major source of the variation, particularly when data are compared between laboratories. Inconsistent data may also result from the use of different strain rates and target loads or variable specimen size and dimensions.

The tensile mechanics of inhomogeneous and anisotropic materials (e.g., heart valves) are expected to vary with test specimen dimensions as different fibers are severed and others retained in the selected test specimen. The absolute size and aspect ratio of the selected speci-

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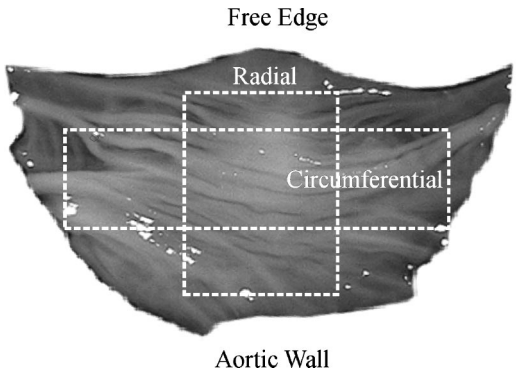

FIGURE 1. Image of a porcine aortic valve cusp showing the orientation of circumferential and radial test strips. Note that collagen fiber bundles originate at the aortic wall, subdivide, fan-out, and coalesce in the belly of the cusp. Fiber bundles are larger and more isolated at the periphery of the cusp. At the center, they are smaller and more uniform in density.

men may, therefore, affect the measured mechanical properties. We note that for the data cited above, Rousseau *et al.*[11] used 3-mm-wide circumferential strips, Leeson-Dietrich *et al.*[10] used 5-mm-wide strips, and Tan and Holt[15] used 6-mm-wide strips.

Regardless of the source of experimental variability, its presence has meant that mechanical test data from various laboratories cannot be easily compared and integrated. This limits the usefulness of such data. We suspect, however, that much of the intergroup variability results from systematic effects such as specimen size and dimensions and can thus be modeled. The objectives of this study, therefore, were to isolate and investigate the effects that specimen size and dimensions have on mechanical properties measured from uniaxial tensile tests. We were specifically interested in determining whether specimen size and aspect ratio affect the measured stiffness and extensibility of fresh porcine aortic valve cusps in a way that can be described via a predictive model.

MATERIALS AND METHODS

Specimen Preparation

Aortic valve cusps were harvested from porcine hearts obtained fresh from the abattoir. Radially and circumferentially oriented rectangular strips of specified dimensions were dissected from the central region of each cusp. The circumferential direction was defined as parallel to the main collagen fiber bundles visible from the outflow aspect, while the radial direction was defined as transverse to these fibers (Fig. 1). A custom-made cutting block with parallel grooves separated by distances equal to the required widths of the specimens was used to ensure that the dissected strips were cut precisely and uniformly. Specimen dimensions were varied between mechanical tests according to the test protocols described below.

Test Protocols

To investigate the effects of varying the aspect ratio, the width of each specimen was progressively reduced by trimming 1 mm of tissue from both sides of the specimen while keeping its length constant. Circumferentially oriented specimens ($n=27$) were trimmed from an initial dimension of 10 mm$\times$10 mm to 10$\times$8, 10$\times$6, 10$\times$4, and 10$\times$2, and radially oriented specimens ($n=29$) were trimmed from 8 mm$\times$10 mm to 8$\times$8, 8$\times$6, 8$\times$4, and 8$\times$2. Specimens were subjected to tensile testing at their initial dimensions and at each new aspect ratio after trimming. To ensure that the memory of previous strain history was completely erased before further trimming and testing, the specimens were allowed to recover for 24 h at 5 °C in Hanks physiologic saline solution between tests.[2] The testing protocol for changes in aspect ratio, therefore, lasted five days (four changes in width). This study was designated Protocol 1 (Table 1).

To investigate the effect of absolute specimen size, each rectangular test specimen was trimmed in both length and width while the aspect ratio was kept constant at 2:1. Circumferential specimens ($n=34$) were trimmed from an initial dimension of 16 mm$\times$8 mm to 14$\times$7, 12$\times$6, 10$\times$5, and 8$\times$4 and radial specimens ($n=21$) were trimmed from 12 mm$\times$6 mm to 10$\times$5, and 8$\times$4. The method of trimming, sequence of testing, the interval between them, and the duration of the test protocol were the same as used in Protocol 1 above, except that the radial specimens were tested over three days (two changes in size). This study was designated Protocol 2 (Table 1).

Since Protocols 1 and 2 were performed over several days, the effects of dimensional changes could not be decoupled from the possible effects of accumulated strain due to specimen handling, dissecting, mounting, and repeated testing. We have shown previously[2] that the memory of strain that results from the handling and testing of heart valve tissues resets after 24 h. However, the effects of repeated testing (accumulated strain) and prolonged storage on mechanical properties are not known. To attribute changes in mechanical properties to changes in dimensions alone, two control studies were performed. In the first control study, circumferential (10 mm$\times$5 mm, $n=32$) and radial (8 mm$\times$10 mm, $n=16$) specimens were tested daily over five days without altering their dimensions. These specimens also remained in their grips between tests to minimize handling artifacts. The rest interval between subsequent tests was the same as used for Protocols 1 and 2. This protocol was, there-

TABLE 1. Experimental protocols and dimensions of test specimens.

Protocols	Circumferential test specimens (mm×mm)	Radial test specimens (mm×mm)
1[a] Variable aspect ratio at fixed length	10×10, 10×8, 10×6, 10×4, 10×2 ($n=27$)	8×10, 8×8, 8×6, 8×4, 8×2 ($n=29$)
2[b] Variable dimensions at fixed aspect ratio (2:1)	16×8, 14×7, 12×6, 10×5, 8×4 ($n=34$)	12×6, 10×5, 8×4 ($n=21$)
3[c] Control: fixed dimensions	10×5 ($n=32$)	8×10 ($n=16$)
4[d] Control: variable aspect ratio at fixed length	10×10, 10×8, 10×6, 10×4, 10×2 ($n=10$)	

[a]Protocol 1: same specimens in each group are tested daily over 5 days, immediately after a change in width.
[b]Protocol 2: same specimens in each group are tested daily over 5 days (circumferential specimens) or 3 days (radial specimens), after a change in length and width at a fixed aspect ratio of 2:1.
[c]Protocol 3 (control): a single group of test specimens is tested daily over 5 days without changing specimen dimensions.
[d]Protocol 4 (control): specimens are randomized, trimmed, and grouped according to aspect ratio and tested only once, all on the same day.

fore, designed to reveal any effects resulting from repeated testing and prolonged storage, and it was designated Protocol 3 (Table 1).

In a second control study that was performed retrospectively based on the findings of the first, we tested circumferential specimens that were grouped ($n=10$) according to aspect ratio, on the same day. Each specimen in a group was therefore tested only once. The specimens were randomized with respect to the type of cusp and the hearts from which they came. Since different specimens were used in each aspect ratio group, biological variability was introduced. However, this study eliminated the effects of repeated testing and prolonged storage. This study was designated Protocol 4 (Table 1).

Tensile Testing

Tensile testing was performed with an Instron 8511 servohydraulic testing machine (Plus series, Instron, Canton, MA), using a 5.0 lb load cell (Sensotec, Columbus, OH). All tests were conducted in a bath of Hanks saline solution at 37 °C. Each specimen was held in sandpaper-lined plastic grips that were inserted between the actuator and the load cell of the testing system. Prior to testing, each specimen was brought to the same reference state by subjecting it to 25 preconditioning cycles under load control. The maximum test load was adjusted as required to give the same maximum tension (force per unit width—N/m) regardless of specimen size. The final loading curve after preconditioning was used for data analysis.

Data Reduction and Material Parameters

Load was converted to tension by multiplying by g/w, where g is acceleration due to gravity and w is the width of the specimen. Displacement was converted to percent strain by multiplying by $100/l_g$ where l_g is the specimen gauge length. Gauge length was estimated using a new method based on identifying the stationary point on the slope of the specimen's load-elongation curve at low loads.[3] For data reduction purposes, the loading data were fitted to a piecewise exponential-cubic function defined as follows:

$$T(\varepsilon)=\begin{cases} a(e^{b\varepsilon}-1)+c\varepsilon, & \varepsilon\leqslant\varepsilon_T, \\ d(\varepsilon-\varepsilon_T)^3+e(\varepsilon-\varepsilon_T)^2+f(\varepsilon-\varepsilon_T)+g, & \varepsilon>\varepsilon_T. \end{cases} \quad (1)$$

$T(\varepsilon)$ represents the loading tension at strain ε; ε_T, g, and f are the strain, tension, and slope, respectively, where the two functions join. The parameters a,c,d,e,f, and g have units of tension while b is nondimensional. We have found this function to fit the loading curves of

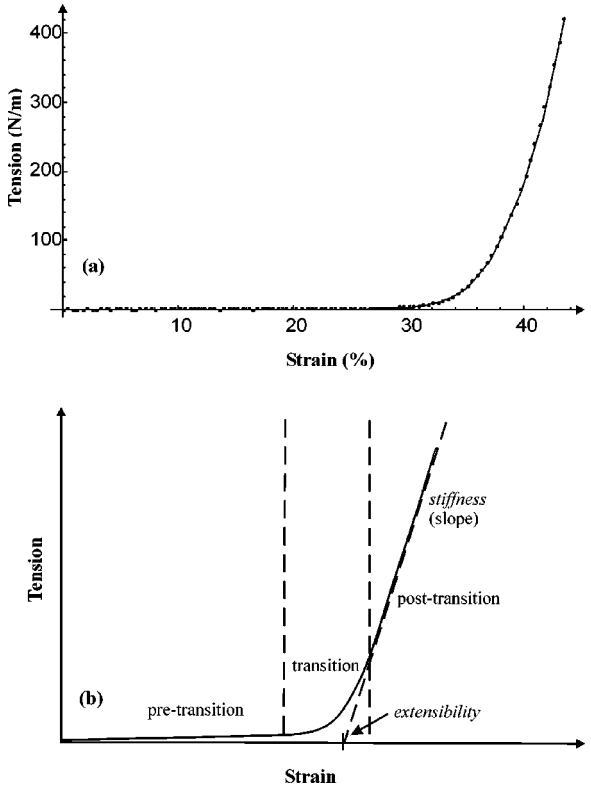

FIGURE 2. (a) Typical fit (data reduction) of porcine aortic valve tensile data by the function defined by Eq. (1). We used tension (force/specimen width) to describe valvular tissues because this avoids the errors associated with measuring tissue thickness. (b) Schematic representation of a typical elongation curve showing the region where material parameters (stiffness and extensibility) are evaluated. Stiffness was defined as the slope of the elongation curve at a particular tension in the post-transition region. Extensibility was taken as the strain where the asymptote to the elongation curve at that tension intersects the strain axis. Stiffness and extensibility were evaluated at a tension of 250 N/m for circumferential specimens and 100 N/m for radial specimens.

heart valve tissues, which typically have a long toe region, extremely well [Fig. 2(a)]. The function was fitted using the Mathematica® regression procedures *Fit and NonlinearRegress* (Wolfram Research, Champaign, IL).

Tension–strain curves of heart valve tissues are characterized by pretransition, transition, and post-transition regions [Fig. 2(b)]. The strain ε_T is typically chosen in the transition region. Each test specimen was characterized by its stiffness and extensibility in the post-transition ''linear'' region of its tension–strain curve. In this region, collagen fibers are thought to have lost their waviness, and the material's behavior is approximately linearly elastic. Stiffness (N/m) was defined as the slope of the linear region of the tension–strain curve. Extensibility was the strain (%) at which the asymptote to the linear region intersects the strain axis [Fig. 2(b)]. For consistency, stiffness and extensibility were computed at a tension of 250 N/m for circumferential specimens and 100 N/m for radial specimens (physiologic values).

Statistical Analysis

Descriptive statistics (mean ± standard deviation) were calculated for each set of data. To account for multiple measurements from the same tissue, longitudinal methods were used. Mixed model repeated measures analysis was performed to assess the effects of aspect ratio, specimen size, and duration of storage on mechanics (stiffness and extensibility). Various variance component structures were considered, and an unstructured variance was assumed in the final models.[6] Different transformations of the independent variables were tested to determine if model fits could be improved. The statistical analysis was performed using SAS® version 8 and a significance level of 0.05 was used for all statistical tests.

RESULTS

Both the specimen size and specimen aspect ratio affected the measured mechanical properties of porcine aortic valve cusps. As specimens of fixed length became narrower (Protocol 1), they also became stiffer and less extensible (Fig. 3). Stiffness and extensibility were associated with the aspect ratio in a nonlinear way ($p < 0.001$). As the aspect ratio increased from 1:1 to 5:1 in the circumferential specimens, the stiffness increased by 36%. As the aspect ratio increased from 0.8:1 to 4:1 in the radial specimens, the stiffness again increased by 36%. The extensibility of the same specimens decreased by 21% and 34%, respectively, for the same changes in aspect ratio.

As the size of test strips was progressively reduced by trimming the length and width while keeping a fixed aspect ratio (Protocol 2), they became less stiff and more extensible (Fig. 4). Stiffness and extensibility were linearly associated with specimen size (circumferential stiffness, $p = 0.05$; circumferential extensibility, $p < 0.001$; radial stiffness, $p = 0.03$; radial extensibility, $p = 0.005$). As the area of the circumferential specimens decreased from 128 to 32 mm^2, the stiffness decreased by 6%. As the area of the radial specimens decreased from 72 to 32 mm^2, the stiffness decreased by 7%. The extensibility of the same specimens increased by 17% and 16%, respectively, for the same changes in specimen size.

Results from the first control study (Protocol 3, Fig. 5) indicated that stiffness and extensibility changed linearly with duration of storage (day of test less one day). Of these changes, the change in radial stiffness was not statistically significant ($p = 0.48$), but the others were: (circumferential stiffness, $p = 0.004$; circumferential extensibility, $p < 0.001$; radial extensibility, $p = 0.03$). In the control specimens, circumferential stiffness and radial extensibility increased (5% and 6%, respectively),

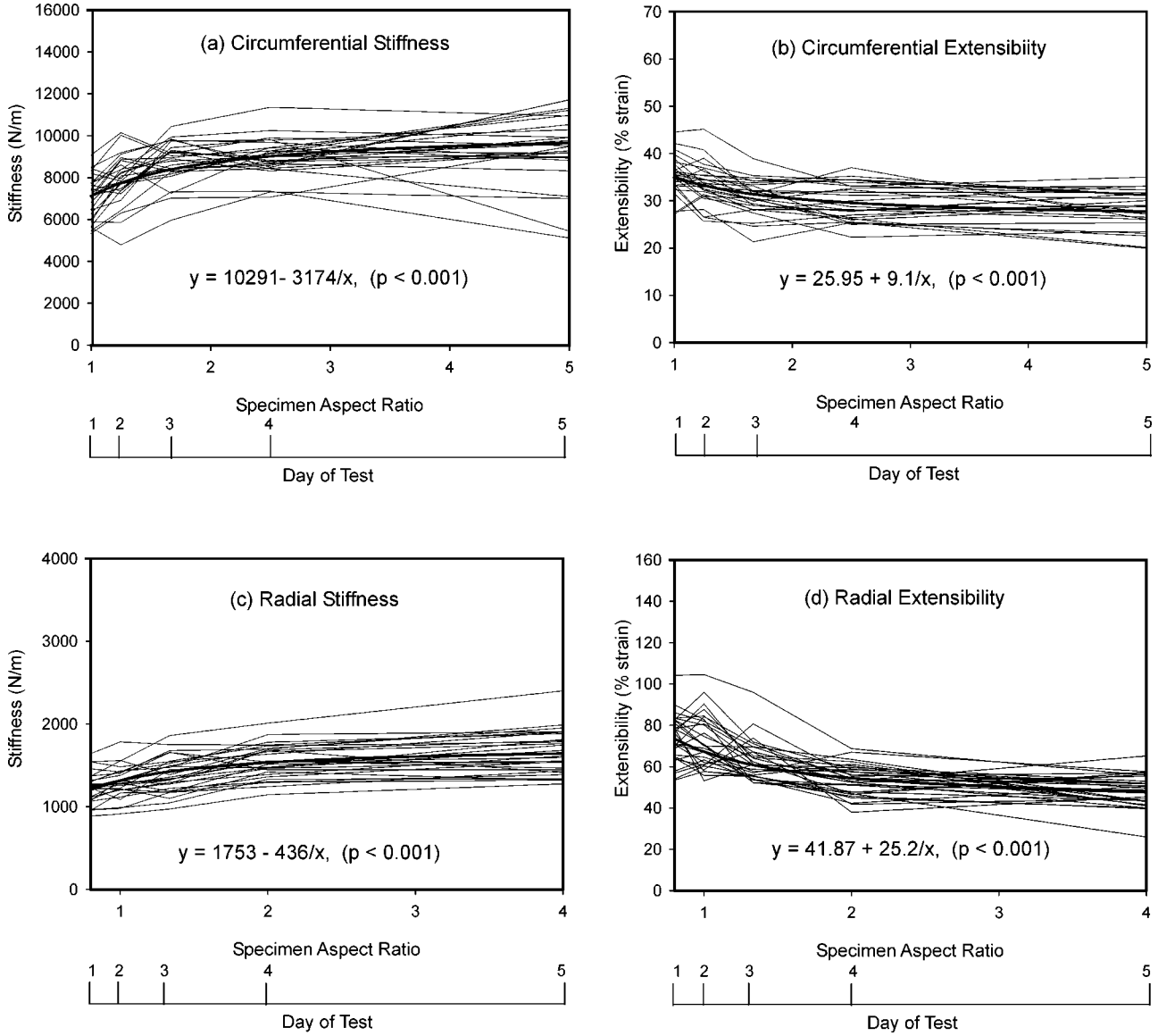

FIGURE 3. Variation of stiffness and extensibility with aspect ratio at fixed length (Protocol 1, Table 1). Bold curves are models (equations given in insets) generated from longitudinal repeated measures statistical methods with unstructured variance (Ref. 6). Nonlinear models relate stiffness and extensibility to aspect ratio ($p<0.001$).

and circumferential extensibility decreased (7%) during the five days of repeated testing and storage. For the circumferential specimens, we concluded that the increase in stiffness and reduction in extensibility that was observed following an increase in specimen aspect ratio (Protocol 1) were likely exaggerated because the controls showed the same trends. Conversely, the reduction in stiffness and increase in extensibility observed following a decrease in specimen size (Protocol 2) was likely underestimated because the controls showed the opposite trends. For the radial specimens, we concluded that the observed reduction of extensibility with increasing aspect ratio was likely underestimated whereas the observed increase in extensibility with decreasing specimen size was likely exaggerated, for the same reasons. We note that the association of mechanics with accumulated strain and duration of storage was stronger in the circumferential direction as indicated by the smaller p values.

The second control study (Protocol 4) was designed to eliminate the effects of accumulated strain and prolonged storage. Since the first control study demonstrated that the effects of accumulated strain and duration of storage were greater in the circumferentially oriented specimens, only specimens of this orientation were tested under Protocol 4. The results confirmed that stiffness indeed increased and extensibility decreased as aspect ratio was increased (Fig. 6). The associations were nonlinear in both cases ($p<0.001$) and similar to those observed in

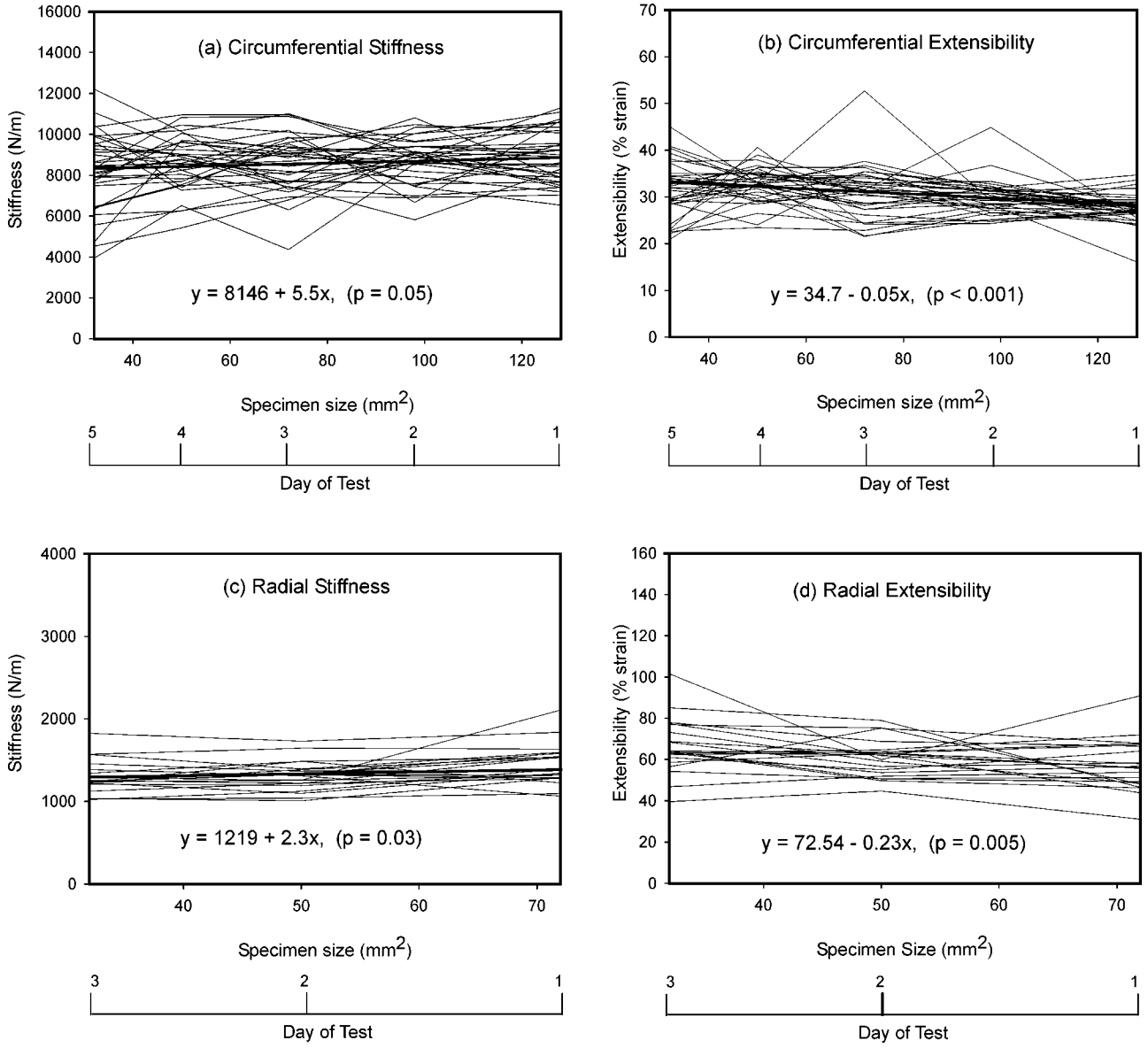

FIGURE 4. Variation of stiffness and extensibility with specimen size at a fixed aspect ratio of 2:1 (Protocol 2, Table 1). Models (bold curves) were obtained as in Fig. 3. Linear models relate stiffness and extensibility to specimen size ($p=0.05$ or better).

Protocol 1 when the same specimens were tested over several days. We, therefore, concluded that even though repeated testing and prolonged storage affected mechanics, the observed changes in material parameters as a function of the aspect ratio and specimen size were real.

DISCUSSION

In this study, we investigated the effects that test specimen size and dimensions may have on the measured mechanical properties of fresh porcine aortic valve cusps. This study was motivated in part by the observation of a large amount of variability in the published data. We wanted to determine specifically if a variable specimen size and aspect ratio could be responsible for some of the discrepancies in the reported data. Indeed, we found that specimen size and dimensions can significantly affect the measured material parameters of porcine aortic valve cusps. As specimens were trimmed in width (increasing aspect ratio), they became stiffer and less extensible regardless of orientation. As specimens were trimmed along both length and width (reducing specimen size) while keeping the aspect ratio constant, they became less stiff and more extensible, again, regardless of orientation. By using statistical methods, we were able to derive predictive models for the above trends.

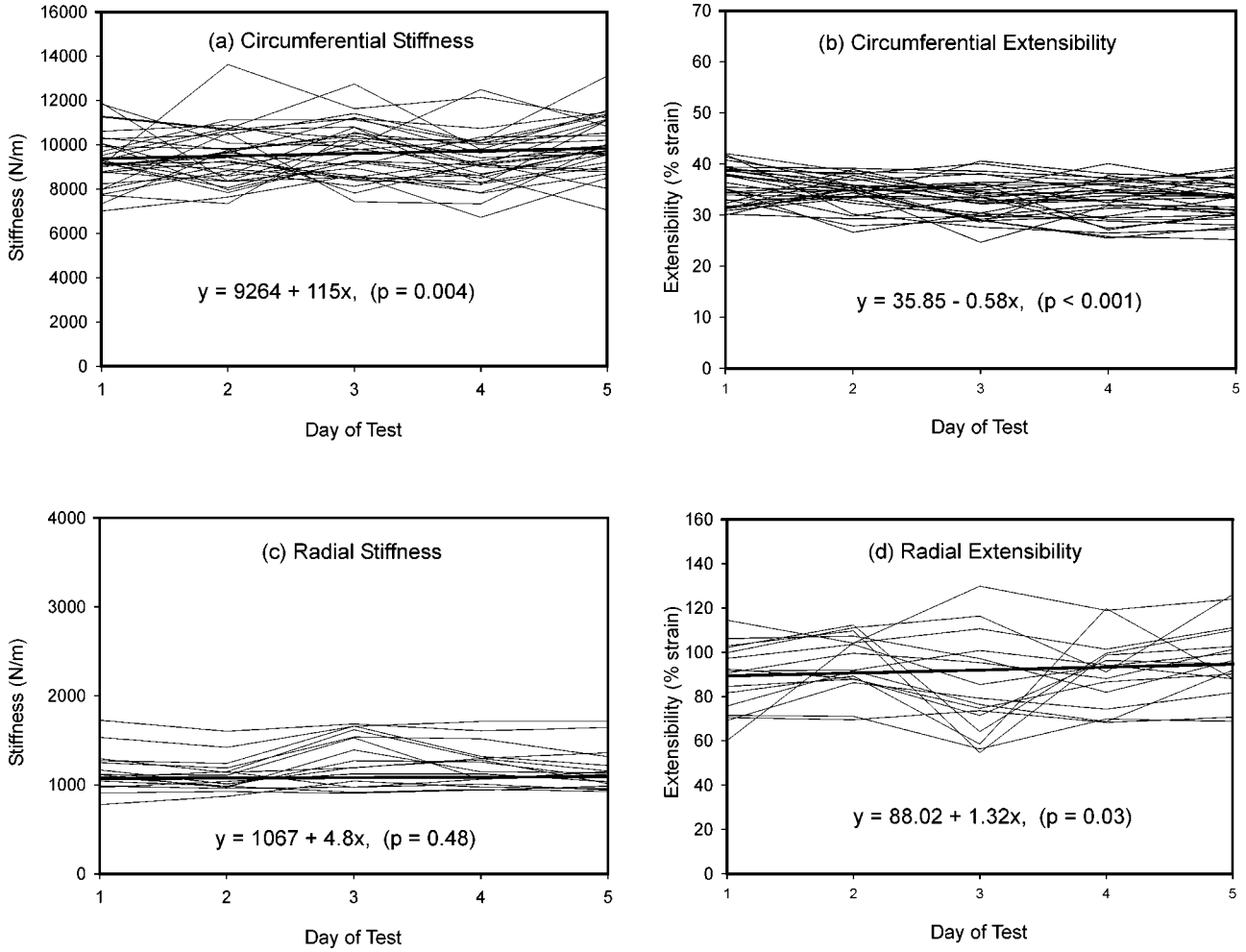

FIGURE 5. Control study: variation of stiffness and extensibility of specimens of the same dimensions tested over a 5-day period (Protocol 3, Table 1). Models (bold lines) were obtained as in Fig. 3. Linear models relate extensibility and circumferential stiffness to duration of storage ($p=0.03$ or better). The relationship between stiffness and duration of storage in the radial direction was not statistically significant ($p=0.48$).

In order to test this predictive ability, we evaluated the stiffness and extensibility at an aspect ratio of 2:1 using the models of Protocol 1. We compared these predictions to the data obtained from independent tests (Protocol 2) on samples different from, but of comparable sizes to, those used for the predictions. The comparisons showed that predictions of circumferential stiffness (8704 N/m), circumferential extensibility (30.5%), and radial extensibility (54.5%), were within one standard deviation of the mean of the measured data (mean$\pm$SD): 8582 $\pm$1336 N/m, 32.6%$\pm$3.8%, and 66.2%$\pm$13.2%, respectively (Table 2). Prediction of radial stiffness (1535 N/m) was within two standard deviations of the mean of the measured data: 1293$\pm$192 N/m (Table 2). These results confirm that the models are indeed predictive.

The reasons for the changes in the mechanical properties of aortic valve tissues as a function of specimen dimensions may be explained by the morphology of the aortic valve cusp. The aortic valve cusp has three distinct layers: the fibrosa, the ventricularis, and the central spongiosa (Fig. 7). The fibrosa is dominated by collagen fiber bundles, the ventricularis contains large amounts of elastin, and the middle spongiosa layer is dominated by loose elastin and collagen fibers, glycosaminoglycans, and water.[4,12,18,19] Clearly visible from the outflow (fibrosa) aspect are collagen fiber bundles that increase in density toward the center of the cusp (Fig. 1). At the center, the fibrosa tends to be nearly isotropic once unfolded.[21] Also, there are considerable interactions between the many components of the valve cusp. We know for example, that the ventricularis is preloaded in tension, whereas the fibrosa is preloaded in compression radially, by virtue of the mutual attachment of the fibrosa and ventricularis.[20] If we assume that the mechanics of a strip of biological tissue are governed by the average fiber content, fiber distribution, and the interaction of the

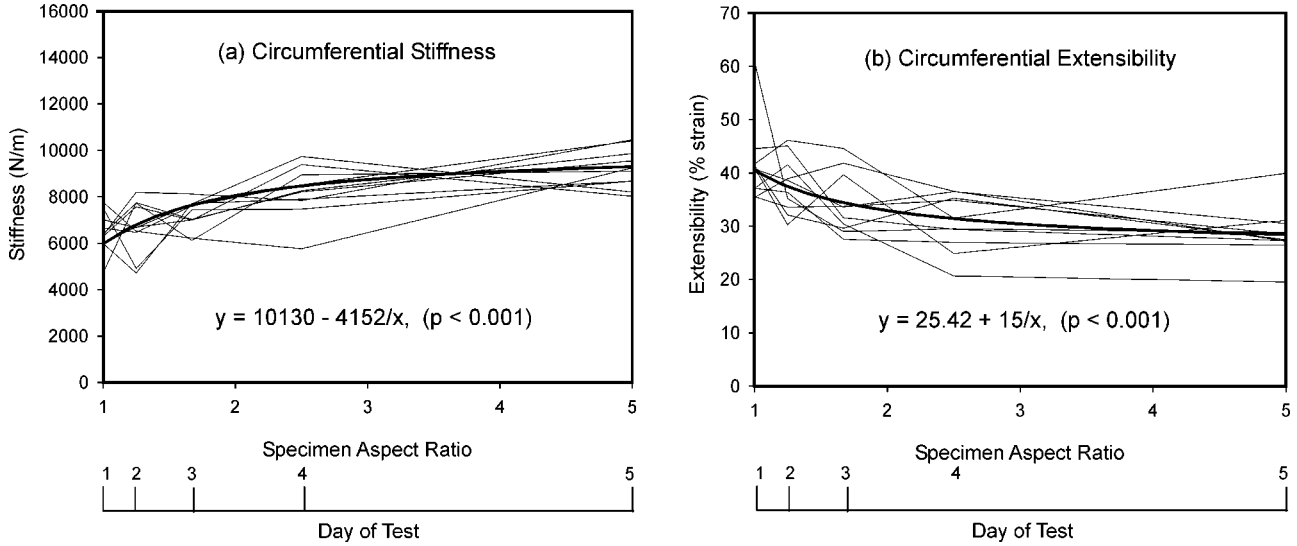

FIGURE 6. Control study: Variation of stiffness and extensibility with aspect ratio at fixed length for randomized circumferential specimens tested on the same day (Protocol 4, Table 1). Models (bold curves) were obtained as in Fig. 3. Nonlinear models relate stiffness and extensibility to aspect ratio ($p<0.001$), similar to the associations identified in Protocol 1 (Fig. 3) when the same specimens were subjected to daily changes in aspect ratio and tested over 5 days.

layers, then trimming away certain structures would alter the mechanics of the resultant tissue strip. We know that fiber density is greater and more uniform near the middle of the cusp and that most fibers are never completely parallel to the testing direction (Fig. 1). In addition, the larger fiber bundles that lie mainly at the periphery of a cusp (Fig. 1) are progressively excluded from a specimen by the trimming process. Thus, as specimens are trimmed to narrower dimensions at fixed length, elastic structures would be cut, possibly releasing some tension. This would result in the lengthening of the collagen fibers whose structures are held in compression by the tension in the elastin fibers. Fiber waviness and extensibility of the tissue would therefore decrease. Stiffness, which is defined as the ratio of the change in tension to the change in strain, would correspondingly increase. On the other hand, as specimens are trimmed along length and width toward the middle of the cusp, the larger fiber bundles that curve at the edges or are positioned off-axis to the main test direction will be cut away. This would certainly apply to circumferentially oriented specimens (Fig. 1). Narrower strips would therefore contain a greater proportion of smaller but nore uniformly oriented fibers and would thus be less stiff and more extensible. Stiffness therefore decreases and extensibility increases as specimen size is reduced.

The changes in the mechanical properties of aortic valve tissues as a function of specimen dimensions have

TABLE 2. Prediction of material parameters from aspect ratio models.

Material parameter	Model and prediction[a] for aspect ratio (x) = 2	Experimental data[b] (mean ± SD)
Circumferential stiffness	$y = 10291 - 3174/x$ 8704 N/m	8582 ± 1336 N/m
Circumferential extensibility	$y = 25.95 + 9.1/x$ 30.5%	32.6 ± 3.8%
Radial stiffness	$y = 17530 - 436/x$ 1535 N/m	1293 ± 192 N/m
Radial extensibility	$y = 41.87 + 25.2/x$ 54.5%	66.2 ± 13.2%

[a]Predictions are those from the models of Protocol 1 (Fig. 3, Table 1).

[b]Experimental data are from the independent experiments of Protocol 2 (Fig. 4, Table 1) on samples equivalent in size to those on which the predictions are based (50 mm^2 circumferential, 32 mm^2 radial). SD, standard deviation.

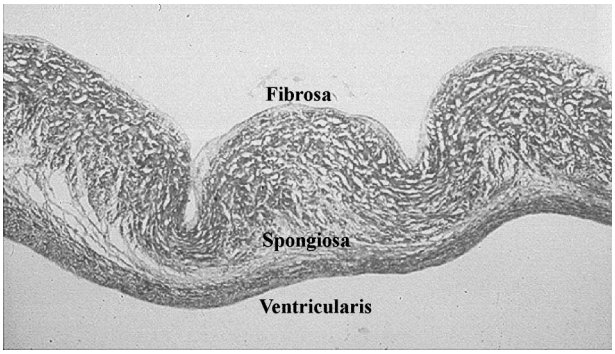

FIGURE 7. Histological image of a radial cross section through a heart valve cusp showing a layered structure. A corrugated fibrosa layer at the outflow surface of the cusp is dominated by circumferentially oriented collagen fibers. A smooth ventricularis exists at the inflow surface and contains large amounts of elastin fibers. A middle gel-like spongiosa layer contains sparse, randomly oriented collagen and elastin fibers. The ventricularis is preloaded in tension and the fibrosa is preloaded in compression radially, by virtue of their physical connection.

been attributed to their complex morphology (Fig. 7). It is, therefore, reasonable to assume that other soft biological materials of similar complexity would also exhibit size-related mechanical properties. On the other hand, materials that are more homogeneous and isotropic (e.g., the aortic wall, pericardium) or materials with a higher degree of fiber alignment (e.g., ligaments and tendons) would be expected to have mechanical properties that are less sensitive to specimen size. In general, extrapolating results from one biological tissue to another is ill advised because of the varying degrees of nonlinear elastic and viscoelastic responses exhibited by biological materials. For example, Atkinson *et al.*[1] showed that human patellar tendon, a material that is approximately transversely isotropic and structurally less complex than the aortic valve, has material parameters (elastic and viscoelastic) that change with cross-sectional area. The reported nonlinear decrease in the modulus of human patellar tendon with increasing cross-sectional area is consistent with the aspect ratio-related changes in stiffness that have been reported here for aortic valve tissues.

This study has also demonstrated that we can successfully precondition and test centered specimens as narrow as 2 mm, regardless of their orientation. This result is consistent with a fibrous structure of increased density toward the center of the cusp, implying that grip-to-grip contiguity of fibers is maintained in the middle 2 mm of the valve cusp. Our results contradict those of Lee *et al.*[8] who observed that circumferential strips less than 4 mm in width and radial strips less than 6 mm cannot yield reproducible preconditioning curves. Most likely, the preconditioning phenomenon is load dependent.

In conclusion, we have shown that the material parameters of heart valve cusps obtained from uniaxial tensile testing vary with test specimen size and dimensions. The observed trends have been explained in terms of internal preload and fiber architecture. Models of the trends were established statistically and were shown to be predictive. Such models will allow data to be scaled between different tests and different laboratories so that they can be effectively compared and integrated. This will considerably enhance the usefulness of published uniaxial tensile test data.

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