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Biaxial Response of Passive Human Cerebral Arteries

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Abstract

The cerebral circulation is fundamental to the health and maintenance of brain tissue, but injury and disease may result in dysfunction of the vessels. Characterization of cerebral vessel mechanical response is an important step toward a more complete understanding of injury mechanisms and disease development in these vessels, paving the way for improved prevention and treatment. We recently reported a large series of uniaxial tests on fresh human cerebral vessels, but the multi-axial behavior of these vessels has not been previously described. Twelve arteries were obtained from the surface of the temporal lobe of patients undergoing surgery and were subjected to various combinations of axial stretch and pressure around typical physiological conditions before being stretched to failure. Axial and circumferential responses were compared, and measured data were fit to a four parameter, Fung-type hyperelastic constitutive model. Artery behavior was nonlinear and anisotropic, with considerably greater resistance to deformation in the axial direction than around the circumference. Results from axial failure tests of pressurized vessels resulted in a small shift in stress-stretch response compared to previously reported data from unpressurized specimens. These results further define the biaxial response of the cerebral arteries and provide data required for more rigorous study of head injury mechanisms and development of cerebrovascular disease.

Key Terms

cerebral artery properties; mechanical testing; traumatic brain injury; head injury; cerebrovascular disease

Introduction

The cerebral blood vessels play a critical role in the health and maintenance of the brain, but their proper function can be disrupted by trauma or by the development of other disease. Approximately 1.4 million people suffer traumatic brain injury (TBI) each year in the United States; of these, 50,000 die¹⁵. Total costs associated with both mortality and morbidity of TBI were estimated at 60 billion dollars in the year 2000⁵. Traumatic brain injury commonly includes damage to the vessels⁸, manifest as hemorrhage following mechanical disruption of the vessel wall. Even in the absence of obvious mechanical damage, trauma may additionally produce physiological dysfunction in the vessels leading to transient breakdown of the blood-brain barrier or to loss of cerebral autoregulation. Stroke, or brain ischemia due to lack of blood supply, occurs in approximately 780,000 people each year, resulting in about 150,000 deaths, and costs an estimated 65 billion dollars annually¹⁸. While stroke is generally considered separate from trauma, it is associated with similar disruption of vessel function. Such conditions may result from rupture of aneurysms or other cerebrovascular malformations, often also leading to vasospasm, or from embolism or thrombosis of the vessels. Efforts to better

understand these injuries and disease processes commonly include modeling techniques requiring knowledge of vessel properties, but only a limited set of data has been available for the cerebral vessels. Definition of cerebrovascular mechanical response is thus a critical step toward enhanced understanding of the mechanisms by which vascular injuries occur and also serves as an important foundation for study of cerebrovascular disease¹³ and treatment.

The few previous studies of the mechanical properties of cerebral arteries have revealed some of the fundamental characteristics of these vessels, including observations that they are stiffer in both the axial^{2, 16} and circumferential^{2, 9} directions than most extracranial arteries and that cerebral arteries are stiffer than cerebral veins¹⁶. This increased stiffness also leads to a relatively low extent of stretch to failure, at least in the axial direction. The increased stiffness may be a result of the presence of an additional layer of adventitial pia-arachnoid tissue the cerebral vessels acquire (and lose) and they pass into (and out of) the subarachnoid space¹. Work from our lab has also shown that large and small cerebral arteries, referring respectively to those in or near the Circle of Willis and those found on the surface of the cortex, behave similarly in the axial direction¹⁷. This is an important consideration given that most other studies of the cerebral arteries have focused on large vessels. We have also demonstrated that arteries from autopsy tend to be less extensible than those obtained fresh from surgery¹⁷. It has additionally been reported that cerebral arteries become less distensible with age^{2, 9}, at least in the circumferential direction.

While previous studies of passive human cerebral arteries have revealed some of their unique characteristics, the multi-axial response of healthy vessels over a broad range of loading conditions has not been defined. Our objective was to provide this information in order to allow more rigorous study of head trauma and other conditions and diseases affecting these vessels.

Procedure

Specimen Preparation and Testing Apparatus

Following procedures described previously¹⁶, 12 normal cerebral arteries were obtained from the lateral surface of the temporal lobe of 6 male and 5 female patients undergoing surgery to treat epilepsy. Patient age ranged from 16 to 62 years, with a mean of 37 years. Specimens were collected and tested over a period of two years. Signed, informed consent for the use of tissue was obtained from each patient using an approved human subjects protocol (UCSF Committee on Human Research).

Prior to resection, a piece of suture was placed next to the specimen to be removed and was cut to identify the vessel's *in vivo* length. Following removal, specimens were immediately placed in a container of cold physiological saline and transported to the laboratory in a cooler. Both specimen and suture lengths were measured to identify zero-load and *in vivo* lengths, respectively, and pia-arachnoid and brain tissue commonly resected along with the artery were carefully dissected away under a stereo microscope. Most arterial segments from the temporal surface include numerous small branches that are intimately associated with the pia-arachnoid tissue that surrounds them in the subarachnoid space. Due to the small size of some branches and the surprising strength of the membranous tissues, it was easy to tear the branches at their connection to the main vessel segment such that the specimen could not be subsequently pressurized. Following removal of surrounding tissue, thin cross-sections were cut from the ends of the main segment, photographed under a microscope, cut radially to release residual stresses, and then photographed again once the section appeared to have reached equilibrium. Branches were ligated with a single fibril of unwound 6-0 silk suture. Ends of the main vessel segment were then tied, using 6-0 silk suture, to blunt-tipped, luer-hub, 25–30 G (depending on specimen diameter) needles attached to acrylic blocks through which holes had been drilled to allow fluid passage and pressure measurement; grooves were machined around the end of

the needles to give purchase to sutures and reduce slippage of the specimen under axial tension. The relative position of the blocks was then fixed with a length-adjustable support arm connecting them, such that the assembly could be moved without risking damage to the vessel, and saline was gently pushed into the vessel lumen at low pressures. Following vessel attachment, the specimen was removed from the saline bath, fluid around it was wicked away with a small strip of paper towel, and a small amount of cyanoacrylate was applied around the vessel-needle interfaces – on the distal sides of the sutures – to ensure a good seal. While the glue was drying, 10 μm microspheres (Polysciences, Inc.; Polybead) were spread over the vessel surface to allow visual tracking of deformations during testing. Attached between one of the needles and its associated block was an acrylic tube over which a square polystyrene cuvette, with a round hole cut out of its bottom, could slide so as to be out of the way during the vessel attachment process. Once the vessel was attached, the assembly was oriented vertically, the cuvette was slid down over the specimen, its base was pressed over a gasket around the hub of the opposite needle, and the cuvette was filled with room-temperature saline to immerse the specimen.

Following specimen preparation, the assembly was connected to the actuator of a computer-controlled servo-hydraulic load frame (MTS, Model 858 Mini-Bionix) on which testing was conducted (Fig. 1). The upper acrylic block was attached to the actuator, and the position of the actuator was carefully lowered until the lower acrylic block was in position to attach to an 11 N load cell (Transducer Techniques, MDB 2.5) fixed to the rigid base of the load frame. The support arm was then released and removed so specimen length would change with actuator position. The chamber of the lower acrylic block was then connected to a saline delivery system, a linear actuator (Ultramotion, Digit) driving the position of a syringe. Pressure was measured with a 200 kPa pressure transducer (Honeywell, MicroSwitch 26PCDFM6G) attached to the upper block. Actuator displacement was monitored through the linear variable displacement transducer (LVDT) built into the load frame, and specimen deformations were recorded (3 frames/sec) using a digital camera (Pixelink, PL-A641), with an attached high-powered zoom lens (Edmund Optics, VZM 450i; FOV: 1.4–17.6 mm), connected to a computer. Typical image resolution was 0.004 mm/pix. All non-video signals were recorded at 100 Hz using the data acquisition (DAQ) card provided with the load frame system.

Mechanical Testing

Since structural and fluidic rearrangements likely occur in vessels between the time of resection – when natural loads are removed – and testing, a preconditioning process was completed prior to testing to restore, as closely as possible, the vessels to their *in vivo* state. The process consisted of a series of typically 3 to 4 inflation tests in which the vessel was stretched axially to a target position, and its length was held constant for approximately two minutes while pressure in the vessel was cycled 5 times between 6.7 and 20.0 kPa (50 and 150 mmHg). The target axial position was gradually increased from one test to another, guided initially by the *in vivo* length measurement, until measured force increased noticeably with pressure (as a result of vessel length exceeding the *in vivo* length²²) and reached peak values near 0.1 N. Between these preconditioning tests, vessel length and pressure were held constant at the estimated *in vivo* length and 13.3 kPa (100 mmHg). Following these initial procedures, a test was conducted in which specimen length was reduced sufficiently to induce buckling and then increased to the maximum value utilized during preconditioning, all while a valve connected to the upper block was open so as to maintain near-zero pressure; this reference test provided for identification of the unloaded specimen length for use during data analysis. The vessel was then subjected to a series of three inflation tests where vessel length was held constant at various values and pressure was increased from near zero to approximately 20 kPa (same range as for preconditioning tests) and three axial stretch tests where pressure was set to a target value and the length of the specimen was cycled between a state of compressive buckling and the

maximum length used in previous tests. Each of these six tests involved five identical cycles, the fifth of which was used for determination of mechanical properties. Typical stretch ratios obtained during the six tests for one specimen are shown in Fig. 2. The illustrated stretch paths are labeled in the order in which they were performed. Following the six physiological-level tests, each specimen was pressurized to approximately 13.3 kPa and stretched axially to failure. All tests were conducted at quasi-static rates.

Data Processing and Analysis

Changes in outer diameter and the axial position of selected microspheres during the tests were determined through subsequent analysis of the recorded video using a program written in LabVIEW (National Instruments) and utilizing tools in its companion Vision Development Module. Actuator displacement recorded in the video was similarly determined and compared to LVDT measurements to allow synchronization of video and DAQ card data. Because only three images were saved during each second of testing, points were added to the video data in order to obtain a one-to-one correspondence with DAQ-recorded measurements; added data values were defined by interpolation between measurements. The resulting video data were then smoothed using the phaseless low-pass digital filter specified in SAE J211¹⁹. Filtered traces were compared to original data to ensure fidelity, though the process often resulted in a waviness, as seen in Fig. 2, that was on the same order of magnitude as the level of noise in the original measurements. Load cell data were similarly processed to filter noise due to machine vibrations. Because the pressure transducer was positioned approximately 8 mm above the center of the specimen, recorded pressure values were increased by a value of 0.8 kPa to account for pressure due to the saline column height.

Measurement software was also used to define cross-section characteristics. Cross-sectional area was measured by tracing the inner and outer contours of each uncut cross-section and taking the difference of their calculated areas. Zero-load outer diameter was defined as the average outer diameter along two perpendicular lines passing through the center of the uncut section. For slightly elliptical sections, these two lines were chosen as the major and minor axes of the ellipse. Outer diameter in the zero-stress configuration was estimated by drawing a circle, having a radius approximately equal to that of the cross-section arc, over the image of the cut section. Using the center of the circle and underlying arc, the associated opening angle was then approximated as half the angle over which the section had receded when cut (applying the definition provided by Chuong and Fung⁴; also see Humphrey¹³, pp. 272–274). Zero-load arc length was then approximated as the product of opening angle and zero-stress outer diameter. Inner and outer arc lengths in both the zero-load (where arc length equals circumference) and stress-free configurations were also measured from images using software. Inner and outer values were averaged for each configuration and the result for the zero-load state was divided by that for the stress-free configuration to determine the zero-load circumferential stretch ratio.

Considering the vessels to be homogenous circular cylinders, mid-wall specimen stretch λ_i was defined using Equations 1,

$$\lambda_\theta = \frac{d_i + d_e}{D_i + D_e}, \quad \lambda_z = \frac{l}{L} \quad (1a, b)$$

where $i = \theta, z$ correspond to the circumferential and axial directions, respectively, and d_i, d_e , and l represent internal and external diameters, and axial distance between two microspheres in the current configuration; D_i, D_e , and L are corresponding values in the zero-load configuration. Internal diameters were approximated using the measured cross-sectional area A and the assumption of incompressibility. Reference length L was measured in the video frame from the unpressurized axial stretch reference test that most closely corresponded to the time

when measured axial force began to increase from zero. Mean Cauchy stress T_i was defined as specified in Equations 2,

$$T_\theta = p_i \frac{d_i}{d_e + d_i}, \quad T_z = \frac{\lambda_z}{A} \left(F + \frac{\pi}{4} d_i^2 p_i \right) \quad (2a, b)$$

where p_i is internal pressure and F is measured axial load, to satisfy global equilibrium.

To characterize the multi-axial response of the vessels, measurements were used to determine best-fit parameters for a candidate hyperelastic constitutive model. Applying the concept of pseudoelasticity, theoretical stresses were calculated for deformations measured during the loading portions of the three inflation and three axial stretch experiments. Theoretical stresses were derived from the Fung-type exponential strain energy function of Equation 3,

$$W = \frac{1}{2} c (e^Q - 1), \quad \text{with } Q = c_1 E_{\theta\theta}^2 + c_2 E_{zz}^2 + 2c_3 E_{\theta\theta} E_{zz} \quad (3)$$

where W is the strain energy, $E_{\theta\theta}$ and E_{zz} are the Green strains (using the zero-load reference configuration) in the circumferential and axial directions, c and c_i are material parameters, and Q is a dimensionless quantity. Incompressibility was enforced directly instead of using a Lagrange multiplier, so despite $W = W(E_{\theta\theta}, E_{zz})$, the formulation is three-dimensional^{13, 21}, though it assumes that principal directions of stress and strain coincide. Using measurements of both the vessel cross-section and the current state of deformation during the tests, the Cauchy stresses t_i , were calculated as

$$t_{\theta\theta} = t_{rr} + \lambda_\theta^2 \frac{\partial W}{\partial E_{\theta\theta}}, \quad t_{zz} = t_{rr} + \lambda_z^2 \frac{\partial W}{\partial E_{zz}} \\ \text{with } t_{rr}(r) = -P_i + \int_{r_i}^r (\lambda_\theta^2 \frac{\partial W}{\partial E_{\theta\theta}}) \frac{1}{r} dr \quad (4)$$

where r is radial position in the wall and $r_i = d_i/2$. These t_i were calculated at multiple radii through the vessel wall and averaged. Coefficients c and c_i were then optimized to minimize differences between these theoretical values and the mean stresses calculated directly from experiments (Eq. 2) using the Levenberg–Marquardt algorithm (Igor Pro, Wavemetrics). Twenty points from each test were used for the best-fit analysis.

In addition to presentation of standard stress-stretch results, values for stretch ratio, stress, and stiffness were estimated for the *in vivo* state. For the axial direction, force from the axial stretch tests was plotted against displacement, and the displacement at which the traces crossed was identified⁷; this displacement was always consistent with the specimen length at which force remained approximately constant while pressure increased during the preconditioning sequence. The axial stretch ratio for the axial stretch test having a pressure nearest 13.3 kPa was then plotted as a function of displacement, and the stretch ratio associated with the identified cross-over displacement was taken as the *in vivo* axial stretch value. The corresponding axial stress was then identified, and a line was fit to the stress-stretch curve over the *in vivo* stress-stretch point to approximate stiffness. A similar approach was applied to define the *in vivo* values for the circumferential direction. In this case, the inflation test having an axial stretch ratio nearest the already-defined *in vivo* value was identified, and the circumferential stress-stretch values corresponding to a pressure of 13.3 kPa in this test were defined as the *in vivo* values. A line-fit was similarly completed about this point on the stress-stretch curve to determine the circumferential stiffness for comparison to that in the axial direction. A paired t-test was used to test the statistical significance of differences between *in vivo* modulus values determined for the circumferential and axial directions for each specimen.

Comparison between specimens was accomplished by plotting one stress-stretch curve (separately for both inflation and axial stretch tests) most closely approximating *in vivo* conditions for each specimen. Because differences between the specimens appeared best described by shifts in stretch values, the potential significance of donor age on *in vivo* stretch values in the data were analyzed using multiple regression (Statacorp; Stata IC 10). Because the stretch ratio in the orthogonal direction of loading at the time of *in vivo* stretch determination was not consistent between specimens, this was used as the second independent variable in the multiple regression analysis. While stretch ratios defined in Equation 1 reference the zero-load configuration, additional analysis was conducted to view some of the measured data using the *in vivo* state as reference configuration. To accomplish this, stretch ratios defined in Equation 1 were divided by the identified *in vivo* stretch values.

Results

Cerebral artery response to biaxial loading was qualitatively consistent with what has been reported elsewhere for similar tests on other types of blood vessels. Figure 3 provides a graphical summary of loading conditions and measurements for three representative specimens. Detailed information for these specimens, and summary data for all specimens, is given in Table 1. As Fig. 3 (Row 1) shows for inflation tests, diameter increased with pressure in characteristic nonlinear, concave-downward fashion. Curves naturally shifted downward with increased fixed specimen length. Degree of nonlinearity in the diameter-pressure curves also tended to decrease with greater axial stretch. Figure 3 (Row 1) additionally demonstrates that axial force values during inflation tests increased more dramatically with pressure for higher levels of fixed axial stretch. Similar to diameter-pressure behavior during inflation tests, the force-displacement relationship in axial stretch tests (Fig. 3, Row 2) was nonlinear, and the arteries developed higher forces at lower axial stretch values for higher imposed pressures. While pressure decreased slightly with displacement for some cases (likely due to fluid leakage), it remained remarkably constant for most specimens, even though it was internal fluid volume, rather than pressure, that was held fixed during the tests. Row 2 of Fig. 3 also demonstrates the tendency for force-displacement traces in these tests to cross one another near the vessels' *in vivo* length^{7, 22}; these crossover displacements were consistent with specimen lengths where axial force remained relatively constant during pressure changes in inflation tests.

Calculated stress-stretch responses (Row 3 of Fig. 3) demonstrate a striking difference between axial and circumferential loading. In particular, axial data from stretch tests generally exhibited significantly higher stresses at much lower stretch ratios compared to circumferential data from inflation tests, suggesting material anisotropy. A relatively wide toe region is also apparent in the circumferential response. The data in Rows 3 and 4 of the figure additionally suggest that the circumferential response was more sensitive to changes in axial stretch during inflation tests than the axial response was to changes in circumferential stretch during axial stretch tests. These stress-stretch observations were generally consistent for all vessels tested, though there was some variability from specimen to specimen, as is illustrated by the stress-stretch plots in Fig. 4.

Further supporting the observation of anisotropy, fits of the selected Fung-type constitutive model (Eq. 3) to data from inflation and axial stretch tests for each specimen resulted in c_2 coefficients that were many times greater than values for c_1 (Table 2). Figure 5 shows experimental and best-fit axial and circumferential stress values in inflation and axial stretch tests for a specimen (A6) with average fit quality. As Table 2 shows, unconstrained fits produced values for c_2 that were particularly high relative to all other coefficient values. Each of the reported coefficient combinations satisfied the requirement that $c_1 * c_2 > c_3^{210, 12}$ but a number of the fits resulted in physically unrealistic negative values for c_3 . Nearly all cases with

a negative c_3 value settled on $c_3 = 0$ when constrained. While it is common to assume orthotropy, as Equation 3 does, for blood vessels, negative and near-zero c_3 values may be a result of the above observation that the circumferential response sometimes appeared to be more sensitive to changes in fixed axial stretch than vice-versa, a non-orthotropic behavior. It is also interesting to note that while Equation 4 was used for results reported in Table 2, a simplified approach assuming $t_{rr} = 0$ produced nearly identical results for all cases, despite the fact that the vessels studied would generally not be considered to have thin walls.

Artery response was further explored by examining behavior around *in vivo* conditions. The points most closely corresponding to the *in vivo* state for both inflation and axial stretch tests for each of the specimens are identified by the “IV” tags given in the stress-stretch plots in Figs. 3 and 4. These points were available for both inflation and axial stretch protocols for 9 of the 12 specimens; in the other 3 specimens, none of either, or both of, the inflation or stretch sequences passed through the *in vivo* state. As the plots suggest, stress values at the approximate *in vivo* state were similar for both directions of loading, each averaging 0.07 MPa. However, stiffness measurements at the same points ranged from 1.8 to 5.5 MPa and from 0.8 to 2.6 MPa for the axial and circumferential directions, respectively. While there is clearly some overlap between these ranges (Fig. 6), paired t-tests, both including and excluding the two specimens with particularly high axial modulus values, suggested a significant difference between the groups ($p < 0.025$). While this result does not support a strong statistical difference between these groups, there is a clear trend of greater stiffness in the axial direction. More significantly, the ratio of axial to circumferential stiffness for each specimen varied from 0.9 to 3.4, with an average of 1.9. Modulus values were not statistically dependent on age.

While the general response of the individual vessels, as shown in Figs. 3 and 4, is similar, it is also clear that there is some variation between specimens. In order to better understand differences in response between vessels and to investigate possible influential factors, we first plotted together one curve from the inflation protocols for each specimen. Circumferential stress-stretch data (from inflation tests) for most of the specimens are plotted together in Fig. 7a. Displayed data are limited to results from inflation test sequences where axial stretch was held near its *in vivo* value, so tests where axial stretch was relatively high were excluded. The figure demonstrates a large range in circumferential stretch among specimens, though general curve shape is similar. Corresponding axial stretch ratios ranged from 1.02 to 1.16 but had no obvious visual correlation with horizontal shift in the circumferential response. Multiple regression of circumferential *in vivo* stretch values with the associated axial stretch ratio, as well as age, suggested a lack of correlation with both parameters ($p > 0.4$). Because the most natural application of these data would utilize an *in vivo* reference, the stress data were re-plotted (Fig. 7b) against the circumferential “IV Ref” stretch ratio, where the reference is the approximated *in vivo*, instead of zero-load, diameter. With this adjustment, response in the circumferential direction is remarkably similar across specimens.

Figure 8 gives a similar presentation of results from axial stretch tests. In this case, a single test from each specimen where the internal pressure was between 10.7 and 16 kPa (80 and 120 mmHg) is included. As shown, there is a significant amount of variation, both in general curve shape and in axial stretch range, in the zero-load reference data (Fig. 8a). Similar to the inflation data, curve characteristics did not exhibit obvious dependence upon corresponding circumferential stretch values (which varied from 0.99 to 1.57). Multiple regression confirmed a lack of axial stretch dependence on the circumferential stretch ratio ($p > 0.8$) but suggested slightly significant negative correlation with age ($p < 0.02$). As with inflation test results, these stress-stretch data were also adjusted to reference the approximated *in vivo* state (Fig. 8b), again resulting in a more consistent set of data, though curve shape still varies somewhat. While it is not customary to utilize a reference configuration associated with a non-zero-stress state, the adjustment to referencing the *in vivo* configuration helps isolate potential causes for

variability among specimens. In the context of the anisotropy presented earlier, it is important to state here that the ratio of axial and circumferential moduli at *in vivo* conditions did not change significantly with the reference adjustment, averaging a value of 1.8.

In contrast to the highly nonlinear response of the cerebral arteries around *in vivo* conditions, most specimens demonstrated approximate linearity at larger deformations (Fig. 9). The transition to linearity is likely attributable to straightening of the previously tortuous collagen fibers in the vessel wall. As with tests around *in vivo* conditions, scatter between specimens was notable, so data are again plotted against axial stretch values referencing the *in vivo* configuration. Although only one failure test could be conducted with each specimen, the influence of pressurization within a specimen is demonstrated by one case that lost its ability to maintain pressure prior to the occurrence of axial failure (rightmost line in Fig. 9). The magnitude of the obvious shift to the right with loss of pressure is consistent with changes in axial stress-stretch data with pressure during *in vivo* level tests. While these experiments were conducted to failure, structural disruption invariably occurred at either a branch point or at the attachment to a needle. In addition, the assumption of incompressibility produced physically invalid predictions for internal diameter at the highest deformations, so the presented data generally do not extend to failure.

As defined in Methods, stress-stretch results presented in this paper reference the zero-load configuration (unless otherwise specified) and are thus mean values. However, measurements of opening angle, required to define the zero-stress state and through-wall stress-stretch distributions, were conducted on these specimens but were found to exhibit a large degree of variability (Table 1; Fig. 10). Upon release of residual stress by radial cut, immersed sections opened immediately, generally reaching their nearly final opening angle within 30 seconds. Some specimens did not open at all and one inverted itself, resulting in overall angle variation from -17 to 180 degrees. Opening angle was also found to be variable along the length of a single specimen. While only one or two sections could be obtained from most specimens prior to testing, one vessel too short to test was cut into multiple sections. Corresponding opening angles varied from 10 – 170 deg. In addition, sections from 2 of the 12 specimens (A8, A12) exhibited elliptical shapes with what might be described as folds at opposing points (see uncut section of Fig. 10b). Radial cuts through these specimens resulted in non-uniform arcs in which the folds were preserved. This is illustrated in Fig. 10b, which shows two open sections cut from nearly identical cross-sections (one shown in the left panel) corresponding to the same specimen. However, one section was cut radially at the location of a fold, while the other was cut radially between folds. Given the variations in opening angle and cut section uniformity, estimation of arc length using these measurements was not considered reliable, so the zero-load state was utilized as the reference configuration. Direct measurement of arc length, however, demonstrated a mean circumferential stretch ratio of 1.06 for the unloaded circumference relative to the stress-free arc length.

Discussion

The objectives of this research were to more fully define the multi-axial behavior of passive human cerebral arteries and to provide data that would be useful in future studies of injury and disease in these vessels. Findings demonstrate that the arteries are anisotropic, typically developing significant stresses at much lower levels of deformation axially than circumferentially. Consideration of vessel response around *in vivo* conditions, rather than at the same levels of deformation from the zero-load reference, reduces the degree of observed directional difference, but axial stiffness values are still typically higher. To our knowledge, the implications of cerebral vessel anisotropy for injury and disease development have not been investigated, but these findings lay the foundation for future research in these areas.

Despite the fact that our previous investigations^{16, 17} of the cerebral arteries indicated that they are particularly stiff axially relative to a number of other extracranial vessels, we did not anticipate that the vessels would be more stiff axially than circumferentially. In fact, based on the observation of Finlay *et al*⁶ that significant bundles of collagen are oriented both axially and circumferentially in the cerebral arteries, there would appear to be no basis for predicting anisotropy. Reflection on the function of the cerebral vessels, however, suggests that there may be good reason for the observed relative laxity in the circumferential direction. The healthy cerebral circulation is particularly adept at regional control of blood flow within the brain. This is accomplished through contraction of the vascular smooth muscle cells to restrict flow. Because the arteries were tested under passive conditions, the measured response represents the most compliant possibility for this direction of loading. Smooth muscle contraction would be expected to stiffen the circumferential response, potentially to a level similar to that seen axially. Whether or not this would bring about an isotropic response is not known, but it is clear that the relaxed response represents only one end of the possible response range. Future study of these vessels should address active contributions of the smooth muscle.

Cerebral artery behavior reported here is generally consistent, in terms of overall response, stress magnitudes, and modulus values, both with our previous findings from uniaxial tests on these vessels^{16, 17} and with the observations of others on large human cerebral arteries^{2, 3, 9}, but the presence of anisotropy in these vessels has not previously been reported. Busby and Burton² conducted tests on large cerebral arteries loaded under conditions of inflation only, with one end of the vessel sealed but unconstrained axially. In these tests, vessel length and diameter typically both increased with pressure, but the researchers observed that the length approached its maximum value at a lower pressure than that required for the diameter to stop increasing significantly. Based on this, it is tempting to conclude that the axial direction was observed to be stiffer. However, circumferential stress was twice the axial stress under the described conditions, and, as Finlay *et al*⁶ note, the predicted response for a linear, isotropic, incompressible material loaded under the same conditions would be for the length to remain constant (a result the Busby and Burton tests approached with increasing donor age). Knowing the vessels to be nonlinear, and suspecting anisotropy based on our findings here, it is not possible to quantitatively interpret and compare the observations of Busby and Burton to the current data without more detail from their experiments. Based on previous work from our laboratory showing similar axial response characteristics for arteries from the Circle of Willis and the surface of the temporal lobe¹⁷, we expect that multi-axial response would also be similar for arteries of different size, but this may not necessarily be true. It thus remains uncertain whether findings of anisotropy in the smaller vessels are also applicable to large arteries such as those tested by Busby and Burton.

While the current results strongly suggest the presence of anisotropy in these vessels, Humphrey and Yin¹¹ have cautioned against drawing conclusions supporting material preferences in the absence of equibiaxial protocols. Similarly, Weizsacker and Pinto²² have pointed out that slight changes in constraints imposed in an orthogonal direction may cause large changes in the measured response and lead to changes in apparent anisotropy. While it is true that the applied protocols did not produce equibiaxial conditions, a few observations help alleviate concern that we've drawn incorrect conclusions. First, uniaxial responses of these vessels for the two directions would clearly be different. No inflation tests were conducted without length constraint, but it is clear that this would result in a circumferential stress-stretch curve shifted even further to the right than seen for the constrained tests. Though results were not reported here, axial stretch tests without pressure constraint were conducted as reference tests, as described in Methods. As expected, these tests similarly resulted in a shift of the axial stress-stretch curve to the right of that from the pressure-constrained axial stretch cases, but the amount of the shift was small, similar in magnitude to the shift seen between the axial stretch tests constrained at different pressure levels. Second, simulation of equibiaxial tests

using the presented constitutive model and the optimized coefficients result in a clearly anisotropic response. Third, the applied experimental protocols explored various combinations of loading conditions, thus allowing evaluation of sensitivity to imposed constraints. Because loading conditions were defined based on pressure and needle displacement, stretch values also naturally varied, yet results related to directional differences were consistent across specimens.

In considering the comparison between responses in the axial and circumferential directions, it is important to recognize that somewhat different ranges of deformation were explored for the two directions. Figures 3 and 4 demonstrate that most of the tested range falls above and below the *in vivo* stretch values for the axial and circumferential directions, respectively. This was a natural consequence both of not pressurizing the vessels too far beyond known physiological levels and of stretching vessels far enough axially to avoid buckling during inflation. Nevertheless, the Fung model coefficients reported in Table 2 are dominated by the comparatively high stresses and accompanying nonlinearity developed in the axial direction, though testing over a different range would not be expected to change the reported *in vivo* modulus values. Although not representative of physiological conditions, experiments including greater pressures and circumferential stretch would lead to increased nonlinearity in the response for that direction and thus larger values for c_1 . Similarly, a decrease in the difference between c_1 and c_2 can be demonstrated by fitting the Fung model to a limited set of data for specimen A9. In this case, data were limited to results from the inflation test having the lowest axial stretch value and to measurements from axial stretch tests not producing axial stress values greater than the maximum circumferential stress produced during the included inflation test. Resulting coefficient values were $c = 5.34$ kPa, $c_1 = 3.44$, $c_2 = 28.96$, and $c_3 = 0.65$, showing a reduced difference between c_1 and c_2 values in comparison to that reported for the full A9 dataset in Table 2. It should be noted that a number of different constitutive equations have been suggested for modeling blood vessel behavior, some of which may model the observed response more accurately than Equation 3, but evaluation of constitutive models was considered beyond the scope of this paper.

Given the previously reported significance of age on the mechanical properties of the cerebral arteries^{2, 9}, it is interesting that this factor did not appear to be as significant in our results. Similar to our previous findings in uniaxial stretch tests on these vessels¹⁷, there was a tendency for vessels obtained from older donors to be less compliant, but this was only found to be true for the axial direction, contrary to the significance of age in the circumferential direction reported by Busby and Burton² as well as by Hayashi *et al*⁹. A few factors may contribute to these differences. As a natural result of the patient population typically undergoing temporal lobectomy surgery, the range of donor age in the present study is not as broad in comparison to the other two investigations. In addition, it may be that large cerebral vessels are more susceptible to age-related disease or changes in the vessel wall. Finally, the previous investigators focus much of their reports on findings observed using parameters not normalized by wall thickness. Both groups, however, note circumferential modulus values to be largely independent of age. Hayashi *et al* additionally found wall stress to be relatively constant over the first 40 years of life. These observations together suggest that age is an important factor in vessel mechanical response, but that its influence is less significant for normalized parameters such as stress and modulus, consistent with the concept that the vessel wall seeks to maintain a homeostatic stress level. There did not appear to be any age-related dimensional changes in the arteries tested here, but limitations inherent to obtaining vessels from surgery, along with the anatomical variability of the vasculature on the surface of the brain, make it impossible to obtain the same specimen from each patient. Nevertheless, given the similarity of the large and small vessels to longitudinal deformation¹⁷, we expect that age does play a role in the response of the cortical arteries, though perhaps a limited one in comparison to larger arteries.

Results reported for large axial deformation are similar to what we have previously reported for unpressurized vessels^{16, 17}, i.e. the initial nonlinear component of the stress-stretch curve quickly becomes linear with greater deformation. Previous comparisons of our unpressurized data to pressurized results from Circle of Willis artery tests by Chalupnik *et al*³ suggested that pressure would produce a leftward shift in response. This is consistent with the data presented here. Although the failure tests here were conducted at quasi-static rates, Chalupnik *et al* reported a lack of strain rate dependence in fluid-filled vessels at rates consistent with trauma, similar to our previous work on unpressurized vessels.

While some natural variation in response between biological specimens is expected, the demonstration of increased uniformity in stress-stretch response as a result of utilizing the *in vivo* state as the reference configuration for stretch values is interesting and would appear to have two possible explanations – one, that variability is introduced in identifying the zero-load reference, or two, that there is some significant natural variability in the sub-*in vivo* behavior of these arteries. While each may contribute, we are confident that some of the observed scatter is associated with zero-load reference definition. Identification of the zero-load reference for the axial direction was often difficult since many of the vessels displayed some degree of curvature along their length and were thus not tested in their natural geometry. In addition, it is possible that vessels were gripped non-uniformly by the suture at the attachment points. Adventitia is a rough, fibrous material, and its fibers can get caught up differently around the suture. Such end effects are expected to have been minimized by preconditioning and by focusing analysis on specimen midsections, but natural curvature and slightly uneven end attachment should be recognized as potentially confounding factors in identification of the zero-load length. Interestingly, there is no similar difficulty in defining the zero-load circumferential reference, since it is measured directly from the specimen cross-section, yet the level of scatter in the circumferential data appears to be at least as great as that for the axial direction. Variation in the circumferential direction would result if cross-sections taken from the end of the vessel did not accurately reflect the geometry of the vessel midsection measured during testing. While this would not be expected for the short vessel lengths tested here, it is possible that preconditioning of the tested segment may produce a slight change in zero-load diameter not reflected in the reference section, especially considering the high compliance of the vessel wall at low pressures. While there appears to be no reason to suspect that sub-physiological response of these specimens would be particularly variable, quantification of error in identifying the circumferential reference would lend insight into whether or not this is the case. In future testing, it is thus recommended that the preconditioned segment outer diameter, measured under conditions of zero pressure, be compared to outer diameter of a cut cross-section.

In considering variations, another potential limitation of the reported data is the unknown influence of vessel branches. The cerebral arteries branch frequently enough that it is uncommon to find a segment of testable length not having branches. As a result, our analysis was focused on regions away from branches, but the extent of any possible branch influence has not been defined. Based on preliminary evaluation of branch effects in our laboratory, however, it appears that the data reported here are not influenced by branches.

While stress-stretch results for specimens A8 and A12 (Fig. 4) do not appear to be particularly different from the other specimens, it is important to note that the presented response does not rigorously represent the behavior of these two vessels, due to their relatively non-circular cross-sections. The equations utilized in the present analysis assume circularity, and uniformity of stress around the circumference (for a given radius), whereas the stress at the mid-wall location for elliptical vessels would be expected to vary around the circumference¹⁴. Calculating an average diameter from measurements of the major and minor axes of an ellipse, as was done here, results in stress predictions between those associated with the two extremes.

The observed variation of opening angle in these specimens was unexpected but not unprecedented; Schulze-Bauer *et al*²⁰ recently reported a large range of opening angles in human iliac arteries. It is unclear, however, whether or not it would be appropriate to model through-wall stresses using these measurements. Further, the observation of sections from two specimens taking on non-circular shapes and demonstrating “folds” in the vessel wall at some locations is similarly puzzling, though this suggests that there may be some variation in properties around the circumference of these vessels, perhaps especially when smooth muscle is not active. Such would be unexpected, but the relatively small number of layers of medial smooth muscle and the thinness of the vessel could potentially contribute to any such inhomogeneity. This requires further investigation.

While the presented findings represent an important step toward a more complete understanding of cerebral vessel response, it is clear that further definition of these vessels is needed. In addition to issues already discussed here, other areas of study that will enhance ability to prevent and treat cerebrovascular injury and disease include accounting for interactions with surrounding tissues, characterizing branch point mechanics, and isolating individual vessel layer response.

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Figure 1. (a) Mechanical testing apparatus and (b) accompanying close-up of specimen A9, with (c) zoomed-in image showing microspheres. Microspheres labeled 1 and 2 were used to determine axial stretch for this specimen. For reference, outer diameter of the specimen as shown in (b) is approximately 0.7 mm.

Figure 2. Stretch ratios for inflation and axial stretch tests on representative specimen (A7). Traces are numbered in the order in which tests were run.

Figure 3.

Measured and calculated values for specimens A2, A6, and A9, selected as representative cases. Row 1 gives outer diameter and axial force as a function of pressure during inflation tests. Row 2 presents axial force and pressure as a function of displacement during axial stretch tests. Row 3 shows calculated Cauchy stresses as a function of stretch, both in the loading direction, for both types of test, and Row 4 shows the combinations of stretch values explored in each case. Traces are numbered in the order in which tests were run for each specimen.

Figure 4.

Cauchy stress-stretch response from inflation and axial stretch tests for all specimens not included in Fig. 3. Specimens are identified at the top of each graph.

Figure 5. Measured and predicted axial and circumferential stress values from inflation (1–3) and axial stretch (4–6) tests on specimen A6.

Figure 6.

Axial and circumferential modulus values at *in vivo* conditions in axial stretch and inflation tests, respectively.

Figure 7. Circumferential Cauchy stress in inflation tests for cases having axial stretch ratios near the axial *in vivo* stretch ratio plotted against the circumferential stretch ratio referencing the (a) zero-load and (b) *in vivo* configurations.

Figure 8.

Axial stress from axial stretch tests conducted at physiological pressures plotted against the axial stretch ratio referencing the (a) zero-load and (b) *in vivo* states.

Figure 9. Axial stress in axial stretch tests to failure plotted against axial stretch referencing the *in vivo* configuration.

(a)

(b)

Figure 10.

Whole and radially cut vessel cross sections in (a) a relatively circular case (A6) and (b) a less circular case (A8) displaying “folds” that persist in the cut sections. Scale bars (0.5 mm) apply to (a) and (b) separately. (Dark, non-uniform coloring of tissue in part a is due to use of black dye to enhance contrast.)

Table 1

Detailed data for specimens A2, A6, and A9, along with summary data for all specimens. From left to right, the table give age, sex, cross-sectional area, zero-load external diameter and wall thickness, opening angle, circumferential stretch between zero-stress and zero-load configuration, and zero-load length.

	Age	Sex	A (mm ²)	D _e (mm)	h (mm)	θ (deg)	λ _{0,0}	L ₀ (mm)
A2	17	M	0.325	0.92	0.14	140	1.05	5.11
A6	51	M	0.090	0.51	0.07	145	1.01	3.23
A9	18	F	0.136	0.61	0.08	105	1.05	4.05
				Summary data for all specimens				
max	62	5 F	0.325	0.92	0.18	180	1.14	6.98
min	16	6 M	0.090	0.51	0.06	-17	1.00	3.23
avg	37.6		0.180	0.67	0.11	107	1.06	5.03

Unconstrained Fung model (Eq. 3) coefficient values resulting from fitting procedure of data from both inflation and axial stretch tests.
 χ^2 is a goodness-of-fit measure which decreases in magnitude with quality of fit.

Spec	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
<i>c</i> (kPa)	0.16	1.16	3.75	1.5	15.77	2.78	6.58	0.98	8.90	6.79	2.48	2.37
<i>c1</i>	11.32	13.54	3.13	2.48	6.73	5.36	32.32	3.51	1.68	2.02	2.27	7.86
<i>c2</i>	126.69	111.25	171.83	47.68	188.92	199.41	105.30	188.27	42.39	26.11	259.48	623.14
<i>c3</i>	14.44	-0.36	-5.51	-0.47	-13.71	6.73	18.05	-12.79	0.01	-0.01	-0.78	-28.49
χ^2	0.05	0.39	0.07	1.94	0.92	0.54	0.15	0.23	0.86	0.96	0.28	0.16