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Failure and Fatigue Properties of Immature Human and Porcine Parasagittal Bridging Veins

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Abstract

Tearing of the parasagittal bridging veins (BVs) is thought to be a source of extra-axial hemorrhage (EAH) associated with abusive traumatic brain injuries (TBIs) in children. However, the pediatric BV mechanical properties are unknown. We subjected porcine adult, porcine newborn, and human infant BVs to either a low rate pull to failure, a high rate pull to failure, or 30 seconds of cyclic loading followed by a pull to failure. An additional subset of human infant BVs was examined for viscoelastic recovery between two cycling episodes. We found that human infant BVs are stronger than porcine BVs, and BV mechanical properties are rate dependent, but not age dependent. Successive cyclic loading to a uniform level of stretch softened BVs with decaying peak stresses, and shifted their stress-stretch relationship. These data are critical in understanding BV tissue behavior in accidental and abusive trauma scenarios, which in turn may clarify circumstances that may be injurious to young children.

Key Terms

brain injury; abusive head trauma; hemorrhage; pediatric trauma; child abuse; mechanical properties

Introduction

Traumatic brain injury (TBI) is a leading cause of death and disability in children aged 0 to 4 years in the United States with over 250,000 emergency department visits, 15,000 hospitalizations, and nearly 1,000 deaths annually¹⁸. Extra-axial hemorrhage (EAH), a collection of blood between the brain and skull (including both subdural and subarachnoid hemorrhage), is one presentation of TBI. While incompletely understood, one etiology of EAH is thought to be the rupture or tearing of the parasagittal bridging veins (BVs) which drain blood from the brain into the superior sagittal sinus. Tearing of BVs may be caused by rapid head motions in which the movement of the brain lags that of the skull, stretching the veins at increased rates of deformation to supraphysiological displacements at their attachment points or within the subdural space.

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EAH is currently an important presenting finding used to distinguish between accidental and abusive injuries in young children^{7, 23}. TBI in infants and toddlers is frequently due to abusive head trauma (AHT)⁶, and these cases often have poor outcomes^{17, 24}. It follows that whether BV rupture and subsequent EAH may be produced from vigorous shaking alone (no impact) is subject to significant debate^{14, 26, 42, 50, 51}. Although previous studies have identified age-dependent properties of brain, skull, and suture^{10, 11, 21, 33, 47, 53}, to date testing has been performed on adult BVs^{12, 30, 31, 36, 38, 40}, but not from subjects under three years of age³⁶ with exception of a limited study of samples from three infant subjects⁴¹. Determining the BV mechanical properties over an age range that includes very young subjects is essential to understanding mechanisms of EAH in children.

In vigorous shaking associated with AHT and in recurring head impacts in sports, BVs are believed to experience repeated elongations, but their behavior has never been evaluated under cyclic or repeated loading. Many engineering materials exhibit fatigue, or changes in stiffness and strength after being exposed to repeated sub-catastrophic deformations. Fatigue is undesirable in biological tissues that are expected to function normally during a lifetime exposure of repeated deformations (e.g. cardiac tissue, pulmonary epithelium). In fact, fatigue has been a proposed mechanism for some repeated-exposure injuries, like bone stress fractures^{3, 8, 16, 27, 34, 45, 54} and a variety of other repeated strain injuries including musculoskeletal disorders (e.g. tendonitis, osteoarthritis, bursitis), peripheral-nerve entrapment (e.g. carpal tunnel syndrome), and vascular syndromes (e.g. Raynaud's syndrome)⁵⁶. Thus, it is important to elucidate whether BVs exhibit fatigue behavior.

In this study, we measure the axial mechanical properties and behavior of parasagittal BVs in three subject types: porcine newborn, porcine adult, and human infant. Subjects were evaluated under three loading protocols: high and low stretch rates to failure, and cyclic loading followed by elongation to failure, with an additional human infant subset evaluated for viscoelastic recovery between two cyclic loading protocols. These data are analyzed for age, species, rate, and cyclic loading contributions to low stretch and linear region moduli, corner stretch, yield, and failure properties of BVs, enhancing the understanding of BV tissue behavior in accidental and abusive TBI in youth.

Materials and Methods

Test Apparatus

A custom device coupling a rotary motor to a gear chain drive, described previously,⁴³ was used to perform elongation tests. BVs were gripped by flat plate-type grips similar to prior investigations,^{12, 30, 31, 38, 40, 41} as cannulation and fluid perfusion risked pre-test damage to the delicate BVs. Hyperterminal scripts for each test type provided appropriate rate and frequency inputs to the device.

Specimen Preparation

All pig euthanasia and procurement procedures were approved by the University of Pennsylvania and Children's Hospital of Philadelphia Institutional Animal Care and Use Committees. Parasagittal BVs were harvested from newborn (1 week old, n=9) and adult

(2 month old, n=12) farm pigs immediately after sacrifice by removing an intact brain-meninges-skull section from the cranium en bloc. Brain tissue was cautiously dissected away from the dura/superior sagittal sinus-skull complex to expose the BVs, which were cut from their brain connection point using surgical scissors. Next, the dura was peeled from the skull, and finally, BVs were cut at their point of attachment to the dura/superior sagittal sinus.

Human infant sample procurement and testing procedures were approved by the Children's Hospital of Philadelphia and University of Pennsylvania Institutional Review Boards. Human infant parasagittal BVs were procured at autopsy by a pathologist within 24 hours of death. Human subject inclusion criteria were an age of 0–12 months and autopsy performed less than 24 hours after death, without bias to race, gender, ethnicity, or socio-economic status. Exclusion criteria were history of head trauma, HIV, or hepatitis.

All BV samples (porcine newborn, porcine adult, and human infant) were cryopreserved (described in the electronic supplement) after excision until testing.

Test Protocol

Once a BV sample was fully fastened in the device, the grip distance was adjusted until the load cell (Model 31, 250g capacity load cell with shock and vibration resistance option, 20 mV/V sensitivity, 740 Hz natural frequency; with UV-10 +/- 10V InLine Amplifier, Honeywell-Sensotec, Columbus, OH) just began to register a load, defined as the point at which the vessel was visibly taut and the output voltage began to increase above the slack vessel baseline reading, and specimen gage length was measured from grip to grip with calipers. Typical length to wide aspect ration of the mounted specimen at gage length was 6:1. Five cycles of preconditioning, similar or equivalent to the number of cycles in other small cerebral vessel testing (5–10)^{2, 39}, to a low nominal stretch ratio of 1.05 were performed, as specimen in vivo length was unknown and stretching to higher stretch ratios may have damaged the tissue prior to testing. Then the grip distance was readjusted until a load signal registered once again and the test gage length was measured to eliminate uncertainty in zero-load length following preconditioning and ensure stretch rates were repeatable between tests, as the device speed input for repeatable stretch rates is dependent on specimen gage length. This adjusted test gage length was used to determine the appropriate inputs to the test device in order to match experimental parameters between tests, and immediately after these adjustments were made in Hyperterminal the specimen was elongated. Specimens were continuously hydrated with Dulbecco's Modified Eagle Media via a transfer pipette up until the elongation test.

One BV specimen from each porcine and human subject was used per loading protocol: high rate pulls to failure (averaging $13.91 \pm 2.84 \text{ }^{-1}$), low rate pulls to failure (averaging $1.36 \pm 0.26 \text{ }^{-1}$), and cyclic loading followed by a pull to failure (averaging $2.56 \pm 0.32 \text{ }^{-1}$). High and low stretch rates were chosen to achieve a difference of approximately one order of magnitude between protocols. In addition, as one objective of these studies was to investigate the presence of stretch rate dependencies in pediatric BV mechanical properties, high and low rates were chosen within the realm of previously observed human adult BV mechanical property stretch rate dependencies for comparison between rates and ages

($\sim 100 \text{ s}^{-1}$, Lowenheim³¹; $< 3.4^{-1}$ and $10\text{--}60^{-1}$, Monea et al.³⁸). If after a failure test, a BV specimen was found to have torn at the grips on visual inspection the test was not used in further analysis, and test parameters were repeated on an additional sample from the same subject. Based on preliminary porcine ultimate stretch ratios, the cyclic loading protocol was conducted at near-failure peak stretch ratios of 1.184 ± 0.009 (Figure 1). A cycling frequency of 3 Hz was chosen to match that of simulated abusive trauma via vigorous shaking of an anthropomorphic surrogate representative of a 1 month old human infant^{15, 46}, while a cycling duration of 30 seconds (~ 90 cycles, Figure 1) was chosen as a hypothetical extreme, and to match the duration of in vivo porcine cyclic head rotational injury studies⁹. To evaluate viscoelastic recovery, a subset of additional human infant specimens ($n=6$) were subjected to two 15-second episodes of cyclic loading separated by 10 minutes of relaxation, during which the specimen was held at its test gage length and continuously hydrated. A 10-minute recovery period was chosen to match the recovery period prescribed in other small cerebral vessel testing². Load and displacement values were sampled at 10 kHz for high rate tests and 1 kHz for low rate and cyclic tests, and recorded on a computer. Cross-sectional area of each specimen was obtained post-test using optical methods (described in the electronic supplement).

Data Analysis

Cyclic Loading Analysis—To obtain appropriate cutoff frequencies for low pass filtering, the power spectral densities of the raw load and displacement signals were compared across all subjects and the highest cutoff frequency was defined as the cutoff for a given test type. Cyclic raw load and displacement signals were low-pass filtered (second order Butterworth) at 10 Hz. As real-time cross-sectional deformation was not recorded, stretch ratio and stress were calculated from the displacement and load traces, as follows:

$$\lambda(t) = \frac{l(t)}{l_0} \quad [1]$$

$$\sigma(t) = \frac{F(t)}{A} \quad [2]$$

where λ is stretch ratio, t is time, l is the instantaneous length of the specimen, l_0 is the gage length, σ is stress, F is instantaneous load, and A is post-test cross-sectional area found as described in the electronic supplement.

Prior studies examining brain, periodontal ligament, and vocal fold lamina propria have approximated transient mechanical tissue responses using a series of exponential decay functions^{22, 28, 57}. In a preliminary analysis, first, second, and third order exponential decay functions were examined to describe the decay of peak stress with successive cycling, and three exponentials provided a good fit visually and statistically (average $R^2=0.985$), with limited improvement over a second order function. Thus, the decay of peak stress with

successive cycles was fit to a third order exponential relationship for each sample using the following equation:

$$\sigma_{peak}(n) = c + \sum_{i=1}^3 a_i e^{-b_i * n} \quad [3]$$

where σ_{peak} is the peak stress, n is cycle number, and a_i , b_i , and c are constant coefficients. Parameter optimization was performed using a custom Matlab program, implementing the Levenberg-Marquardt algorithm to determine convergence of coefficients a_i , b_i , and c . = “Instantaneous” peak stress, $\sigma_{peak,0}$ ($a_1 + a_2 + a_3 + c$ in Equation 3), and steady state peak stress, $\sigma_{peak,SS}$ (c in Equation 3) were determined for each fit. Differences between $\sigma_{peak,0}$ and $\sigma_{peak,SS}$ across subject types (newborn porcine, adult porcine, and infant human) were evaluated by repeated measures ANOVA and post hoc Tukey-Kramer analyses with significance defined as $p < 0.05$. For the subset of human infant BVs subjected to two consecutive 15-second cycling protocols separated by 10 minutes of relaxation, σ_{peak} , measured in the last cycle of the first cycling episode was compared to σ_{peak} measured in the first cycle of the second cycling episode using a Wilcoxon signed-rank test with significance defined as $p < 0.05$, to determine if any viscoelastic recovery of peak stress had occurred.

Failure Analysis—For each failure test type (high rate, low rate, or post-cyclic), the power spectral densities of the raw load and displacement signals were compared across all subjects and the highest cutoff frequency was chosen as the cutoff for a given test type, similar to cyclic loading analysis. Raw load signals were low-pass filtered (second order Butterworth) at 150Hz for high rate tests and 35Hz for both low rate and post-cyclic tests. Raw displacement signals were filtered at 60Hz for high rate tests and 20Hz for both low rate and post-cyclic tests.

Stretch ratio and stress were calculated in the same manner as cyclic loading tests, using Equations 1 and 2. Traces were then cropped from the point where stretch ratio began monotonically increasing above 1 to the point of maximum stress. Stretch rate was defined as the slope of the stretch-time relationship. Ultimate stress (σ_u) and ultimate stretch (λ_u) were defined at the point of peak stress.

Most previous studies investigating parasagittal BVs only reported ultimate failure properties^{30, 31, 36, 41}, but recent work has also measured modulus and yield properties^{12, 38, 40}. A multi-step custom algorithm (detailed in the electronic supplement) was employed to isolate the most linear region (high stretch) of the stress-stretch curve for linear elastic modulus, E_{linear} , determination⁴⁴. The last point in the linear region, or proportional limit, was defined as the yield point from which yield stress (σ_y) and yield stretch (λ_y) were extracted. To capture stress-stretch nonlinearity, a similar algorithm was employed to describe a low stretch region modulus (~1.05 stretch ratio), E_{low} (detailed in the electronic supplement). The corner stretch, λ_{corner} , was defined as the stretch ratio value of the point at which the E_{low} and E_{linear} lines of best fit intersected.

Yield stress, yield stretch, linear elastic modulus, low stretch region modulus, corner stretch, ultimate stress, and ultimate stretch were compared between failure test type (high rate, low

rate, and post-cyclic), subject type (newborn porcine, adult porcine, and infant human), and their interactions using a series of two-way ANOVAs with statistical significance defined for $p < 0.05$. When statistical significance was detected, post-hoc Tukey-Kramer analyses were conducted to determine group-wise differences.

Results

Cyclic Loading

The stress-stretch behavior of the BVs was nonlinear (Figure 2, inset, representative human infant and newborn porcine curves), with the vessel displaying stiffer behavior at higher stretches. In all 30-second cycling cases, peak stress decayed exponentially (Eq. 3, Figures 1 and 2), with coefficients of determination (R^2) averaging 0.985, and never less than 0.917. Instantaneous peak stresses ($\sigma_{\text{peak},0}$) were significantly higher than steady state peak stresses ($\sigma_{\text{peak,SS}}$, Table 1). There was no significant variation in either instantaneous or steady state peak stress with subject type nor an interaction effect between subject type (porcine newborn, porcine adult, and human infant) and time point (instantaneous and steady state).

In the subset of human infant BVs subjected to two 15-second cyclic loading protocols separated by 10 minutes of relaxation, peak stress decayed exponentially with continued cycling in both the first (R^2 averaging 0.998 and never less than 0.995) and second (R^2 averaging 0.940 and never less than 0.832) loadings (Figure 3). Furthermore, σ_{peak} in the last cycle of the first cycling episode (1.244 ± 0.338 MPa) was statistically indistinguishable ($p = 0.31$) to σ_{peak} in the first cycle of the second cycling episode (1.208 ± 0.372 MPa) indicating an absence of viscoelastic recovery of axial peak stress after 10 minutes of prescribed relaxation (Figure 3).

Failure Tests

As expected, during failure tests (low rate, high rate, post-cyclic) up to the yield point, we observed nonlinear stress-stretch curves with stiffer behavior as stretch increases (Figure 4, representative human infant and newborn porcine curves), similar to the nondestructive cyclic tests.

BV behavior was not dependent on age, but did differ with species (Table 3). Specifically, for all of the outcome metrics (λ_y , σ_y , E_{linear} , E_{low} , λ_{corner} , λ_u , and σ_u), there were no differences between porcine newborn and porcine adult BVs, but human infant BV values were always significantly higher than porcine tissue for all metrics except E_{linear} , E_{low} , and λ_{corner} , where they were indistinguishable. Human infant stretch rates were also slightly higher than porcine newborn rates, while porcine adult rates were indistinguishable from both human infant and porcine newborn. The custom device did not provide an instantaneous constant stretch rate, taking a fraction of the total test time to ramp up to final speed, and rates were benchmarked from expected failure stretch of porcine tissue. Because the ultimate stretch of human infant BVs was higher than porcine tissue, this resulted in modestly, but significantly, higher stretch rates in human infant BV failure testing than in porcine newborn tests (Table 2). Though statistically significant, this stretch rate difference was small compared to those between low rate and high rate loading protocols, and no

interaction effect between failure loading protocol and subject type was found. Thus, the differences in stretch rate between subject types are unlikely to explain the finding that human infant BV behavior differed significantly from porcine tissue. This same phenomenon accounts for the variability of stretch rates between trials of the same subject and test type as individual samples did not have the exact same failure stretch as others from the same subject type undergoing the same test protocol. Similarly, variability of stretch rate within a given subject-test type group is unlikely to have contributed to differences in mechanical property findings between test groups as the overall difference in stretch rate between low and high rate protocols was much larger than within-protocol variability. Future studies with finer stretch rate control may examine more minute differences in BV behavior with changes in rate.

BV behavior changed significantly between test types (low rate, high rate, post-cyclic) for both human and porcine tissues. As planned, stretch rates prescribed to high, low, and post-cyclic failure pulls differed significantly, with $\dot{\lambda}_{\text{high}} > \dot{\lambda}_{\text{post-cyclic}} > \dot{\lambda}_{\text{low}}$ (Table 2), though $\dot{\lambda}_{\text{high}}$ was much higher than both $\dot{\lambda}_{\text{post-cyclic}}$ and $\dot{\lambda}_{\text{low}}$ which were of the same order of magnitude. Significant differences were detected between test types for λ_y , σ_y , E_{linear} , E_{low} , λ_{corner} , and σ_u , as might be expected for viscoelastic materials that demonstrate fatigue-like behavior, but interestingly, not for λ_u (Table 3). Specifically, yield stretches were greater in post-cyclic failure pulls than in low and high rate tests, which were indistinguishable from one another (Figure 4, black 'x' indicates yield point). Yield stress and ultimate stress were higher in high rate tests than low rate and post-cyclic (Figure 4, black 'x' indicates yield point, black circle indicates ultimate parameter point), which were similar. E_{linear} was larger in high rate tests than low rate tests, while post-cyclic E_{linear} was not significantly different from both high and low rate (Figure 4, black line indicates linear region over which E_{linear} was calculated). E_{low} was highest in high rate test, and lowest in post-cyclic tests, while low rate E_{low} was between high rate and post-cyclic. Corner stretch values in high rate and post-cyclic tests were both significantly greater than those in low rate experiments. In all cases, the shape of the stress-stretch curve for post-cyclic failure pulls was also qualitatively different than those for high rate and low rate tests, with a longer low-stress "toe" regime (Figure 4). These findings indicate that both rate and load-history affect BV behavior in both species.

Discussion

This study deepens our understanding of potential biomechanical and biological factors that contribute to BV rupture. First, BV mechanical properties did not display any age dependency. Second, except for E_{linear} , E_{low} , and λ_{corner} , BV mechanical properties are species dependent, with human tissue producing higher values than porcine. Third, we find that load-related mechanical properties (σ_y , σ_u , E_{linear} , E_{low} , λ_{corner}) of BVs are rate-dependent, with values increasing with rate. However, parameters that are purely displacement-related (λ_y , λ_u) do not differ with changes in stretch rate. Fourth, prior load history also has a significant effect on BV failure loading such that in post-cyclic tests, stress-stretch toe regions are longer, leading to a decrease in low stretch modulus as well as an increase in yield and corner stretches, but interestingly not in ultimate stretch, compared to tests without prior cyclic loading. Yield and ultimate stresses found in post-cyclic failure

loading are similar to those performed at lower stretch rates without a prior history of cyclic loading. Indeed, during cyclic loading to a common level of displacement, peak stress achieved by the BVs decreased exponentially with successive cycles. Together, these data will increase the understanding of biomechanical tissue property factors contributing to BV rupture in pediatric TBI.

Our finding that BV properties are independent of age between newborn and adult porcine tissue is in agreement with studies performed by Meaney on human BV tissue from toddler through adult aged subjects that showed ultimate stress, ultimate tension, and ultimate stretch did not differ across this large range of ages (groups: 3–9 years, 27–47 years, and >62 years)³⁶. Monea et al also investigated age effects on biomechanical parameters in adults and the elderly, and only found weak effects, indicating decreases in elastic modulus and yield stress with age over a comparatively smaller range of advanced age subjects (63 to 92 years)³⁸. Taken together with our study, we conclude BV mechanical properties remain unchanged from infant through advanced age, and that any predisposition to BV rupture and subsequent extra-axial hemorrhage in certain age groups may be due to the relative ease by which the brain and skull can move independently (e.g. with brain atrophy in the elderly), or likelihood of rapid head rotations with or without impact (e.g. child abuse in the young, falls in children and the elderly, playing football in childhood to young adulthood, etc.).

We found that human BVs produced significantly higher values than porcine tissue for all failure test mechanical parameters except E_{linear} , E_{low} , and λ_{corner} . Prior to this study, only Morison had examined the mechanical properties of both human and porcine BVs⁴¹, though only two porcine and three human subjects were evaluated, and the porcine vessels included other pial and arachnoid veins in addition to BVs. While no statistics were performed, Morison's anecdotal findings showed that ultimate stress was higher in human BVs than the array of porcine veins⁴¹, consistent with this study. However, BVs from the three human subjects had lower ultimate stretches than cerebral veins from the two porcine subjects, which differs from our much larger subject population⁴¹. We conclude it is important to consider species dependencies in BV properties in animal models of TBI. Although species differences in failure properties have implications for translating hemorrhage findings from animal studies to humans, the similar moduli across pigs and humans is a convenient finding for constitutive modeling.

We observed no differences in displacement-related mechanical parameters (λ_y , λ_u) with changes in stretch rate. In an early study investigating rates of approximately 1–1000 s⁻¹, Lowenheilm found much larger ultimate strains at low strain rates than at high strain rates, but this trend was only observed for rates $\sim <100\text{s}^{-1}$ ³¹, which is within the range of rates tested in our study. Monea et al found the reverse pattern with modest significant increases of ultimate strain and yield strain with increasing rates ($<3.4\text{s}^{-1}$, 10–60s⁻¹, and 100–200s⁻¹)³⁸. In further contrast, Meaney, Delye et al, and Lee and Haut found no dependence of the mechanical properties of BVs on strain rate^{12, 30, 36}. In conclusion, most studies find no rate dependence in BV displacement-related parameters (λ_y , λ_u) with single studies reporting properties increase or decrease with rate.

In contrast, we detected rate dependency in load-related mechanical properties (σ_y , σ_u , E_{linear} , E_{low} , λ_{corner}) of BVs, with values increasing at higher rates of deformation. Lowenhielm also observed higher ultimate stresses at higher strain rates ³¹. Monea et al recently found modest significant increases in yield stress with higher rates, and this small increase was also observed in ultimate stress, but was not significant ³⁸. Monea et al also commented that (linear region) elastic modulus increased between low ($<3.4\text{s}^{-1}$) and intermediate ($10\text{--}60\text{s}^{-1}$) strain rates (similar to the low and high rate groups in this study, respectively, Table 3), with a subsequent decrease from intermediate to high ($100\text{--}200\text{s}^{-1}$) rates, and these differences were trending towards significance ($p=0.059$) ³⁸. However, Monea et al only obtained ultimate stress data from three BVs and yield and (linear region) elastic modulus data from four BVs in their lowest rate group ($<3.4\text{ s}^{-1}$) ³⁸. In a study involving BVs, middle cerebral arteries, and cortical arteries and veins, Monson et al found that strain rate (groups: $0.03\text{--}0.21\text{ s}^{-1}$, and $20\text{--}138\text{s}^{-1}$) did have a significant effect on mechanical properties, but the authors state that these results were not consistent within various groups of data (e.g. surgical- versus autopsy-obtained vessels, small versus large vessels, etc.) ⁴⁰. Conversely, several studies did not find rate dependence in BV load-related mechanical properties ($0.1\text{--}250\text{s}^{-1}$) ^{12, 30, 36}. We conclude that our results regarding load-related mechanical properties agree with many studies in the literature, with increases in BV ultimate stress, yield stress, and linear region elastic modulus with increasing stretch rates.

We compared our measured values for human infant BV linear region elastic modulus, ultimate stress, yield stress, ultimate stretch and yield stretch with those previously reported in literature. The human infant linear region elastic modulus value calculated at low rates in this study ($30.173\pm 18.492\text{ MPa}$) is remarkably similar to those in Delye et al ($30.69\pm 19.40\text{ MPa}$) ¹² and also close to those in Monea et al ($23.26\pm 14.08\text{ MPa}$, low rate) ³⁸, both of whom tested at rates similar to the low rate group presented here. The ultimate stresses found in this study ($7.204\pm 4.613\text{ MPa}$ for low rates, $9.885\pm 6.752\text{ MPa}$ for high rates) are between those reported by Meaney ($12.02\pm 5.9\text{ MPa}$ for 3–9 year olds, $18.57\pm 14.2\text{ MPa}$ for 27–47 year olds, $8.02\pm 3.95\text{ MPa}$ for subjects >62 years old; no age significant differences observed) ³⁶ and those reported by Delye et al ($4.99\pm 2.55\text{ MPa}$) ¹² and Monea et al ($3.60\pm 0.76\text{ MPa}$ for low rates $<3.4\text{s}^{-1}$, and $4.78\pm 2.82\text{ MPa}$ for intermediate rates $10\text{--}60\text{s}^{-1}$) ³⁸. However, yield stress in this study is consistently higher than what has been found previously, as is high rate modulus (Meaney reported neither yield properties nor elastic modulus.). Yield and ultimate stretch values for human infant BVs found in this study (1.248 ± 0.073 across similar low and high rate tests, and 1.448 ± 0.159 across similar low, high and post-cyclic tests, respectively) are similar to those reported for human BVs from older subjects ($1.13\text{--}1.29$ yield stretch; $1.25\text{--}1.67$ ultimate stretch) ^{13, 30, 36, 38, 40}. Furthermore, because these displacement-related mechanical parameters conveniently did not vary with stretch rate in our study, we support their use in future modeling endeavors.

To our knowledge, ours is the first study to examine the axial mechanical properties of BVs under cyclic loading. Furthermore, there have been few other studies to measure axial behavior of any blood vessel under cyclic loading ^{19, 20, 25, 32, 43}, with most focused on the first five cycles ^{19, 20, 25, 32}. Our study was designed to mimic scenarios of repeated single loads or cyclic head rotations in both sports (e.g. boxing, football, heading a soccer ball, etc.) and child abuse (shaking or repeated blows to the head) resulting in numerous BV

elongations. With exposure to prolonged cyclic elongation to the same subcatastrophic peak displacement, we observe softening of the BVs characterized by decreasing peak stresses (Figures 1–3), and lengthening of the low-stress “toe” region, such that stiffer behavior occurs at increasingly higher stretch ratios (Figure 4), also observed in immature porcine common carotid arteries⁴³, and in agreement with findings from a longitudinal over-stretch study of ovine middle cerebral arteries². This softening is also exemplified by significantly smaller low stretch regime moduli and larger corner stretches in post-cyclic failure testing compared to low rate failure testing, performed at similar though significantly *lower* stretch rates than post-cyclic tests. While, surprisingly, corner stretches in high rate tests were similar to those measured in post-cyclic failure tests, this is likely a stretch rate dependent result. In addition, ultimate stretch was unaffected by a history of cyclic loading and no viscoelastic recovery of peak stress was observed with 10 minutes of relaxation between episodes of cyclic loading, again similar to ovine middle cerebral artery overstretch observations². Overall, these results show that, like the middle cerebral artery, subcatastrophic stretching along the longitudinal axis of the BVs, produces an unrecoverable change in properties, which may lead to disruption or impairment in function or structural integrity due to brain injury.

The nonlinear shape of the stress-stretch curves obtained in these experiments is common for single elongation to failure in many soft tissues. At low stretches, crinkled collagen fibers straighten and elastin fibers elongate from the traction-free state. At higher levels of stretch, the stiff collagen fibers become taut and dominate the mechanical response of the vessel yielding much stiffer behavior. In an investigation into the ultrastructure of BVs, Yamashima and Freide found that the majority of collagen fibers are oriented circumferentially rather than longitudinally⁵⁵. Thus, we posit that the observed changes in E_{low} , λ_y , λ_{corner} , and toe region length with cyclic loading may be due to irreversible changes to the vessel wall as collagen fibers are pulled apart from one another transverse to their axes, or as individual longitudinally-oriented fibers are damaged during cyclic loading. Our observation of post-cyclic failure pull linear region elastic moduli similar to those calculated for both low and high rate tests may in turn be due to reorientation of some collagen fibers in the precise direction of loading. Indeed, progressive collagen fiber alignment in the direction of loading has been observed in cyclic testing of tendons and ligaments^{37,49}. Furthermore, because low stretch moduli were smaller, yield and corner stretches were higher, and toe regions were longer after cycling, we conclude that cyclic loading conferred damage to the mechanical integrity of the BVs prior to failure loading. To assess these hypotheses, future studies should include: rate-matched failure tests with and without prior cyclic loading, observation of collagen fiber orientation with successive cycling, and histological investigations of the unloaded and cyclically loaded vessel wall.

The presented studies include several limitations. First, due to small vessel size, particularly in the newborn pig vessels, the use of flat plate-style grips may have led to stress concentrations in the tissue near the grip attachment points. While samples that tore at the level of the grip were eliminated from analysis, specimens that tore near, but not level with the grip were included. Thus, the presence of stress concentrations near the grips may have led to underestimations of BV strength. Furthermore, displacement was measured from grip to grip to capture the average BV response, which may not be representative of local tissue

displacements near the point of rupture. Second, it is important to note that because cross-sectional area was obtained post-test from both specimen fragments, calculated stress may be an overestimate of Cauchy and engineering stress values. This area measurement method was utilized because the small, delicate newborn porcine vessels risked damage from drying and manipulation if cross-sectional area measurement had been obtained prior to study. Investigators interested in more localized stress and stretch behavior may use speckle patterning and video capture techniques. In addition, the flat plate grips did not allow for investigation of the effects of fluid perfusion, as would be encountered in vivo. Third, only axial direction mechanical properties were investigated in this study, as these are considered to be the most relevant in traumatic rupture of the BVs as the brain and skull move relative to one another. Circumferential wall stresses caused by internal pressurization, and circumferential direction mechanical property measurements were thus absent from this investigation, and their combined effect with longitudinal stretch is an important area for future investigation.

Fourth, testing was conducted at room temperature. Future investigations may evaluate BV properties at body temperature to evaluate any temperature dependencies and ensure biofidelity of properties. Fifth, all BV tissue was cryopreserved between excision and testing. While several studies employing similar cryopreservation techniques have found no difference in mechanical properties of fresh and cryopreserved blood vessels,^{1, 4, 5, 29, 35, 48, 52} testing of fresh BVs is required to confirm that this pattern holds for BVs. Sixth, no histological analysis was performed on the BV tissue and tests were not imaged with high speed video. In Figure 4, the low rate human infant failure curve shown includes a transient decrease in stress at high levels of stretch. This is the most extreme example of transient high stretch decreases in stress observed in all of our testing. We posit that this may be indicative of failure of individual vessel wall components; however, as no histological testing was done on post-test BV specimens and the tests were not filmed with high speed video, we cannot exclude slippage at the device grips, though none was observed on visual inspection. Future testing of BV mechanical properties with histological analysis of elastin and collagen fiber content and orientation before and after different stretch protocols may reveal structural changes that result in mechanical property changes with rate and load history, and high speed video is encouraged to capture local vessel displacements and possible slippage of the specimens from the grips. Seventh, we were unable to measure the biomechanical influence of biological remodeling after a single or a cyclic sub-failure elongation test. In vivo, remodeling may further soften or possibly stiffen BV mechanical properties and alter the vein behavior prior to a subsequent elongation. We expect that the remodeling response may vary depending on whether the next load occurs minutes, hours, or days later. Eighth, the cyclic peak stretch level was benchmarked from preliminary low rate porcine failure tests. Due to the higher ultimate stretch values found for human infant compared to porcine BVs, the human infant tissue was cycled to a proportionately lower stretch compared to its ultimate value than porcine tissue (Figure 2). Although loading differences could contribute to observations that porcine BVs were more likely to incur damage during cyclic loading than human BVs, similar observations of softening BV behavior were observed across species (Tables 1 and 3). Finally, the cyclic loading protocol was limited to a 30 second duration as a hypothetical extreme for abusive shaking scenarios

and to match the duration of in vivo porcine cyclic head rotational injury studies⁹. It is possible that steady state was not achieved in this timeframe and that peak stress would continue to decay if cycling had continued beyond 30 seconds in our experiments. Future studies may examine both longer and shorter cycling durations to determine the convergence to steady state more precisely.

In this study, we have shown that the load-related failure properties of parasagittal BVs are sensitive to rate within the range of $\sim 1.2\text{--}16\text{s}^{-1}$, with high rates yielding higher yield stress, ultimate stress, and elastic modulus than low rates. We have also shown that, with the exception of elastic modulus, human BV mechanical parameters (λ_y , λ_u , σ_y , σ_u) are consistently higher than porcine. Thus, investigators utilizing animal models to describe hemorrhage thought to stem from rupture of the BVs should interpret results according to species-appropriate thresholds for BV failure. In addition, we find a lack of age dependency in BV mechanical properties. We observe that BVs display persistently altered mechanical behavior demonstrated by continuously decreasing peak stresses, longer low stress “toe” regions in the stress-stretch curve with successive loading to a repeated level of stretch, and an absence of recovery after a prescribed period of relaxation, implying that BVs experience mechanical fatigue with recurring loads. Future studies should evaluate this altered BV behavior with precision by elucidating BV vessel wall mechanical disruption through histological analysis and assessment of biaxial tissue mechanics.

This is the first study to examine the behavior of parasagittal BVs under cyclic loading. However, repeated loading of the BVs may be a common mode of injury (e.g. repeated tackling in football or heading of a soccer ball, and shaking-type incidences of child abuse). The data presented here will inform future studies of BV failure during singular and repeated TBI events, and is an important first step in investigating the presence of mechanical fatigue in the parasagittal BVs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Stretch vs time and stress vs time plots of a typical cyclic loading protocol followed by post-cyclic failure test. Cyclic loading peak stretch ratios are highly repeatable between cycles. Peak stresses decay exponentially with continued cycling.

Figure 2.

Representative human infant and porcine newborn curves showing the exponential decay of peak stresses with continued cycling for 30 seconds. Peak stress points are blue dots and exponential curve fits (Eq. 3) are indicated by solid red lines. Representative human infant and porcine newborn cyclic stress vs stretch curves are inset showing cyclic loading and post-cyclic failure curves. Note that the y-axis limit of the inset stress vs stretch curves differ between human and porcine subjects. While human and porcine cyclic loading was conducted to the same level of peak stretch, because human failure stretches occurred at significantly higher values than porcine, human specimens experienced cyclic loads further from their failure limit than porcine tests.

Figure 3.

Representative curve from anecdotal human infant BV tests in which two 15-second cycling episodes were separated by 10 minutes of relaxation, during which the specimen was held at its test gage length and continuously hydrated. In both the first and second cycling episodes, peak stress decays exponentially with continued cycling, and no viscoelastic recovery in axial BV peak stress is observed after 10 minutes of relaxation.

Figure 4.

Failure test stress vs stretch plots for representative human infant and porcine newborn subjects. Yield points are indicated by “x” and ultimate points are indicated by circles. Solid black lines indicate the determined linear region over which E_{linear} was calculated.

Table 1

Average $\pm$ standard deviation instantaneous peak stress ($\sigma_{\text{peak},0}$) and steady state peak stress ($\sigma_{\text{peak,SS}}$) for 30-second cycling episodes within subject types and across all subjects. No differences were observed between subject types, but instantaneous peak stresses were significantly higher than steady state peak stresses, indicated by differing bracketed letters.

Subject Type	$\sigma_{\text{peak},0}$ (MPa)	$\sigma_{\text{peak,SS}}$ (MPa)
Porcine Newborn (n=9)	6.091 $\pm$ 3.689	0.566 $\pm$ 0.389
Porcine Adult (n=12)	2.964 $\pm$ 3.048	0.792 $\pm$ 0.809
Human Infant (n=7)	3.677 $\pm$ 2.426	0.962 $\pm$ 1.058
Overall (n=28)	4.052 $\pm$ 3.129 [B]	0.751 $\pm$ 0.598 [A]

Table 2

Average stretch rates for low rate, high rate, and post-cyclic failure tests by subject type. Porcine newborn rates were significantly, though modestly, lower than human infant rates, while porcine adult rates were indistinguishable from both porcine newborn and human infant. High stretch rates were significantly higher than post-cyclic stretch rates, which in turn were significantly higher than low stretch rates. Significant differences are indicated by bracketed letters A–E. Values with A and B show differences between rate and subject type, and values with C, D, and E reveal differences between test-type and rate.

Subject Type	$\dot{\lambda}_{\text{low}} \text{ (s}^{-1}\text{)}$	$\dot{\lambda}_{\text{high}} \text{ (s}^{-1}\text{)}$	$\dot{\lambda}_{\text{post-cyclic}} \text{ (s}^{-1}\text{)}$
Porcine Newborn (n=9) [A]	1.248±0.203	12.745±2.046	2.552±0.387
Porcine Adult (n=12) [AB]	1.257±0.126	13.825±2.659	2.453±0.154
Human Infant (n=7) [B]	1.677±0.242	15.692±3.446	2.747±0.384
Overall (n=28)	1.358±0.258 [C]	13.911±2.845 [E]	2.556±0.318 [D]

Table 3

Average yield stretch (λ_y), yield stress (σ_y), ultimate stretch (λ_u), ultimate stress (σ_u), linear elastic modulus (E_{linear}), low stretch modulus (E_{low}), and corner stretch (λ_{corner}) across subject and failure test types. For λ_y , σ_y , λ_u , and σ_u , human infant values were significantly higher than porcine. These differences are indicated by bracketed letters A–B. Load-related metrics (σ_y , σ_u , E_{linear} , E_{low} , λ_{corner}) are significantly higher in high rate tests than in low rate tests. Specifically, for E_{low} post-cyclic values were significantly lower than low rate values, while for λ_{corner} , post-cyclic values were surprisingly similar to high rate values. Post-cyclic σ_y and σ_u were similar to low rate tests and significantly smaller than those in high rate tests, while post-cyclic E_{linear} was indistinguishable from those calculated in both low and high rate tests. Ultimate stretch (λ_u) did not vary with test type, but (λ_y) was greater in post-cyclic tests than in both low and high rate tests, which were indistinguishable. Differences in failure test mechanical parameters by test type are indicated by bracketed letters C–E.

	λ_y	σ_y (MPa)	λ_u	σ_u (MPa)	E_{linear} (MPa)	E_{low} (MPa)	λ_{corner}
<i>Porcine Newborn (n=9)</i>							
Low	1.111±0.021 [A]	1.521±0.539 [A]	1.257±0.110 [A]	3.237±1.524 [A]	25.288±8.544 [A]	15.814±7.022 [A]	1.085±0.020 [A]
High	1.162±0.086 [A]	3.366±1.868 [A]	1.261±0.036 [A]	4.842±2.456 [A]	37.277±29.137 [A]	27.492±21.376 [A]	1.105±0.071 [A]
Post-Cyclic	1.249±0.028 [A]	2.308±1.303 [A]	1.361±0.154 [A]	3.043±1.741 [A]	24.262±16.663 [A]	0.277±1.733 [A]	1.147±0.015 [A]
<i>Porcine Adult (n=12)</i>							
Low	1.164±0.080 [A]	2.391±1.427 [A]	1.235±0.069 [A]	3.215±1.474 [A]	21.858±11.085 [A]	12.899±7.056 [A]	1.057±0.110 [A]
High	1.198±0.076 [A]	4.147±1.907 [A]	1.295±0.132 [A]	5.799±2.927 [A]	33.101±9.596 [A]	17.011±9.864 [A]	1.126±0.063 [A]
Post-Cyclic	1.226±0.028 [A]	2.215±0.670 [A]	1.250±0.035 [A]	2.446±0.789 [A]	24.439±7.886 [A]	0.886±1.089 [A]	1.137±0.014 [A]
<i>Human Infant (n=7)</i>							
Low	1.240±0.085 [B]	5.424±4.252 [B]	1.489±0.183 [B]	7.204±4.613 [B]	30.173±18.492 [B]	11.620±9.028 [B]	1.106±0.063 [B]
High	1.256±0.065 [B]	7.837±6.240 [B]	1.428±0.172 [B]	9.885±6.752 [B]	49.044±39.764 [B]	20.018±18.864 [B]	1.144±0.043 [B]
Post-Cyclic	1.296±0.052 [B]	5.758±4.479 [B]	1.427±0.134 [B]	7.645±4.755 [B]	48.106±32.908 [B]	0.835±1.213 [B]	1.170±0.011 [B]
<i>Overall (n=28)</i>							
Low	1.166±0.082 [C]	2.870±2.708 [C]	1.306±0.157 [C]	4.219±3.063 [C]	25.039±12.611 [C]	13.516±7.474 [D]	1.078±0.080 [D]
High	1.201±0.082 [C]	4.818±3.799 [D]	1.318±0.136 [C]	6.513±4.418 [D]	38.429±26.129 [D]	21.132±16.597 [C]	1.124±0.061 [C]
Post-Cyclic	1.251±0.044 [D]	3.130±2.745 [C]	1.330±0.131 [C]	3.938±3.316 [C]	30.299±21.398 [CD]	0.678±1.334 [E]	1.148±0.019 [C]