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Abstract
Traumatic brain injury (TBI) is the most prominent cause of death and disability in infants. Extra-axial hemorrhage (EAH) is a common finding among infants diagnosed with abusive head trauma, but is confounded by similar findings in accidental injuries. We conducted detailed biomechanical studies to determine whether the kinematics of vigorous shaking could rupture the parasagittal bridging veins (BVs), one cause of EAH. We performed BV mechanical property tests, in situ and in vivo rapid head rotation animal experiments, infant surrogate head kinematic studies, and finite element (FE) model simulations of the neonatal porcine and infant human heads to reveal whether BV rupture may occur due to vigorous shaking. Under longitudinal tension, we found that BV mechanical properties and behavior do not depend on age, but vary with species, stretch rate, and a history of cyclic loading. In situ brain-skull displacements in the neonatal porcine head under rapid nonimpact sagittal rotations are lower than those observed in axial rotations, and altering BV properties yielded no appreciable difference in FE model brain-skull displacement. We correlated FE-predicted BV element failures during sagittal rapid nonimpact head rotations in the piglet with EAH pathology from corresponding animal studies, and identified and independently validated a threshold of 6 failed BV elements in our model were associated with detectable EAH. Finally, using this threshold and human infant BV mechanical properties in a human infant head FE model, we determined a response corridor for BV rupture, and found that rotational acceleration influences BV rupture. Our results suggest that it may be possible to generate a combination of sagittal head kinematics during vigorous shaking that could produce EAH in the human infant. Prior to legal application, additional analyses are required to validate these predictions of BV rupture against real-world data, such as torn BVs at autopsy in cases of admitted shaking without impact. Together, the integrated studies presented in this dissertation illuminate biomechanical factors that may cause BV rupture in the infant.

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CAN VIGOROUS SHAKING CAUSE EXTRA-AXIAL HEMORRHAGE IN NEWBORNS? A DETAILED
HUMAN AND PORCINE STUDY

Stephanie Ann Pasquesi
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CAN VIGOROUS SHAKING CAUSE EXTRA-AXIAL HEMORRHAGE IN NEWBORNS? A DETAILED HUMAN AND PORCINE STUDY

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In loving memory of my grandparents, Carlo and Costanza Pasquesi, who understood hard work and the value of education.
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ABSTRACT
CAN VIGOROUS SHAKING CAUSE EXTRA-AXIAL HEMORRHAGE IN NEWBORNS? A DETAILED HUMAN AND PORCINE STUDY

Stephanie Ann Pasquesi
Susan S. Margulies, PhD

Traumatic brain injury (TBI) is the most prominent cause of death and disability in infants. Extra-axial hemorrhage (EAH) is a common finding among infants diagnosed with abusive head trauma, but is confounded by similar findings in accidental injuries. We conducted detailed biomechanical studies to determine whether the kinematics of vigorous shaking could rupture the parasagittal bridging veins (BVs), one cause of EAH. We performed BV mechanical property tests, in situ and in vivo rapid head rotation animal experiments, infant surrogate head kinematic studies, and finite element (FE) model simulations of the neonatal porcine and infant human heads to reveal whether BV rupture may occur due to vigorous shaking. Under longitudinal tension, we found that BV mechanical properties and behavior do not depend on age, but vary with species, stretch rate, and a history of cyclic loading. In situ brain-skull displacements in the neonatal porcine head under rapid nonimpact sagittal rotations are lower than those observed in axial rotations, and altering BV properties yielded no appreciable difference in FE model brain-skull displacement. We correlated FE-predicted BV element failures during sagittal rapid nonimpact head rotations in the piglet with EAH pathology from corresponding animal studies, and identified and independently validated a threshold of 6 failed BV elements in our model were associated with detectable EAH. Finally, using this threshold and human infant BV mechanical properties in a human infant head FE model, we determined a response corridor for BV rupture, and found that rotational acceleration influences BV rupture. Our results suggest that it may be possible to generate a combination of sagittal head kinematics during vigorous shaking that could produce EAH in the human infant. Prior to legal application, additional analyses are required to validate these predictions of BV rupture against real-world data, such as torn BVs at autopsy in

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CHAPTER 1: BACKGROUND AND SIGNIFICANCE

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 (the highest of any age group), over 15,000 hospitalizations, and nearly 1,000 deaths annually [1]. While accidental falls are the most common cause of TBI among young children, motor vehicle accidents and abusive head trauma (AHT) produce more severe injuries and fatalities [2]. More serious TBIs in infants and toddlers are frequently due to AHT [3], and these cases often have worse outcomes [4]. Extra-axial hemorrhage (EAH), a collection of blood between the brain and skull (including both subdural and subarachnoid bleeds), is a common presentation among children who have suffered AHT [2-4]. Unilateral EAH is more common in older children, while young children display bilateral EAH more frequently [5], suggesting that the mechanism and pattern of injury in AHT may vary with age. Past studies from our laboratory have shown that children do indeed have injury mechanisms and tissue (brain, skull, and suture) material properties that are distinct from adults [6-16].

One mechanism of injury responsible for EAH is thought to be the rupture or tearing of the parasagittal bridging veins (BVs), which drain blood from brain tissue into the superior sagittal sinus, housed in the falx cerebri, which is attached to the inner surface of the skull. Tearing of the BVs may be caused by rapid head motions in which the movement of the brain lags that of the skull, stretching the veins at high rates of deformation at their attachment points or within the subdural space. Indeed, over forty years ago rotational accelerative trauma was identified as the cause of diffuse brain injuries that were most severe at the periphery [17], whereas translational accelerations produced more focal lesions. Thus, together with the prevalence of EAH in cases of AHT, we hypothesize that vigorous shaking without impact in the human infant may indeed cause BV rupture leading to EAH. Questions include whether vigorous shaking alone can cause serious TBI including BV rupture and subsequent EAH, whether the mechanical properties of BVs are rate and/or age-dependent, and whether BVs exhibit fatigue type wear due
to cyclic stretching which may occur in shaking type insults. In summary, the injury mechanism of EAH and the contribution of shaking to EAH in pediatric TBI are not well characterized, generating an abundance of scientific and legal debate [18-22]. As a consequence, prevention of misdiagnosis, improvement of diagnosis criteria, and protection of abused children continue to be clinical, societal, and legal challenges. The over-arching objective of this dissertation was to determine whether BV failure and subsequent EAH may result from episodes of vigorous shaking in the human infant. This goal was achieved by combining human and porcine bridging vein mechanical property testing at low rates, high rates, and under repeated loading (Chapter 2), determination of a sagittal plane rotational brain-skull displacement boundary condition (Chapter 3), porcine and human computational modeling to relate head kinematics to BV elongations (Chapters 4 and 5), and anthropomorphic dummy studies to determine real world AHT loading conditions (Chapter 5). A flowchart of research activities is shown in Figure 1.1.

![Flowchart of research activities](image)

**Figure 1.0.1** Flowchart of studies contained in and questions answered by this dissertation

As background, the axial mechanical properties of BVs have been examined previously in the adult [23-28], but to date no testing has been performed on specimens from subjects under
three years of age [24], with exception of an anecdotal report of bridging veins from three infant subjects [29]. Furthermore, BVs have never been tested during longitudinal cyclic loading. Many materials display fatigue-type failures due to cyclic loading, in which localized microscopic damage is amplified with each successive loading cycle. While undesirable in biological tissues that are expected to function normally over a lifetime of repeated loading, several repeated-strain injuries are reported to stem from mechanical fatigue including bone stress fractures, carpal tunnel syndrome, and tendonitis, among other ailments [30-36]. It is possible that BVs also display similar fatigue-like behavior. Characterizing BV behavior under repeated cyclical loading is especially relevant for understanding whether BVs incur damage or rupture from shaking-type insults. In Chapter 2, we report mechanical properties and behavior of porcine newborn, porcine adult, and human infant bridging veins elongated to failure at two different stretch rates, and under cyclic loading followed by an elongation to failure.

Finite element modeling is a popular computational tool used by many researchers to predict factors that contribute to traumatic brain injury (TBI). To mimic tissue responses, finite element models must include appropriate representations of the brain, skull, and other cranial contents in terms of geometry, material properties, and the contact conditions between different structures. Computational models of adult traumatic head injury have begun incorporating BV structures to determine the likelihood of EAH from TBI [37-44]. However, only three finite element models have attempted to evaluate the potential for infant BV rupture in shaking-type insults [45-47], but these models did not include BV elements, and instead assessed rupture potential from BV insertion and attachment points determined in a previous adult model [39, 45], the relative motion of the brain and skull along the falx cerebri [46], or approximated BVs as springs [47]. In addition, biological fidelity of the interface between the brain and skull, defining the ease with which the brain may translate relative to the skull, is critical to predicting bridging vein damage or failure accurately in finite element simulations of TBI. The anatomical makeup of this space between the brain and skull includes the pia and arachnoid mater, cerebrospinal fluid, arachnoid trabeculae and vasculature, which collectively constitute what is referred to as the pia-arachnoid
complex (PAC). While sophisticated models usually represent the PAC boundary condition using solid or fluid elements, the effects of the chosen boundary conditions often have not been validated against geometric, material property, or other experimental data. Recently, Coats et al [48] optimized a representation of this brain-skull boundary condition against the relative displacement of the brain and skull observed in axial plane rotational motion of an axially transected piglet head [49]. While Coats et al found both linear elastic connectors and solid isotropic elements between the brain and the skull provided good correlation of finite element model brain-skull displacement with those observed in the physical transections, linear elastic connectors resulted in better predictive capabilities for extra-axial hemorrhage [48]. While a few microscale models have begun to address the individual and combined components of the PAC [38, 50, 51], only one study has validated hemorrhage predictions by a full head finite element model using PAC representative elements, but the model response was not validated against in situ brain-skull displacements [50]. In Chapter 3, we measure porcine infant sagittal plane brain-skull displacements at low level rotational velocities using head transection methods similar to Ibrahim et al and Sullivan et al [49, 52], and determine an appropriate representation of this boundary in a sagittal-transection configuration of our previously published porcine infant finite element model [48] including bridging vein elements and properties determined in Chapter 2.

In Chapter 4, to identify the relationship between BV failure and EAH, we combine our porcine bridging vein mechanical properties and behavior from Chapter 2, sagittal plane brain-skull finite element model boundary condition determined in Chapter 3, and previously published newborn piglet head rotation studies [52-54]. We simulated the animal experiments in our whole head newborn porcine finite element model [48], and correlated findings of bridging vein failure with the presence of EAH in experimental pathology. From this, we were able to determine a threshold value for the number of failed bridging vein elements associated with detectable EAH.

In Chapter 5, to predict if vigorous shaking without impact can cause EAH, we modify a finite element model of the human infant head previously developed in our lab [55, 56] to include bridging vein elements with properties found in Chapter 2 for human infant vessels, and the
sagittal plane brain-skull boundary condition determined from newborn porcine transection studies in Chapter 3. Utilizing an instrumented 1.5 month old human infant anthropomorphic surrogate previously developed in our lab [16] with biofidelic neck improvements [57], we measured head kinematics associated with vigorous shaking without impact. These shaking episodes were then simulated in the human infant finite element model to estimate if the angular velocities and accelerations associated with shaking an infant may cause bridging vein failure. This study reveals important insight into the likelihood of EAH as a presenting injury associated with abusive shaking without impact.

Finally, in Chapter 6, the major findings of this dissertation are summarized, the limitations are addressed, and suggestions are given for future studies to aid in understanding and identifying injury scenarios in infants that may produce bridging vein rupture and subsequent EAH, with consideration towards objectively informing the biomechanical engineering science that is critical to accurate clinical differential diagnoses of abusive head trauma.

References


18. Rorke-Adams, L.B., *The triad of retinal haemorrhage, subdural haemorrhage and encephalopathy in an infant unassociated with evidence of physical injury is not the result of shaking, but is most likely to have been caused by a natural disease*: No. J Prim Health Care, 2011. 3(2): p. 161-3.

19. Squier, W., *The triad of retinal haemorrhage, subdural haemorrhage and encephalopathy in an infant unassociated with evidence of physical injury is not the result of shaking, but is most likely to have been caused by a natural disease*: Yes. J Prim Health Care, 2011. 3(2): p. 159-61.


CHAPTER 2: MECHANICAL PROPERTIES OF INFANT HUMAN AND NEWBORN PORCINE PARASAGITTAL BRIDGING VEINS

Abstract

In distinguishing between accidental and abusive traumatic brain injuries (TBIs) in children, extra-axial hemorrhage (EAH, including both subdural and subarachnoid bleeds) is often considered a critical presenting feature. Tearing of the parasagittal bridging veins (BVs) is thought to be one source of EAH associated with TBI. However, there is a dearth of pediatric bridging vein mechanical property data, preventing accurate biomechanical predictions of EAH in accidental and abusive scenarios. Porcine adult, porcine infant, and human infant BVs were subjected 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. We find that human infant BVs are stronger than porcine BVs, and that 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. Importantly, BVs that had been cyclically loaded displayed a shifted stress-stretch behavior. These data will inform future computational modeling efforts to enhance understanding of injury scenarios causing bridging vein failure. Clarifying the role of BV failure in the realm of TBI will significantly impact the public health, clinical, and legal aspects associated with improving the welfare of young children.

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 (the highest of any age group), over 15,000 hospitalizations, and nearly 1,000 deaths annually [1]. 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.

EAH is currently an important presenting finding used to distinguish between accidental and abusive injury etiologies in young children [2, 3]. TBI in infants and toddlers is frequently due to abusive head trauma (AHT) [4], and these cases often have poor outcomes [5, 6]. It follows that whether BV rupture and subsequent EAH may be produced from vigorous shaking alone (no impact) is subject to significant debate [7-11]. Axial mechanical properties and behavior of BVs have been examined previously in the human adult [12-17] with conflicting reports on whether properties depend on stretch rate. Although previous studies have identified age-dependent properties of brain, skull, and suture [18-25], to date no testing has been performed on BV specimens from subjects under three years of age [12] with exception of a limited study of samples from three infant subjects [26]. Thus it is important to elucidate whether BVs also exhibit age-dependent properties and/or behavior.

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 [27-33] 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) [34]. 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.

In this study, we measure the axial mechanical properties and behavior of parasagittal bridging veins 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. These data are analyzed for age, species, rate, and cyclic loading contributions to the elastic modulus, yield, and failure properties of bridging veins, establishing the foundation for future computational studies of accidental and abusive TBI in youth.

**Methods**

**Test Apparatus**

A custom device coupling a rotary motor to a gear chain drive described previously [35] was used to perform elongation tests. Briefly, a computer-controlled closed loop stepper motor (Vexta #AS46AAP, Oriental motor, Braintree, MA) was used to rotate threaded shafts coupled to a gear-chain system to move a plate up and down in a linear fashion. The bottom grip was attached to the moving plate, while the top grip was attached to a 250g capacity load cell (Model 31 with shock and vibration resistance option, Honeywell-Sensotec, Columbus, OH). The load cell was fastened to a lab jack mounted on the ceiling of a cage surrounding the entire device which allowed for gage length adjustment of the specimen. A laser displacement sensor (LC 1607-50, Micro-Epsilon, Ortenburg, Germany) measured the global elongation of the vessel by tracking the position of the translating plate. The lab jack was adjusted until the BV specimen was taut and the load cell just began to register a load. Bridging veins were gripped by flat plate-type grips similar to previous investigations [13-17, 26] as cannulation and fluid perfusion were overly cumbersome and risked pre-test damage to the delicate BVs. Hyperterminal scripts for low rate, high rate, and cyclic elongation provided appropriate rate and frequency inputs to the motor driver (Vexta #ASD13A-AP, Oriental motor, Braintree, MA).

**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 bridging veins 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 bridging veins, which were cut from their brain connection point using surgical scissors. Next, the dura was peeled from the skull, and finally, bridging veins 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 bridging veins were procured at autopsy by a pathologist within 24 hours of death. Care was taken to minimize distortion of the veins during excision. 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.

After excision, bridging vein samples were placed in serial dilutions of 2.5, 5, and 7.5% dimethyl sulfoxide (DMSO) in Dulbecco’s Modified Eagle Media (DMEM) for ten minutes each, followed by a final concentration of 10% DMSO-90% DMEM for 20 minutes prior to freezing in Eppendorf tubes at -80°C. Samples remained frozen until testing at a later date. Previous studies have shown that cryopreservation does not significantly alter the material properties of blood vessels [36-42]. In a preliminary study of n=10 immature porcine saphenous veins using our cryopreservation method, we confirmed that there was no significant difference between the moduli of fresh and frozen vessels (Appendix A).

On the day of testing, samples were removed from -80°C storage and thawed at room temperature until the medium melted, then rinsed of DMSO by placement in serial dilutions of 7.5, 5, 2.5, and 0% DMSO in DMEM for ten minutes each. After rinsing, specimens were placed in fresh DMEM prior to testing.
**Test Protocol**

Bridging veins were first secured in the top grip, which was then attached to the test apparatus. The lab jack was lowered until the opposite end of the bridging vein could be secured in the bottom grip. Once the bridging vein sample was fully fastened, the lab jack was adjusted until the specimen was taut and the load cell just began to register a load. Specimen gage length was measured from grip to grip with calipers and five cycles of preconditioning to a stretch ratio of 1.05 were performed. Then the lab jack was readjusted until a load signal was registered once again and the test gage length was measured from grip to grip with calipers. The specimen was tested immediately after the appropriate adjustments were made to match experimental parameters between tests in the Hyperterminal loading protocols. Specimens were continuously hydrated with DMEM via a transfer pipette up until the elongation test.

One bridging vein specimen from each porcine and human subject was used per loading protocol. Three different test protocols were conducted: high rate pulls to failure (averaging 13.91±2.84 s⁻¹), low rate pulls to failure (averaging 1.36±0.26 s⁻¹), and cyclic loading followed by a pull to failure (averaging 2.56±0.32 s⁻¹). Based on preliminary porcine ultimate stretch ratios, the cyclic loading protocol was conducted at near-failure peak stretch ratios of 1.188±0.005 (Figure 2.1). A cycling frequency of 3Hz was chosen to match that of simulated abusive trauma via vigorous shaking of an anthropomorphic surrogate representative of a 1 month old human infant [43, 44], while a cycling duration of 30 seconds (~90 cycles) was chosen as a hypothetical extreme (Figure 2.1). 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.
Figure 2.1 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.

Cross-sectional area of each specimen was obtained post-test using optical methods. Each specimen fragment was placed on a microscope slide and covered with coverglass. Bridging vein fragments were imaged at 10x magnification (TCS SP5 MP, Leica Microsystems, Wetzlar, Germany), and image slices were taken every 1µm from slide to coverglass (Figure 2.2). Specimen thickness was determined by multiplying the number of slices containing the bridging vein specimen by the 1µm step resolution. Using a projection of all slices, bridging vein width was measured at two points along the length of the specimen contained in each frame. Most often, a bridging vein fragment was too long to fit in a single frame. As such this protocol was repeated from end to end of each fragment. Each measurement (thickness, width) was averaged across all frames and both fragments of a specimen. The circumference was defined as twice the specimen width and wall thickness was half of the measured specimen thickness. From the average
measurements of wall thickness (h) and circumference (C), cross-sectional area (A) was approximated as that of a hollow cylinder as follows:

\[ r_o = \frac{C}{2\pi} \quad [1] \]

\[ r_i = r_o - h \quad [2] \]

\[ A = \pi(r_o^2 - r_i^2) \quad [3] \]

where \( r_o \) is the outer radius and \( r_i \) is the inner radius.

\[ \text{Figure 2.2 Diagram of vessel microscopy setup for dimension measurements} \]

\textbf{Data Analysis}

\textit{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. Stretch ratio and stress were then calculated from the displacement and load traces, as follows:

\[ \lambda(t) = \frac{l(t)}{l_0} \quad [4] \]
\[ \sigma(t) = \frac{F(t)}{A} \] [5]

where \( \lambda \) is stretch ratio, \( t \) is time, \( l \) is the instantaneous length of the specimen, \( l_0 \) is the gage length, \( \sigma \) is stress, \( F \) is instantaneous load, and \( A \) is cross-sectional area as defined in Eq. 3.

The decay of peak stress with successive cycles was fit to a mono-exponential relationship for each sample using the following equation:

\[ \sigma_{\text{peak}} = ae^{-bn} + c \] [6]

where \( \sigma_{\text{peak}} \) is the peak stress, \( n \) is cycle number, and \( a, b, \) and \( c \) are constant coefficients.

Parameter optimization was performed using a custom Matlab program. A nonlinear least-squares formulation was used to fit the data to Equation 6 (above) by implementing the Levenberg-Marquardt algorithm to determine convergence of coefficients \( a, b \) and \( c \). A series of fits with initial values of \( a, b, \) and \( c \) ranging from 0 to 1 in increments of 0.05 was performed, and a combination of initial values which minimized the sum of squared errors was chosen for the final fit. Average values for \( a, b, \) and \( c \) were calculated within each subject group, as well as across all subjects. “Instantaneous” peak stress, \( \sigma_{\text{peak,0}} \) (\( a + c \) in Equation 6), and steady state peak stress, \( \sigma_{\text{peak,SS}} \) (\( c \) in Equation 6) were determined for each fit. Differences between \( \sigma_{\text{peak,0}} \) and \( \sigma_{\text{peak,SS}} \) 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 \( p \leq 0.05 \).

**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 4 and 5. 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 ($\sigma_u$) and ultimate stretch ($\lambda_u$) were defined at the point of peak stress.

Most previous studies investigating parasagittal bridging veins only reported ultimate failure properties [12, 14, 16, 26], but recent work has also measured modulus and yield properties [13, 15, 17]. Often, yield is defined at the point of maximum modulus along the stress-stretch ratio or stress-strain curve [13, 15, 17, 45]. The maximum modulus has been identified as elastic modulus [13], or the elastic modulus at yield [15, 45], but it is unclear whether reported values were calculated over a region of the stress-stretch/strain curve or derived from a single point. Elastic modulus has also been reported over the most linear region of the stress-strain curve [17], but again, determination of the end-points of the linear region remains ambiguous.

Thus, we sought to provide a well-defined method for determining the yield point and linear region of the stress-stretch curve, with guidance from previous investigations. A multi-step protocol was employed. First, to ensure the selection of a linear region after the “toe” region and before plastic deformation, the point of peak modulus occurring above a stretch ratio of 1.075 and before which instantaneous modulus did not fall below -10MPa was identified. This negative modulus cut-off was used to systematically analyze a load trace that was not always monotonically increasing (Figure 2.4, human low rate, representative example), as large transient negative moduli may be indicative of failure of individual fibers or other components of the vessel wall [13, 17]. A linear regression was fit to the point of peak modulus and the four points immediately preceding it. This linear fit was then applied to the entire length of the stress-stretch ratio curve, and the magnitude of the residuals (or absolute value of the difference) between the measured stress values and the linear fit were extracted. The linear region was defined as the segment, including the point of peak modulus, where residual magnitudes remained within 0.05 MPa. The last point in the linear region, or proportional limit, was defined as the yield point from which yield stress ($\sigma_y$) and yield
stretch ($\lambda_y$) were extracted. A second linear regression was performed, this time over the defined linear region, and the elastic modulus (E) was defined as the slope of this fit.

Yield stress, yield stretch, elastic modulus, 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**

Qualitatively similar to previous blood vessel mechanical property studies, the stress-stretch behavior of the bridging veins was nonlinear (Figure 2.3, inset, representative human infant and newborn porcine curves), with the vessel displaying stiffer behavior at higher stretches. In all cases, peak stress decayed exponentially (Eq. 6, Table 2.1, Figures 2.1 and 2.3), with coefficients of determination ($R^2$) averaging 0.913, and never less than 0.791. Instantaneous peak stresses ($\sigma_{\text{peak},0}$) were significantly higher than steady state peak stresses ($\sigma_{\text{peak,SS}}$, Table 2.2). 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). All bridging vein cyclic loading stress-stretch and peak stress decay plots are presented in Appendix B.

**Table 2.1** Average exponential decay equation coefficients (Eq. 6) and coefficients of determination ($R^2$) within subject types and across all subjects.

<table>
<thead>
<tr>
<th>Subject Type</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porcine Newborn (n=9)</td>
<td>2.759±4.708</td>
<td>0.326±0.596</td>
<td>0.822±0.829</td>
<td>0.906±0.044</td>
</tr>
<tr>
<td>Porcine Adult (n=12)</td>
<td>0.880±0.341</td>
<td>0.087±0.031</td>
<td>0.898±0.371</td>
<td>0.918±0.032</td>
</tr>
<tr>
<td>Human Infant (n=7)</td>
<td>1.148±0.820</td>
<td>0.106±0.040</td>
<td>1.110±1.145</td>
<td>0.894±0.055</td>
</tr>
<tr>
<td><strong>Overall (n=28)</strong></td>
<td>1.551±2.737</td>
<td>0.189±0.397</td>
<td>0.866±0.593</td>
<td>0.913±0.037</td>
</tr>
</tbody>
</table>
Figure 2.3 Representative human infant and porcine newborn curves showing the exponential decay of peak stresses with continued cycling. Peak stress points are blue dots and exponential curve fits (Eq. 6) 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.

Table 2.2 Average instantaneous peak stress ($\sigma_{peak,0}$) and steady state peak stress ($\sigma_{peak,SS}$) 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 bracketed letters, alphabetized from low to high values.

<table>
<thead>
<tr>
<th>Subject Type</th>
<th>$\sigma_{peak,0}$ (MPa)</th>
<th>$\sigma_{peak,SS}$ (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porcine Newborn (n=9)</td>
<td>3.581±4.705</td>
<td>0.822±0.829</td>
</tr>
<tr>
<td>Porcine Adult (n=12)</td>
<td>1.778±0.878</td>
<td>0.898±0.371</td>
</tr>
<tr>
<td>Human Infant (n=7)</td>
<td>2.258±1.786</td>
<td>1.110±1.145</td>
</tr>
<tr>
<td>Overall (n=28)</td>
<td>2.248±2.645 [B]</td>
<td>0.927±0.751 [A]</td>
</tr>
</tbody>
</table>

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, similar to the nondestructive cyclic tests (Figure 2.4, representative human infant and newborn porcine curves).

BV behavior was not dependent on age, but did differ with species. Specifically, for all of the outcome metrics ($\lambda_y$, $\sigma_y$, $E$, $\lambda_u$, and $\sigma_u$), there were no differences between porcine newborn and porcine adult bridging veins, but human infant bridging vein values were always significantly
higher than porcine tissue for all metrics except elastic modulus, 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 bridging veins was higher than porcine tissue, this resulted in modestly, but significantly, higher stretch rates in human infant bridging vein failure testing than in porcine newborn tests (Table 2.3). Though statistically significant, this stretch rate difference was small compared to those between low rate, high rate and post-cyclic failure loading protocol rates, 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.

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 2.3), 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 $\lambda_y$, $\sigma_y$, $E$, and $\sigma_u$, as might be expected for viscoelastic materials that demonstrate fatigue-like behavior, but, interestingly, not for $\lambda_u$ (Table 2.4). Specifically, yield stretches were greater in post-cyclic failure pulls than in low and high rate tests, which were indistinguishable from one another (Figure 2.4, black ‘x’ indicates yield point). Yield stress and ultimate stress were higher in high rate tests than low rate and post-cyclic (Figure 2.4, black ‘x’ indicates yield point, black circle indicates ultimate parameter point), which were similar. Elastic modulus was larger in high rate tests than low rate tests, while post-cyclic elastic modulus was not significantly different from both high and low rate (Figure 2.4, black line indicates linear region over which $E$ was calculated). 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 regime, often referred to as the “toe” region (Figure 2.4). All bridging vein failure pull stress-stretch curves are presented in Appendix B. These findings indicate that both rate and load-history affect BV behavior in both species.

![Figure 2.4](image)

**Figure 2.4** 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 elastic modulus was calculated.

**Table 2.3** 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.

<table>
<thead>
<tr>
<th>Subject Type</th>
<th>$\dot{\lambda}_{low}$ (s$^{-1}$)</th>
<th>$\dot{\lambda}_{high}$ (s$^{-1}$)</th>
<th>$\dot{\lambda}_{post-cyclic}$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porcine Newborn (n=9) [A]</td>
<td>1.248±0.203</td>
<td>12.745±2.046</td>
<td>2.552±0.387</td>
</tr>
<tr>
<td>Porcine Adult (n=12) [AB]</td>
<td>1.257±0.126</td>
<td>13.825±2.659</td>
<td>2.453±0.154</td>
</tr>
<tr>
<td>Human Infant (n=7) [B]</td>
<td>1.677±0.242</td>
<td>15.692±3.446</td>
<td>2.747±0.384</td>
</tr>
<tr>
<td>Overall (n=28)</td>
<td>1.358±0.258 [C]</td>
<td>13.911±2.845 [E]</td>
<td>2.556±0.318 [D]</td>
</tr>
</tbody>
</table>
Table 2.4: Average yield stretch ($\lambda_y$), yield stress ($\sigma_y$), ultimate stretch ($\lambda_u$), ultimate stress ($\sigma_u$), and elastic modulus (E), across subject and failure test types. For all metrics except elastic modulus, human infant values were significantly higher than porcine. These differences are indicated by bracketed letters A-B, alphabetized from lowest to highest values. Load-related metrics ($\sigma_y$, $\sigma_u$, and E) are significantly higher in high rate tests than in low rate tests. Post-cyclic yield and ultimate stresses were similar to low rate tests and significantly smaller than those in high rate tests, while post-cyclic elastic moduli were indistinguishable from those calculated in both low and high rate tests. Ultimate stretch did not vary with test type, but yield stretch 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-D, alphabetized from lowest to highest values.

<table>
<thead>
<tr>
<th></th>
<th>$\lambda_y$</th>
<th>$\sigma_y$ (MPa)</th>
<th>$\lambda_u$</th>
<th>$\sigma_u$ (MPa)</th>
<th>E (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Porcine Newborn</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=9)</td>
<td>[A]</td>
<td>[A]</td>
<td>[A]</td>
<td>[A]</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>1.111±0.021</td>
<td>1.521±0.539</td>
<td>1.257±0.110</td>
<td>3.237±1.524</td>
<td>25.288±8.544</td>
</tr>
<tr>
<td>High</td>
<td>1.162±0.086</td>
<td>3.366±1.868</td>
<td>1.261±0.036</td>
<td>4.842±2.456</td>
<td>37.277±29.137</td>
</tr>
<tr>
<td>Cyclic</td>
<td>1.249±0.028</td>
<td>2.308±1.303</td>
<td>1.361±0.154</td>
<td>3.043±1.741</td>
<td>24.262±16.663</td>
</tr>
<tr>
<td><strong>Porcine Adult</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=12)</td>
<td>[A]</td>
<td>[A]</td>
<td>[A]</td>
<td>[A]</td>
<td>[A]</td>
</tr>
<tr>
<td>Low</td>
<td>1.164±0.080</td>
<td>2.391±1.427</td>
<td>1.235±0.069</td>
<td>3.215±1.474</td>
<td>21.858±11.085</td>
</tr>
<tr>
<td>High</td>
<td>1.198±0.076</td>
<td>4.147±1.907</td>
<td>1.295±0.132</td>
<td>5.799±2.927</td>
<td>33.101±9.596</td>
</tr>
<tr>
<td>Cyclic</td>
<td>1.226±0.028</td>
<td>2.215±0.670</td>
<td>1.250±0.035</td>
<td>2.446±0.789</td>
<td>24.439±7.886</td>
</tr>
<tr>
<td><strong>Human Infant</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=7)</td>
<td>[B]</td>
<td>[B]</td>
<td>[B]</td>
<td>[B]</td>
<td>[A]</td>
</tr>
<tr>
<td>Low</td>
<td>1.240±0.085</td>
<td>5.424±4.252</td>
<td>1.489±0.183</td>
<td>7.204±4.613</td>
<td>30.173±18.492</td>
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<tr>
<td>High</td>
<td>1.256±0.065</td>
<td>7.837±6.240</td>
<td>1.428±0.172</td>
<td>9.885±6.752</td>
<td>49.044±39.764</td>
</tr>
<tr>
<td>Cyclic</td>
<td>1.296±0.052</td>
<td>5.758±4.479</td>
<td>1.427±0.134</td>
<td>7.645±4.755</td>
<td>48.106±32.908</td>
</tr>
<tr>
<td><strong>Overall</strong> (n=28)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>1.166±0.082</td>
<td>2.870±2.708</td>
<td>1.306±0.157</td>
<td>4.219±3.063</td>
<td>25.039±12.611</td>
</tr>
<tr>
<td>High</td>
<td>1.201±0.082</td>
<td>4.818±3.799</td>
<td>1.318±0.136</td>
<td>6.513±4.418</td>
<td>38.429±26.129</td>
</tr>
<tr>
<td>Cyclic</td>
<td>1.251±0.044</td>
<td>3.130±2.745</td>
<td>1.330±0.131</td>
<td>3.938±3.316</td>
<td>30.299±21.398</td>
</tr>
</tbody>
</table>
Discussion

This study deepens our understanding of potential biomechanical factors that contribute to bridging vein rupture, and provides data essential to modeling bridging vein behavior under scenarios in which a young child may sustain a head injury. First, bridging vein mechanical properties did not display any age dependency. Second, except for elastic modulus, bridging vein mechanical properties are species dependent, with human tissue producing higher values than porcine. Third, we find that load-related mechanical properties ($\sigma_y$, $\sigma_u$, E) of bridging veins are rate-dependent, with values increasing with rate. However, parameters that are purely displacement-related ($\lambda_y$, $\lambda_u$) do not differ with changes in stretch rate. Fourth, prior load history also has a significant effect on bridging vein failure loading such that in post-cyclic tests, stress-stretch toe regions are longer, leading to an increase in yield stretch, 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 bridging veins decreased exponentially with successive cycles. Together, these data will increase the biofidelity of future computational modeling simulations of 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 bridging vein 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)[12]. 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) [17]. Based on the properties measured in infant through advanced age, we conclude that there is no evidence for age dependence of bridging vein mechanical properties, and that any predisposition to bridging vein 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 bridging veins produced significantly higher values than porcine tissue for all failure test mechanical parameters except elastic modulus. Prior to this study, only Morison had examined the mechanical properties of both human and porcine bridging veins [26], though only two porcine and three human subjects were evaluated, and porcine vessels included other pial and arachnoid veins in addition to bridging veins. Though no statistics were performed, Morison’s anecdotal findings showed that ultimate stress was higher in human bridging veins than the array of porcine veins [26], consistent with this study. However, bridging veins from the three human subjects had lower ultimate stretches than cerebral veins from the two porcine subjects, which differs from our much larger subject population [26]. We conclude it is important to incorporate species dependencies in bridging vein properties in order to accurately communicate findings of extra-axial hemorrhage in animal models of TBI. Although species differences in failure properties have implications for translating hemorrhage findings from animal studies to humans, the similar elastic moduli across pigs and humans is a convenient finding for computational modeling.

We observed no differences in displacement-related mechanical parameters ($\lambda_y$, $\lambda_u$) 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 $\sim<100$s$^{-1}$ [14], 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 ($<3.4$s$^{-1}$, 10-60s$^{-1}$, and 100-200s$^{-1}$) [17]. In further contrast, Meaney, Delye et al, and Lee and Haut found no dependence of the mechanical properties of bridging veins on strain rate [12, 13, 16]. In conclusion, most studies find no rate
dependence in displacement-related parameters ($\lambda_y$, $\lambda_u$) in bridging veins with single studies reporting properties increase or decrease with rate.

In contrast, we detected rate dependency in load-related mechanical properties ($\sigma_y$, $\sigma_u$, $E$) of bridging veins, with values increasing at higher rates of deformation. Lowenhielm also observed higher ultimate stresses at higher strain rates [14]. 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 [17]. Monea et al also commented that elastic modulus increased between low ($<3.4\text{s}^{-1}$) and intermediate (10-60s$^{-1}$) strain rates (similar to the low and high rate groups in this study, respectively, Table 2.3), with a subsequent decrease from intermediate to high (100-200s$^{-1}$) rates, and these differences were trending towards significance ($p=0.059$) [17]. However, Monea et al only obtained ultimate stress data from three bridging veins and yield and elastic modulus data from four bridging veins in their lowest rate group ($<3.4 \text{s}^{-1}$) [17]. Additional data at this rate level may provide better statistical power. In a study involving bridging veins, middle cerebral arteries, and cortical arteries and veins, Monson et al found that strain rate (groups: 0.03-0.21 s$^{-1}$, and 20-138s$^{-1}$) did have a significant effect on artery and vein 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.) [15]. Conversely, several studies did not find rate dependence in bridging vein load-related mechanical properties (0.1-250s$^{-1}$) [12, 13, 16]. 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 elastic modulus with increasing stretch rates.

We compared our measured values for human infant bridging vein elastic modulus, ultimate stress, yield stress, ultimate stretch and yield stretch with those previously reported in literature. The human infant elastic modulus value calculated at low rates in this study (30.173±18.492 MPa) is remarkably similar to those in Delye et al (30.69±19.40 MPa) [13] and also close to those in Monea et al (23.26±14.08 MPa, low rate) [17], both of whom tested at rates similar to the low rate group presented here. The ultimate stresses found in this study
(7.204±4.613 MPa for low rates, 9.885±6.752 MPa for high rates) are between those reported by Meaney (12.02±5.9 MPa for 3-9 year olds, 18.57±14.2 MPa for 27-47 year olds, 8.02±3.95 MPa for subjects >62 years old; no age significant differences observed) [12] and those reported by Delye et al (4.99±2.55 MPa) [13] and Monea et al (3.60±0.76 MPa for low rates <3.4s⁻¹, and 4.78±2.82 MPa for intermediate rates 10-60s⁻¹) [17]. 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 bridging veins 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 bridging veins from older subjects (1.13-1.29 yield stretch; 1.25-1.67 ultimate stretch) [12, 13, 15-17]. Furthermore, because these displacement-related mechanical parameters conveniently did not vary with stretch rate in our study, we encourage their use in future modeling endeavors.

To our knowledge, ours is the first study to examine the longitudinal mechanical properties of bridging veins under cyclic loading. Furthermore, there have been few other studies to measure longitudinal behavior of any blood vessel under cyclic loading [35, 46-49], and most have not reported cyclic responses beyond five cycles [46-49]. 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 multiple bridging vein elongations. With exposure to prolonged cyclic elongation to the same peak displacement, we observe softening of the bridging veins characterized by decreasing peak stresses (Figures 2.1 and 2.3), and lengthening of the low-stress “toe” region, such that stiffer behavior occurs at increasingly higher stretch ratios (Figure 2.4), also observed in immature porcine common carotid arteries [35] (Appendix C).

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, typically referred to as the “toe” region, 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. Yield stretches were higher in elongation to failure tests performed after cyclic loading than failure tests without prior cyclic loading, but interestingly, cyclic loading and its associated higher yield stretches and shifted behavior did not significantly affect any other property measured. In summary, although elastic modulus and failure properties did not differ between lower rate elongations to failure with or without 30 seconds of cycling prior, because yield stretch was higher and toe regions were longer after cycling, we conclude that cyclic loading conferred damage to the mechanical integrity of the bridging veins prior to failure loading. The increased length of the toe region may imply that the brain will continuously achieve greater momentum (and thus higher stretch rates) when bridging veins are repeatedly elongated because they are unable to supply the same level of mechanical resistance to motion at low stretch ratios as their previously unloaded counterparts.

In an investigation into the ultrastructure of bridging veins, Yamashima and Freide found that the majority of collagen fibers are oriented circumferentially rather than longitudinally [50]. Thus, we posit that the observed changes in yield and toe region length of bridging vein behavior 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. It is plausible that comparable damage may have occurred during cyclic loading, but was not as readily detectable due to the relatively fast loading-unloading frequency. Our observation of post-cyclic failure pull 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 [51, 52]. In comparison to our cyclic bridging vein experiments, Bell et al found that longitudinally over-stretching ovine middle cerebral arteries to subcatastrophic levels beyond in vivo stretches resulted in decreased toe region slope and increased high-stretch slope of the stress-stretch curve [53], similar to our findings regarding longer toe regions and elastic moduli indistinguishable from high rate tests in
post-cyclic failure tests. Bell et al also found that ultimate stretch values obtained from a failure test after over-stretch and a relaxation period were unaffected by the amount of over-stretch applied to a middle cerebral artery specimen [53], similar to our finding of indistinguishable ultimate stretches between post-cyclic failure tests and failure tests involving specimens with no previous load history. Finally in the Bell et al study, after over-stretch, an increased amount of stretch was required to obtain a target level of stress, and there was no viscoelastic recovery within 60 minutes [53]. We hypothesize that analogous experiments investigating viscoelastic effects in bridging veins would yield results similar to those found by Bell et al. 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, histological investigations of the unloaded and cyclically loaded vessel wall, and studies of multiple cyclic loading protocols with prescribed periods of relaxation to account for any viscoelastic effects.

Limitations

The presented studies include several limitations. First, testing was conducted at room temperature. Future investigations may evaluate bridging vein properties at body temperature. Second, we measured grip to grip displacement to capture the average bridging vein response. Investigators interested in more localized stretch behavior may use speckle patterning and video capture techniques. Third, cyclic loading was displacement-controlled, and yielded decreasing peak stress and longer low-stress toe regions in the stress-stretch curve with successive cycling. Creep response may be evaluated in future stress or force-controlled studies; and/or multiple cyclic loading protocols may be prescribed with periods of relaxation between them to assess the presence of mechanical fatigue under repeated loading. A limitation of these proposed ex vivo studies is that we are 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 bridging vein 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. Finally, 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 bridging veins, the human infant tissue was cycled to a proportionately lower stretch compared to its ultimate value than porcine tissue (Figure 2.3). Although loading differences could contribute to observations that porcine bridging veins were more likely to incur damage during cyclic loading than human bridging veins, similar observations of softening bridging vein behavior was observed across species (Tables 2.1, 2.2, and 2.4).

Conclusions

In this study, we have shown that the load-related failure properties of parasagittal bridging veins are sensitive to rate within the range of \( \sim 1.2-16 \text{s}^{-1} \), 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 bridging vein mechanical parameters \((\lambda_y, \lambda_u, \sigma_y, \sigma_u)\) are consistently higher than porcine. Thus, investigators utilizing animal models to describe hemorrhage thought to stem from rupture of the bridging veins should interpret results according to species-appropriate thresholds for bridging vein failure. In addition, we find a lack of age dependency in bridging vein mechanical properties. We observe that bridging veins display persistently altered mechanical behavior demonstrated by continuously decreasing peak stresses and longer low stress “toe” regions in the stress-stretch curve with successive loading to a repeated level of stretch, implying that bridging veins may experience mechanical fatigue with recurring loads. Computational simulations of repeated insults that may cause TBI, similar to those seen in sports and child abuse, should employ this altered BV behavior in order to accurately describe incidences of BV failure and subsequent EAH.

This is the first study to examine the behavior of parasagittal bridging veins under cyclic loading. However, repeated loading of the bridging veins 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 finite element and computational studies of bridging vein failure during singular and repeated TBI events, and is an important first step in investigating the presence of mechanical fatigue in the parasagittal bridging veins. Future studies may use the data and observations shown in this study to simulate a variety of mechanical insults and predict the occurrence or likelihood of bridging vein mechanical disruption and subsequent extra-axial hemorrhage.

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**References**


7. Rorke-Adams, L.B., The triad of retinal haemorrhage, subdural haemorrhage and encephalopathy in an infant unassociated with evidence of physical injury is not the result of shaking, but is most likely to have been caused by a natural disease: No. J Prim Health Care, 2011. 3(2): p. 161-3.

8. Squier, W., The triad of retinal haemorrhage, subdural haemorrhage and encephalopathy in an infant unassociated with evidence of physical injury is not the result of shaking, but is most likely to have been caused by a natural disease: Yes. J Prim Health Care, 2011. 3(2): p. 159-61.


CHAPTER 3: MEASUREMENT OF SAGITTAL PLANE BRAIN-SKULL DISPLACEMENTS AND VALIDATION OF A CORRESPONDING FINITE ELEMENT MODEL BOUNDARY CONDITION IN THE NEONATAL PIG

Abstract

Computational models are valuable tools for studying tissue level mechanisms of traumatic brain injury, but in order to produce more accurate estimates of tissue deformation, these models must be validated against experimental data. In this study, we present in situ measurements of brain-skull displacement in the neonatal piglet head (n=3) at the sagittal midline during six rapid nonimpact rotations (two rotations per specimen) with peak angular velocities averaging 51.7±1.4 rad/s. Each rotation was captured via high speed video, marks on the sagittally cut brain and skull/rigid potting surfaces were tracked throughout motion, and peak values of relative brain-skull displacement were extracted. Maximum physical model brain-skull displacements in the second rotation were significantly greater than those measured during the first rotation, but this difference was not greater than our measurement error and thus it is unknown whether this difference is meaningful. Comparison of maximum physical model brain-skull displacements in this sagittal transection study were significantly less than values extracted from a previous axial plane physical transection model. In a finite element model of the sagittally transected neonatal porcine head, the brain-skull boundary condition was matched to the measured physical experiment data, and the corresponding finite element boundary condition optimized for sagittal plane rotations is far less stiff than its axial counterpart. Finally, bridging veins were included in the finite element model and varying the bridging vein mechanical behavior over the range of those observed in Chapter 2 had no influence on the brain-skull boundary displacements. This direction-specific sagittal plane boundary condition will be employed in future finite element models designed to develop thresholds for and predictions of bridging vein rupture and extra-axial hemorrhage resulting from rapid sagittal head rotations in Chapters 4 and 5.
Introduction

Finite element modeling is a popular computational tool used by many researchers to aid in understanding and prediction of traumatic brain injury (TBI). To simulate injurious occurrences accurately, finite element models must include appropriate representations of the brain, skull, and other cranial contents in terms of geometry, material properties, and contact interactions between different structures. Extra-axial hemorrhage is thought to stem from the rupture or tearing of the parasagittal bridging veins that drain blood from the brain into the superior sagittal sinus, which is housed within the falx cerebri, attached to the skull. Thus the boundary condition between the brain and skull is of utmost importance when using finite element modeling to simulate and predict the occurrence of extra-axial hemorrhage.

Anatomically, three meningeal layers lie between the brain and skull: the dura mater, which is firmly attached to the skull, the arachnoid mater in the middle, and the pia mater which adheres to the surface of the brain following all of the gyri and sulci. Together the arachnoid and pia mater constitute the leptomeninges, or pia-arachnoid complex (PAC), consisting of cerebrospinal fluid, vasculature, arachnoid trabeculae, and the membranes themselves. While numerous sophisticated finite element models designed to investigate head injury are described in the literature, most have not replicated properties of the PAC with precision. Our lab recently found that linear elastic spring connectors imposed between the brain and skull provided good agreement between axial plane physical model brain-skull displacements [1] and finite element model brain-skull displacements [2]. Furthermore, this PAC representation predicted the occurrence of extra-axial hemorrhage in a rapid nonimpact rotation model of TBI in porcine neonates with 80% sensitivity and 85% specificity based on the peak strain levels observed in the connector elements [2].

However, we have also shown that physiological and histopathological responses, clinical presentations, and behavioral outcomes, are all dependent on the directional plane of rotational injury in our porcine TBI model [3-5], and similar phenomena have been noted in other studies in pigs [6, 7], rats [8], non-human primates [9, 10], and humans [11-13]. Thus it is critical to
investigate whether the brain-skull interface is also dependent on the direction of rotation or region of the cranium, and if so, determine appropriate direction or region specific boundary representations for finite element models of head rotation.

In this study, we determined brain-skull displacements from a series of sagittally transected piglet heads subjected to sagittal plane rotations, developed a finite element model mimicking the geometry of the sagittally transected piglet head, and determined a brain-skull boundary condition in the finite element model that matched finite element displacements to experimentally-derived values. We also compared our results to those found previously in axial plane physical and finite element model transections [1, 2]. The optimized sagittal brain-skull boundary condition will be used in future finite element models of the entire porcine head when sagittal plane rotations are simulated in order to find estimated elongations of the parasagittal bridging veins and determine the likelihood of extra-axial hemorrhage.

**Methods**

**Physical Model**

**Construction**

Using methods similar to those outlined in a previous studies [1, 2, 14], 3-5 day old female piglets (n=3) were used to construct physical transection models to study sagittal plan brain movements during rapid head rotation. Each piglet was anesthetized with isoflurane, and then sacrificed by administration of a lethal dose (150 mg/kg) of sodium pentobarbital, approved by the University of Pennsylvania Institutional Animal Care and Use Committee. Immediately after sacrifice, the piglet was decapitated at the cervical spine, and all exterior soft tissue and the mandible were removed. A cutting plane was marked in the sagittal plane along the midline of the skull, and the skull was cut using a Dremel rotary tool with diamond wheel attachment with care taken to leave the meninges and brain intact. Once the skull was breached, the meninges and brain were sliced with a brain sectioning knife. The left side of the skull, meninges, and brain was discarded.
The right side of the transected head was embedded in a 5-inch top inner diameter aluminum pan using polymethylmethacrylate (PMMA, Dentsply, York, PA) and attached to a custom aluminum canister via three set screws inserted through the side of the canister and aluminum pan and into the PMMA. Due to heat release during the PMMA curing process, preliminary tests were conducted to determine whether these high temperatures may affect the mechanical integrity of the tissue. The similar thermal conductivity ($k=0.42$ W/mK) Plaster of Paris compared to bone allowed the use of Plaster of Paris as a bone surrogate. Hemispherical molds ($n=2$) of Plaster of Paris (Red Devil Inc., Union, NJ) with thickness similar to that of the porcine infant skull (4-5mm) were embedded in curing PMMA and filled with water. Across both tests, the peak temperature recorded on the inner surface of the Plaster of Paris mold during curing was 37.1°C, which is below the normal average body temperature of infant piglets (39°C [15]), and therefore we conclude that no mechanical damage to the brain tissue occurred during PMMA curing.

Figure 3.1 Cross-sectional diagram of the physical sagittal plane transection configuration.
Immediately after mixing 120 g of powder and 80 ml of liquid PMMA components, the curing PMMA was poured into the tart pan/set screw/canister structure and the right side of the transected head was potted in the PMMA with the cut surface facing outwards, and the skull edge level with that of the aluminum canister. Once the PMMA had hardened, 24-48 dots, ~2-3 mm in diameter, were applied to the exposed surface of the brain with India Ink (Speedball Art, Statesville, NC). An additional 6-16 dots were placed on the skull and PMMA and used to assess rigid body motion. A border of clear silicone caulk was applied on top of the exposed skull edge to provide a seal around the brain and between the skull and a clear acrylic cover plate. The silicone caulk and India Ink were allowed to dry for ~45 minutes. A thin layer of transparent lubricant (KY Brand, Skillman, NJ) was applied to fill the space between the brain and the cover plate to minimize friction between the brain and the plate. Finally, the cover plate was secured to the canister. Throughout the preparation, the brain tissue was kept moist by periodic application of 1X phosphate-buffered saline on the cut surface. All sagittal transection models were prepared and tested within 6 hours of sacrifice. A schematic of the transection experimental configuration is shown in Figure 3.1.

Rotation

The canister assembly was mounted on one side arm of a custom linkage that converts linear motion generated by a HYGE device (Bendix Corporation, Southfield, MI) into a 65 degree angular rotation [16]. The center of rotation of the linkage was within the piglet cervical spine relative to the center of mass of the brain in the canister assembly. Each specimen was subjected to two consecutive rotations with peak velocities averaging 51.3 ± 1.2 rad/s (rotation 1) and 52.0 ± 1.7 rad/s (rotation 2). Experiments were filmed with a high speed video camera (HG TH, Redlake, Tallahassee, FL) at 2500 frames per second with a resolution of 320 x 480 (~0.4 mm/pixel). Angular velocity was measured by two angular rate sensors (ARS-06, Applied Technology Associates, Albuquerque, NM) mounted to the linkage side arm, and recorded at 10,000 Hz using LabView software (National Instruments, Austin, TX).
**Image Processing**

To enhance the visibility of the marker dots, image contrast and brightness were batch adjusted in Adobe Photoshop CS4 (San Jose, CA) prior to analysis. A previously-developed custom MATLAB script (The Mathworks, Natick, MA) was used to isolate and track the marker dots frame-by-frame [1, 14]. Marker dots on the brain, skull, and PMMA surfaces were segmented and labeled as objects in a binary black and white image. The perimeter and centroid of each dot was calculated and superimposed on the original image for each frame to verify segmentation accuracy. To match marker dots between frames, marker centroid coordinates in a given frame were compared to those of the preceding frame and the marker centroid nearest to and within a 10 pixel distance of a confirmed marker location in the preceding frame was assigned the corresponding dot label. In each frame, the centroid x and y pixel coordinates for each successfully segmented dot were defined in a global stationary reference system and recorded.

**Data Analysis**

After marker dots were successfully isolated, dots on the periphery of the cut brain cortex surface (Figure 3.2), excluding those on the cerebellum and brainstem, were matched to the nearest skull or PMMA dot to assess brain displacement relative to the rigid skull throughout the canister rotation. The distance between brain dot centroids and their respective rigid dot centroids was calculated for each video frame from fifty frames before motion began until the frame in which the brain had stopped moving. For each brain-rigid dot pair, the calculated distances over the fifty frames before motion began was averaged to find a baseline distance for each brain-rigid dot pair. The average baseline distance for each pair pre-motion was subtracted from the respective brain-rigid dot pair’s calculated distance in each frame to find the relative displacements between brain and rigid dots over the fifty frames before motion and the duration of the rotation. The maximum relative displacement found in each brain-rigid dot pair in the fifty frames prior to motion was extracted as an error measure correlating with the maximum
experimental displacements. The maximum relative displacement achieved by each brain-rigid dot pair during rotation was extracted over the experimental frames.

Figure 3.2 Example of an initial cropped video frame of a physical transection experiment. A) Brain dots around whole cortex periphery are selected. B) Sagittal-only brain dots are selected.

The maximum experimental displacements and respective error measures for each brain-rigid dot pair were pooled between subjects into two groups: those found in the first rotation, and those found in the second rotation. A series of Wilcoxon signed-rank tests were conducted to determine whether significant differences existed between the maximum displacements found during a rotation and their respective pre-motion errors, maximum displacements observed in the first and second rotations, and errors in the first and second rotations. Maximum displacements observed during the first rotations were also compared to those from other transection experiments performed previously in the axial plane at similar angular velocities [1, 2], again using a Wilcoxon signed rank test.
Finite Element Model

Construction and Simulation

To create finite element model geometry similar to the sagittal transection physical configuration, we modified a previously reported piglet finite element model [2, 14]. To mimic the sagittal transection experimental configuration, the converged 3-5 day old piglet model was transected at the sagittal midline, discarding the left side of the model. The resulting transection model (Figure 3.3) had 6,509 brain elements, 732 falx elements, and 8,858 skull elements. The frictionless boundary condition between the transected surface of the brain and the acrylic cover plate was replicated by imposing a kinematic coupling constraint on the corresponding finite element model surface such that the brain nodes on the cut surface were free to translate in the cut plane, but were prevented from out of plane deformations.

Figure 3.3 Sagittally transected finite element model of the 3-5 day old piglet, brain (blue), skull (green), and falx (yellow).
The brain elements were defined as a homogenous, isotropic, nonlinear hyperelastic material according to a first-order Ogden strain energy density function (Eq. 1):

\[
W = \frac{2\mu(t)}{\alpha^2} (\lambda_1^\alpha + \lambda_2^\alpha + \lambda_3^\alpha - 3) \quad [1]
\]

where the \( \lambda \)'s are the principal stretch ratios, \( \alpha \) is a nonlinear tissue-specific parameter dependent on strain magnitude, and \( \mu(t) \) is the brain shear relaxation modulus. The brain shear relaxation viscoelastic response was modeled using a two-term Prony series function (Eq. 2):

\[
\mu(t) = \mu_0 \left( 1 - \sum_{i=1}^{2} C_i \left( 1 - e^{-t/\tau_i} \right) \right) \quad [2]
\]

where \( \mu_0 \) is the brain shear modulus, \( C_i \) are the relaxation moduli, and \( \tau_i \) are time constants. The relaxation moduli and time constants were selected based on those published in our previous study investigating high strain deformation of porcine brain [17]. Brain shear modulus was selected as the low end of an optimized range of 3-5 day old piglet brain shear moduli (553 Pa) determined from prior in situ axial transection experiments [14]. This value is similar to previously reported measures of in vitro shear modulus (526.9 Pa) [17] and in situ shear modulus (541 Pa) [18]. The falx was defined as an isotropic, linear elastic material with elastic modulus half that of adult human dura [19], based on a comparison of adult and fetal human dura [20] stiffness values [2]. The skull was modeled as a rigid body. All material properties and constants are reported in Table 3.1.

<table>
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<th>Material Property</th>
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<td></td>
<td>( \alpha = 0.01 )</td>
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</tr>
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<td></td>
<td>( C_1 = 0.3322 )</td>
<td>Prange and Margulies, 2002 [17]</td>
</tr>
<tr>
<td></td>
<td>( C_2 = 0.3890 )</td>
<td>Prange and Margulies, 2002 [17]</td>
</tr>
<tr>
<td></td>
<td>( \tau_1 = 2.9572 \text{ s} )</td>
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</tr>
<tr>
<td></td>
<td>( \tau_2 = 0.1813 \text{ s} )</td>
<td>Prange and Margulies, 2002 [17]</td>
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<tr>
<td></td>
<td>( \rho = 1.04 \text{ g/cm}^3 )</td>
<td>Sullivan et al, 2015 [14]</td>
</tr>
<tr>
<td></td>
<td>( \nu = 0.49999 )</td>
<td>Sullivan et al, 2015 [14]</td>
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</tbody>
</table>
A previous study using the axial transection and full head configurations of this newborn porcine computational model examined the boundary condition between the brain and skull during axial direction rotations, and found that linear elastic spring connectors linking every brain surface node to the nearest skull node provided an approximate combined response of the pia-arachnoid complex and cerebrospinal fluid such that brain tissue strain and brain-skull displacement were similar between physical and finite element estimations [2]. Not including the sagittal cut plane, the sagittally transected model had 1,398 brain surface nodes. Each brain surface node was matched to the nearest node on the inner surface of the skull or falx. Eleven of these node pairings were reserved for elements representing bridging veins, according to the average number of bridging veins found per hemisphere in a study of human adult cadavers [21], leaving 1,387 linear elastic connector elements to define the boundary condition between the brain and skull. We hypothesize that brain-skull displacement in the axial plane may differ from that in the sagittal plane, and our objective was to optimize the elastic stiffness of the connectors based on measured brain-skull displacements from the physical transection experiments (Results). A total of 15 different elastic stiffnesses ranging from 34.6 to 4,000 N/m for the general brain connectors were simulated in all six sagittal transection simulations (n=3 piglets x 2 rotations/piglet, Table 3.4, Results).

In axial and coronal plane rotations, the falx cerebri may prevent lateral brain movement near the superior cortical surface. Conversely, in sagittal plane rotations, the falx cerebri does little to prevent brain-skull relative displacement and resulting bridging vein elongation and it is important to include bridging vein elements to account for their potential tethering influence at the brain-skull boundary. The eleven bridging veins were chosen from brain nodes that connected to nodes on the inner surface of the falx as the superior sagittal sinus, into which the bridging veins drain, is housed inside the falx. In the same study of adult cadavers, the bridging veins were found to enter the superior sagittal sinus in two clusters, and this clustering also matched findings from digital subtraction angiography patients [21]. Briefly, from anterior to posterior, the superior sagittal sinus was divided into four segments, the first two of which each comprised about 20% of
the total length while the last two comprised 30% of the total length each [21]. The first and third segments were tributary, while the second and fourth had few bridging connections [21]. Finally, the first segment contained about five bridging veins and the third contained six bridging veins on average per hemisphere [21]. Based on this observed clustering, the inner surface of the porcine falx in our computational model was divided into four segments from anterior to posterior along its length corresponding to 20%, 20%, 30%, and 30% of the total length of the falx. Falx nodes that were part of brain tethering pairs in the first and third segments were selected as potential bridging vein connection points. The transected falx inner surface nodes occurred in five medio-lateral columns. Any connection points on the midline of the falx were rejected as bridging vein connection points, as these were likely unrealistic connection points since bridging veins enter from the hemispheres more laterally. From the remaining falx connection points eligible for inclusion as bridging vein elements, bridging vein connection points were selected such that bridging vein elements were evenly distributed in an anterior-posterior sense within their respective falx segments (the first and third segments described earlier). Five bridging vein connection locations were selected in the first falx segment, and six bridging vein connection points were selected in the third falx segment. Together with their corresponding brain connection points, these brain-falx node pairs comprised the eleven bridging vein elements in this sagittal transection model.

Bridging veins were represented by nonlinear axial connector elements, with force-displacement curves prescribed according to averaged post-cyclic porcine newborn stress-stretch curves obtained Chapter 2. Post-cyclic stress-stretch curves were chosen as a worst case scenario in terms of bridging vein ability to provide any mechanical resistance to displacement between the brain and skull, due to the observed longer toe region in post-cyclic bridging vein tests. After optimization of the brain-skull boundary condition with post-cyclic bridging vein behavior, a simulation was performed using high rate bridging vein behavior to determine whether the optimized boundary condition was reasonable for the span of bridging vein behaviors measured in Chapter 2. To create averaged stress-stretch curves, the stretch ratio was
discretized in intervals of 0.005 from 1 until the maximum ultimate stretch ratio obtained over all post-cyclic tests. Then, for individual stress-stretch curves, stress values at each discretized stretch ratio point were found by linearly interpolating the original stress-stretch ratio curve points immediately before and after each discretized stretch ratio value. Next, stresses at each stretch ratio point were averaged across all subjects. Once the discretized stretch ratio point was beyond an individual stress-stretch curve ultimate point, the subject’s stress-stretch curve was no longer included in the average stress calculations. The initial length of each bridging vein element was found based on the undisturbed positions of their respective brain and falx nodes. Averaged stress-stretch curves for post-cyclic and high rate bridging vein failure tests are presented in Appendix D. To create a force-displacement response for input into Abaqus, the discretized stretch ratio curve was multiplied by the initial length of each bridging vein element and the initial length of the bridging vein element was subtracted from this product to obtain individual displacement curves for each bridging vein element. All bridging vein elements were assumed to have the same cross-sectional area, assigned as the average cross-sectional area of all porcine newborn bridging veins tested in Chapter 2. The averaged stress curve values were multiplied by this average cross-sectional area to obtain an average force response. These individual force-displacement curves were assigned to each bridging vein element. Bridging vein failure was designated at the average ultimate stretch ratio of post-cyclic newborn porcine bridging veins from Chapter 2, expressed as a failure position in Abaqus by multiplying this number by the individual initial bridging vein lengths. If during a simulation, a bridging vein element exceeded this length, it was eliminated from the simulation, as if ruptured.

Every brain perimeter and rigid ink dot centroid used in analysis of brain-skull displacement in the physical transection experiments was matched to a corresponding node in the finite element model by overlaying images of segmented dots in the physical experiment with an image of the sagittal cut surface of the finite element model in Adobe Photoshop CS4 (San Jose, CA). If an ink dot centroid did not align exactly with a sagittal plane finite element node, up to four surrounding nodes were assigned such that their coordinates may average to the
approximate location of the ink dot. For rigid ink dots in the physical model that were outside the
boundary of the finite element skull, a circle was drawn with the ink dot centroid at the center of
the circle and the radius was expanded until the circle touched the sagittal edge of the finite
element model skull. The skull sagittal edge node closest to the point at which the circle
intersected the finite element model skull edge was matched to the corresponding rigid dot
centroid at the center of the circle. Again, if the circle intersection point did not align exactly with a
skull edge node, up to two surrounding skull edge nodes were assigned such that their
coordinates may average to the approximate location of the circle intersection point. The brain
and skull nodes corresponding to brain and rigid ink dot coordinates were tracked throughout the
simulation, and subsequently analyzed for maximum displacement for comparison with values
determined by the physical transection models (Section 2.2.2). These displacements were used
to refine the general linear elastic connector boundary condition by adjustment of the assigned
elastic stiffness such that finite element maximum displacements were representative of those
observed in the physical experiments.

The angular velocity-time histories from the physical transection experiments filtered at
CFC 200 were used as load inputs for the corresponding finite element model simulations. All
simulations were conducted in ABAQUS Explicit version 6.11 (Simulia, Providence, RI) with
double precision. To eliminate excessive brain deformation and negative element volumes,
distortion and enhanced hourglass control were activated for brain elements. While brain mass is
variable between individual animals, the finite element brain was not scaled as we were unable to
record individual brain masses from the physical transection experiments.

Data Analysis

For each general connector stiffness tested, the maximum relative displacement across
time between brain and skull nodes corresponding to physical experiment brain and rigid ink dots
was extracted. The maximum relative displacements were pooled across all simulations and
compared to the corresponding physical model maximum relative displacements using a linear regression with intercept at zero:

\[ d_{FEM} = a \cdot d_{EXP} \] [3]

where a is the slope of the linear regression. If the 95% confidence interval for slope contained one, the stiffness was deemed to be a good choice for boundary condition between the brain and skull. As several of the tested stiffnesses fit this criterion, the same linear regression analysis was repeated on brain-skull node and brain-rigid dot pairs located on the superior surface of the brain only (Figure 3.2), since the superior surface displacement determines bridging vein displacement. The stiffness that resulted in slope values within the narrowest range of one for both all node and dot pairs and superior only node and 95% confidence intervals for slope including one, was chosen as the optimal boundary condition stiffness for future analyses.

In a final analysis, to determine if the bridging veins supplied some of the tethering between the brain and skull, the averaged newborn porcine high rate bridging vein stress-stretch curve and resulting force-displacement curves were specified in the bridging vein elements. General connector stiffness was defined as the optimized stiffness. The resulting slope values between finite element and experimental displacements for all brain-skull/rigid dot pairs and superior-only brain-skull/rigid dot pairs were compared between the use of post-cyclic bridging vein curves and high rate bridging vein curves.

**Results**

**Physical Model**

**Measurement Error**

Displacement measurement errors between dot pairs captured on video prior to motion averaged across all dot pairs in each rotational experiment are reported in Table 3.2, as are average measurement errors pooled across all subjects for first rotations, second rotations, and all experiments combined. The overall average displacement measurement error was
0.31±0.23mm, which corresponded to <1 pixel difference in the video frames. No significant differences were noted between the first and second rotations. We conclude that our ink dot pair relative displacement measurement error is about 0.3 mm.
Table 3.2 Peak angular velocities, peak angular accelerations, peak angular decelerations, average maximum brain-skull displacements during motion, and average brain-skull displacement errors are shown for each transection experiment. Values are pooled among first and second rotations, as well as across all experiments. Groups sharing the same symbol (*, ‡, †) are significantly different from one another, p<0.01.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Rotation Number</th>
<th>Peak Angular Velocity (rad/s)</th>
<th>Peak Angular Acceleration (krad/s²)</th>
<th>Peak Angular Deceleration (krad/s²)</th>
<th>Average of Maximum Brain-Skull Displacements (mm)</th>
<th>Average Brain-Skull Displacement Error (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>52</td>
<td>9.6</td>
<td>6.8</td>
<td>1.00±0.41</td>
<td>0.32±0.16</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>51</td>
<td>10.3</td>
<td>7.4</td>
<td>1.07±0.40</td>
<td>0.28±0.17</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>50</td>
<td>8.4</td>
<td>8.6</td>
<td>0.63±0.36</td>
<td>0.28±0.19</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>51</td>
<td>9.5</td>
<td>8.1</td>
<td>0.68±0.29</td>
<td>0.31±0.37</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>52</td>
<td>6.2</td>
<td>9</td>
<td>0.58±0.27</td>
<td>0.33±0.29</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>54</td>
<td>7.7</td>
<td>11.2</td>
<td>0.66±0.26</td>
<td>0.31±0.21</td>
</tr>
<tr>
<td>Rotation 1 Avg.</td>
<td>51.3±1.2</td>
<td>8.1±1.7</td>
<td>8.1±1.2</td>
<td>0.73±0.39 ‡</td>
<td>0.31±0.21 *</td>
<td></td>
</tr>
<tr>
<td>Rotation 2 Avg.</td>
<td>52.0±1.7</td>
<td>9.2±1.3</td>
<td>8.9±2.0</td>
<td>0.80±0.37 ‡ †</td>
<td>0.30±0.25 ‡</td>
<td></td>
</tr>
<tr>
<td>Overall Avg.</td>
<td>51.7±1.4</td>
<td>8.6±1.5</td>
<td>8.5±1.5</td>
<td>0.76±0.38</td>
<td>0.31±0.23</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3.4 An example of angular velocity and angular acceleration time histories for one transection rotation and the associated brain-rigid dot displacement for 13 dot pairs. Pre-motion frames include time points up to 0.02s. Note the persistent increased displacements post motion, possibly indicating damage to the brain-skull boundary. While angular velocity and acceleration plots are on the same time scale as the displacement plot, the velocity and video camera data recording triggers were not simultaneous, thus the beginning of the angular velocity and acceleration time histories were aligned based on the displacement plot.

**Brain-Skull Displacement**

Brain-skull displacement appears to peak at two time points during the angular velocity pulse, with maximal values at the end of rotation deceleration (Figure 3.4). All physical transection brain-skull displacement time histories are presented in Appendix E. Average maximum relative displacements between brain and rigid dots in each rotational experiment, pooled across first rotations, pooled across second rotations, and across all experiments combined, are shown in Table 3.2. Maximum relative displacements in each brain-rigid dot pair during the first and second rotations were significantly greater than their respective error measurements ($p<0.000001$ for both tests). Thus we conclude that there was measurable relative
translation between the brain and skull during rotational events. Furthermore, the maximum displacements in each brain-rigid dot pair were greater in the second rotation than in the first rotation (p=0.0094). Coupled with our previous finding that the error measures were not different between the first and second rotations, we conclude that translation between the brain and skull is greater in the second rotation than the first rotation, indicating possible damage to the tethering leptomeninges between the brain and skull had occurred during the first rotation. However, the difference in average maximum relative displacements between the first and second rotations was less than our measured error level of 0.3mm. Thus, the finding that more translation between the brain and skull occurred during the second rotation may be of negligible importance; however, additional consecutive rotations (beyond 2) should be performed in future transection preparations to determine if increasingly greater relative displacements occur with sequential rotations.

Finally, maximum relative displacements that occurred during the first rotation were also compared to those analyzed from previous experiments of axially transected piglet heads rotated in the axial plane at similar levels of peak angular velocity [1, 2], reported here for the first time. Average maximum displacements recorded in each axial transection experiment are shown in Table 3.3. Maximum displacements in the axial transection experiments were significantly higher than those recorded in the sagittal transections in this study (p<0.000001, Figure 3.5).

**Table 3.3** Previously conducted newborn piglet axial transection experiment peak angular velocities, peak angular accelerations, peak angular decelerations, and average maximum brain-skull displacements. These axial transection experiments were conducted at peak velocities similar to those used in our sagittal transection experiments and had no prior load history. Kinematic data was previously reported in Ibrahim et al [1], and while brain-skull displacements were used in Coats et al [2], this is the first time they are being reported explicitly.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Peak Angular Velocity (rad/s)</th>
<th>Peak Angular Acceleration (krad/s²)</th>
<th>Peak Angular Deceleration (krad/s²)</th>
<th>Average of Maximum Brain-Skull Displacements (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Axial – 1</td>
<td>52</td>
<td>6.2</td>
<td>6.9</td>
<td>1.7±0.40</td>
</tr>
<tr>
<td>Axial – 2</td>
<td>53</td>
<td>6.7</td>
<td>6.3</td>
<td>1.50±0.56</td>
</tr>
<tr>
<td>Axial – 3</td>
<td>54</td>
<td>5.5</td>
<td>6.6</td>
<td>1.31±0.56</td>
</tr>
<tr>
<td>Overall</td>
<td>53.0±1.0</td>
<td>6.1±0.6</td>
<td>6.6±0.3</td>
<td>1.50±0.53</td>
</tr>
</tbody>
</table>
Box and whiskers plot of peak brain-skull displacements measured in first rotation sagittal transections from this study and first rotation axial transections detailed in previous work. The middle line in each box indicates median value, while the bottom and top edges of the box are the 25th and 75th percentile values. Finally, the whiskers extend to the most extreme high and low values in each data set. Axial plane brain-skull displacements were significantly higher than sagittal plane brain-skull displacements, p<0.000001 as indicated by the *.

Finite Element Model Optimization

Several general linear elastic connector stiffnesses produced 95% confidence intervals for slope including one when all peripheral brain-skull/rigid dot pairs were analyzed as well as when only superior brain-skull/rigid dot pairs were analyzed (Table 3.4). However, a stiffness of 46.133 N/m produced slopes for all dot pairs and superior only pairs that were within the narrowest range of one (Table 3.4, Figure 3.6). Thus, 46.133 N/m was selected as the optimum stiffness for the general linear connector boundary condition in the finite element model. When high rate bridging vein properties were implemented with this optimized general connector stiffness, no appreciable difference in slope was found between finite element and experimental displacements (Table 3.4), indicating that in comparison with other structures in the pia-arachnoid complex, the bridging veins likely do not contribute much mechanical resistance to translation between the brain and skull. To bracket the potential for bridging vein element failures, simulations with the stiffest (4,000 N/m), least stiff (34.6 N/m) and optimized stiffness (46.133 N/m).
both post-cyclic and high rate bridging vein behavior) were investigated for the bridging vein element failures, and across all of these simulations, no bridging vein element failures occurred.

Table 3.4 All stiffness values tested for general brain-skull/falx connectors in the sagittally transected finite element model, and corresponding slopes between finite element and physical transection experiment brain-skull displacements in Equation 3 over all dots, \(a_{\text{all}}\) and superior-only dots \(a_{\text{sup}}\). 95% confidence intervals for slope are indicated in parentheses to the right of the derived slope value. † indicates the general connector stiffness used in previous porcine finite element models, optimized to axial brain-skull displacements [1, 2]. * indicates stiffnesses for which the 95% confidence intervals for both \(a_{\text{all}}\) and \(a_{\text{sup}}\) contain one. ‡ indicates that finite element simulations were run with high rate bridging vein properties. The optimal general connector stiffness chosen for further analysis is 46.133 N/m as the slopes for all brain-skull dots and superior-only brain-skull dots are within the narrowest range of one.

<table>
<thead>
<tr>
<th>Stiffness (N/m)</th>
<th>(a_{\text{all}})</th>
<th>(a_{\text{sup}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>4000</td>
<td>0.769 (0.684, 0.854)</td>
<td>0.665 (0.582, 0.749)</td>
</tr>
<tr>
<td>3750</td>
<td>0.769 (0.684, 0.855)</td>
<td>0.666 (0.582, 0.749)</td>
</tr>
<tr>
<td>3460†</td>
<td>0.770 (0.685, 0.855)</td>
<td>0.666 (0.582, 0.750)</td>
</tr>
<tr>
<td>3250</td>
<td>0.770 (0.685, 0.855)</td>
<td>0.666 (0.583, 0.750)</td>
</tr>
<tr>
<td>3000</td>
<td>0.771 (0.686, 0.856)</td>
<td>0.667 (0.583, 0.750)</td>
</tr>
<tr>
<td>2595</td>
<td>0.772 (0.687, 0.857)</td>
<td>0.668 (0.584, 0.751)</td>
</tr>
<tr>
<td>2000</td>
<td>0.774 (0.700, 0.859)</td>
<td>0.669 (0.586, 0.753)</td>
</tr>
<tr>
<td>1730</td>
<td>0.775 (0.690, 0.861)</td>
<td>0.670 (0.587, 0.754)</td>
</tr>
<tr>
<td>692</td>
<td>0.789 (0.703, 0.875)</td>
<td>0.678 (0.596, 0.761)</td>
</tr>
<tr>
<td>346</td>
<td>0.812 (0.724, 0.899)</td>
<td>0.687 (0.608, 0.766)</td>
</tr>
<tr>
<td>138.4</td>
<td>0.930 (0.826, 1.034)</td>
<td>0.784 (0.695, 0.874)</td>
</tr>
<tr>
<td>69.2*</td>
<td>1.002 (0.879, 1.125)</td>
<td>0.883 (0.753, 1.014)</td>
</tr>
<tr>
<td>46.133*</td>
<td>1.082 (0.939, 1.226)</td>
<td>0.984 (0.8230, 1.144)</td>
</tr>
<tr>
<td>46.133‡</td>
<td>1.081 (0.938, 1.225)</td>
<td>0.981 (0.821, 1.142)</td>
</tr>
<tr>
<td>34.6*</td>
<td>1.149 (0.990, 1.308)</td>
<td>1.056 (0.874, 1.239)</td>
</tr>
</tbody>
</table>
Figure 3.6 Optimized connector stiffness finite element versus physical model peak brain-skull displacements for post-cyclic and high rate bridging vein behavior as well as all and superior-only dot pairs. Data shown by blue dots, linear regressions by red lines, and black line of identity.

Discussion

In this study we measured the sagittal plane brain-skull displacement of the newborn porcine head when subjected to sagittal plane rotations, and provided an appropriate brain-skull boundary condition for finite element modeling of sagittal plane head rotations in the porcine newborn. We observed significant brain-skull relative displacements in an in situ model with sagittal plane rotations at peak velocities of ~52 rad/s. Brain-skull displacements during head rotations are direction-dependent with those measured in the sagittal plane in this study significantly lower than those measured previously in the axial plane. Interestingly, the brain-skull
boundary condition prescribed in order to match respective physical transection experimental brain-skull displacements was far less stiff in a sagittally transected finite element model of the porcine newborn head than that in a corresponding axial transection finite element model. Finally, it appears that bridging veins do not dominate the mechanical tethering between the brain and skull, while other structures may play a larger role. These data will inform future full head finite element model simulations of rotational injury by providing a validated boundary condition for sagittal plane motion.

Despite relatively large error measures in comparison to some maximum brain-rigid dot displacements, paired Wilcoxon signed-rank tests consistently showed that maximum brain-rigid dot displacements during rotation were higher than their respective error measures. This error may be attributable to inconsistent lighting as the frame rate of the high speed camera was faster than the frequency of ambient light in the laboratory, or small frame-to-frame discrepancies with dot edge detection. Regardless, measurement error averaged slightly less than one pixel distance (~0.3 mm versus ~0.4 mm/pixel). While additional Wilcoxon signed-rank tests revealed that the maximum relative displacements between brain-rigid dots in the second series of rotations were higher than those in the first set of rotations, indicating that some structural damage may have occurred as a result of the first rotation. However, the difference in maximum displacements between the first and second rotations was less than our measurement error, rendering this finding statistically significant, but of low importance. Future studies may examine more than two consecutive rotations to the same load level to investigate if maximum brain-skull displacements get progressively larger with an increasing number of rotations, such that they are greater than measurement error. Findings from these proposed studies would provide valuable information for modeling the brain-skull boundary condition in incidences of shaking or repeated insults.

We observed that axial plane brain-rigid dot displacements from a previously studied axial piglet head transection preparation (1.50±0.53 mm) are significantly higher than those measured in this sagittal plane study (0.76±0.38 mm). Indeed, a plethora of previous human and
animal studies have shown that brain material properties [17, 22], TBI pathophysiological [3, 8] and histopathological responses [3-7, 9, 10], TBI clinical sequelae [3, 5-7, 9-13] and required treatment [13], and TBI behavioral outcomes [4, 8], vary with local direction of strain (material properties) and direction of rotational injury plane/impact force (responses, sequelae, treatments, and outcomes). Thus our finding of differing maximum brain-skull displacement by direction is not surprising. Interestingly, although measured maximum brain-skull displacements in sagittal plane physical transections were less than those measured in axial plane preparations, the general connector stiffness prescribed to match the observed maximum displacements was much lower in the sagittal plane transection finite element model than the corresponding axial plane transection finite element model. This phenomenon is not explained by general connector element density as both models had a similar number of connectors per square millimeter of brain surface area over which the general connectors were imposed; in fact, connector density was slightly less in the sagittal plane transection (0.37 connectors/mm²) than in the axial plane transection (0.40 connectors/mm²). Rather, we postulate that the irregular geometry of the sagittal cross-section results in smaller brain-skull displacements than in the axially sectioned brain and skull (Figure 3.7). Because the irregular geometry restricts motion of the brain, the geometric boundary dominates the brain-skull motion response in the sagittal transection and a lower connector stiffness (46.133 N/m) was required than in the axial section (3,460 N/m) [2]. Specifically, the axial plane cross-section is smoother and rounder, with few impediments to movement. In contrast the sagittal brain-skull boundaries near the cerebellum, brainstem, inferior frontal boundary near the porcine olfactory bulbs, and smooth superior surface, may each uniquely and naturally restrain (possibly near the cerebellum, brainstem, olfactory bulbs) or facilitate (possibly near the cerebellum or superior cerebrum surface) motion of the brain relative to the skull. The relatively coarse resolution of the physical model ink dots prevents detailed regional analysis of brain-skull displacements around the sagittal transection perimeter. Nonetheless, we physical transection experiment plotted brain-skull/rigid dot pair displacements with brain dots around the periphery of the transected cerebrum from inferior-posterior to
superior-posterior, and found increased maximum displacements near the brainstem/foramen magnum, and near the superior surface (Figure 3.8). Future studies may attempt to obtain higher resolution high speed videos for better analysis of regional brain-skull displacement in the sagittal plane, and refinement of finite element model boundary condition.

Figure 3.7 Comparison of sagittal (left) and axial (right) transection physical models (top) and finite element models (bottom). Note the more symmetric shape of the axial cross-section compared to the sagittal cross-section.
The use of high rate bridging vein properties compared to post-cyclic bridging vein properties (both from the neonatal pig in Chapter 2) with the optimized general connector stiffness did not provide an appreciable difference in sagittal transection finite element model brain-skull tethering at peak angular velocities around ~52 rad/s. Because of the much longer toe region in the post-cyclic bridging vein stress-stretch curves, and decreased ability in this low stretch ratio realm to provide mechanical resistance to movement, we expected that their use would necessitate a stiffer boundary condition than other bridging vein properties, but this was not the case. Instead, we posit that the mechanical tethering response between the brain and skull is likely governed by other elements of the pia-arachnoid complex (PAC), or again, the sagittal plane shape of the brain and skull (Figures 3.7 and 3.8). Few material property tests have examined the PAC, but the available data reveal that normal traction [23] and transverse shear [24] moduli of the bovine PAC, where arachnoid trabeculae determine the mechanical response, are lower than the longitudinal bridging vein elastic moduli measured for both pigs and humans in Chapter 2. On the other hand, under in-plane tension, the elastic modulus of the bovine PAC (6-40 MPa) [25] is similar to that of the porcine and human bridging vein longitudinal elastic moduli.
from Chapter 2 (22-49 MPa), and both tissues display similar nonlinear elastic and rate-dependent behaviors [25]. Furthermore, a microstructural imaging study of the neonatal porcine PAC revealed high regional variability in arachnoid trabeculae and subarachnoid vasculature volume fraction, indicating that PAC material properties may also vary across the brain surface [26]. While a few microscale finite element models have begun to address the individual and combined components of the PAC [27-29], only one study developed a corresponding macroscale PAC representation based on regional and inter-individual PAC structural distributions [26], validated in a full head model against the presence of hemorrhage [27]. While some material properties of the PAC representative elements were based on literature-reported values [23-25], the model response was not validated against in situ brain-skull displacements [27]. Future combining of representations of the PAC with measured material properties and biofidelic regional and inter-individual structural density ranges, and observed in situ brain-skull displacements may provide an even more accurate simulation of the brain-skull boundary condition in finite element models. Finally, in all material property and finite element studies, the PAC is assumed to be transversely isotropic, but only in-plane tension tests have confirmed this along both the sagittal and coronal planes [25]. Future studies should investigate the transverse shear properties of the PAC by loading in the approximate directions of the sagittal and coronal planes.

Limitations

The presented studies include several limitations. First, it was impossible to capture brain-skull displacements orthogonal to the sagittal plane of rotation (and axial plane of rotation for prior axial studies) in the physical model transections. However, we assume any orthogonal displacements are likely minimal compared to those within the plane of rotation. Second, physical model construction may have caused structural alterations to the transected cranial contents, most notably the probable draining of cerebrospinal fluid, though care was taken to preserve the mechanical integrity of the brain, skull, and interfacing tissues and fill the structures with buffered
saline. Third, brain-skull displacements were matched uniformly around the periphery of the cerebrum in the sagittal plane, but coarse resolution data suggest there may be regional dependencies. Future studies may investigate regional differences in brain-skull displacement using higher resolution video and determine finite element model sagittal plane boundary conditions by region. Finally, physical measurements of maximum brain-skull displacement and the corresponding finite element boundary condition were only optimized for peak angular velocities of ~52 rad/s in the sagittal plane. Whether the prescribed finite element model boundary condition also holds for other load levels is unknown. Future physical sagittal plane transection studies at different levels of peak angular velocity and acceleration and brain-skull displacement analysis of previously conducted higher load level axial transection preparations [14] may address this question.

It is also important to note two differences in analysis. In the axial physical transection experiments, the baseline distance between brain and rigid dots was determined as the distance found in the first frame before motion, rather than the first fifty frames before motion in this series of sagittal physical transection experiments. We do not believe this contributed to our finding of smaller displacements in the sagittal rotations. In the axially transected finite element model, brain-skull displacement was matched to physical experiments using a linear regression with 95% confidence intervals for slope containing one, and intercept containing zero, rather than assuming an intercept of zero as was done in the sagittally transected studies. Similarly, we do not believe this contributed to our finding of lower finite element connector stiffness in sagittal rotations. Finally, in the axial transection studies, the boundary condition was validated separately in all three axial transection studies, rather than by pooled analysis, and it was not optimized, but rather estimated and found to be within acceptable criteria for linear regression similarity. Future studies may optimize the axial transection general connector stiffness and pool all studies together for improved statistical power, and this may result in a small change in the axial plane boundary condition stiffness, but it is still doubtful that any changes would be drastic.
Conclusions

In summary, we have measured sagittal plane brain-skull displacements from an in situ model of the neonatal piglet head, and determined a brain-skull boundary condition to match these displacements in a neonatal pig head finite element model. In completing this correlation, we also found that axial plane brain-skull displacements at similar load levels are larger than those measured in the sagittal plane in this study. However, the finite element boundary condition required to replicate the sagittal plane brain-skull displacements is far less stiff than that employed in its axial plane finite element counterpart, possibly attributable to differences in sagittal and axial plane brain and skull shape, or similarly, regional differences in brain-skull displacement. We also found this boundary condition is unaffected by the use of the range of bridging vein properties determined in Chapter 2, with high rate bridging vein properties yielding no appreciable difference in brain-skull boundary condition compared to post-cyclic bridging vein properties. This indicates that the sagittal plane brain-skull boundary condition may be governed by other PAC structures, or, once more, the irregular shape of the brain and skull in the sagittal plane (Figures 3.7 and 3.8). Accordingly, we encourage the use of appropriate brain-skull boundary conditions in finite element models of the head, dependent on the direction of simulated rotation. The data presented in this study provide critical insight into directional differences in rotational brain-skull displacement, and will be employed in finite element models of the whole neonatal porcine and human infant heads simulating sagittal plane rotational insults to best predict tissue response, in particular the incidence of bridging vein failure.

References


CHAPTER 4: RAPID HEAD ROTATIONS CAUSE BRIDGING VEIN RUPTURE AND EXTRA-AXIAL HEMORRHAGE IN THE NEONATAL PIG

Abstract

Finite element modeling is a technique often used to aid in understanding and prediction of traumatic brain injury, and estimating tissue deformations during traumatic events provides insight into kinematic injury thresholds. In this study, we used a finite element model of the newborn porcine head, including species-appropriate bridging vein elements and properties to simulate rapid nonimpact rotational brain injuries using our well-established model of single rotation traumatic brain injury as well as a newer model of cyclic rotational traumatic brain injury in the neonatal piglet. We found that as peak rotational acceleration magnitudes increased, the number of bridging vein failures predicted by the finite element model also increased, indicating that rotational acceleration levels may govern the occurrence of bridging vein rupture and subsequent extra-axial hemorrhage. In addition, we determined a threshold of six failed bridging vein elements predicted the occurrence of extra-axial hemorrhage with 100% sensitivity and 100% specificity under single rapid nonimpact rotation, and this critical value of failed bridging vein elements performed with 90% accuracy in simulations of cyclic rotational head injuries. These findings will be used in the development of a finite element model of the human infant head to simulate real-world trauma such as household accidental falls and abusive shaking-type insults.

Introduction

The most consistent clinical finding in cases of childhood abusive head trauma is the presence of subdural and subarachnoid hemorrhage [1], but diagnosis is confounded by similar occurrences of hemorrhage in accidental trauma. Biomechanical studies can provide insight into the kinematic thresholds required to produce injury, and injury prediction capabilities to inform clinical differential diagnosis strategies.
Finite element modeling is a common tool used to estimate or predict tissue deformations during traumatic events. Indeed, previous finite element model studies have correlated brain-skull boundary deformation thresholds with the presence of extra-axial hemorrhage in our porcine TBI model [2, 3], but these simulations did not include elements representative of the parasagittal bridging veins, which are thought to tear or rupture as they are stretched in the subdural space during rapid head rotations when the movement of the brain lags that of the skull. More accurate representations of the bridging veins are required in order to facilitate improved injury prediction, and until now, progress toward this goal has been limited by a lack of pediatric bridging vein mechanical property data.

In this study, we developed and validated a prediction criterion relating the amount of bridging vein element failures with the presence of extra-axial hemorrhage using a finite element model of the neonatal porcine head and a retrospective collection of newborn porcine rotational brain injury experiments conducted previously in our lab [4-7]. Using neonatal porcine bridging vein properties measured in Chapter 2, and the optimized finite element sagittal plane brain-skull boundary condition determined in Chapter 3, we first simulated 24 rapid nonimpact rotations in the sagittal plane over a wide range of load levels, and found the number of failed bridging vein elements associated with the presence of extra-axial hemorrhage after in vivo injuries having the highest sensitivity and specificity. We then validated our criterion in simulations of an additional 12 rapid nonimpact rotations in the sagittal plane and 10 cyclic rotational injuries in both the sagittal and axial planes. Data from this study will inform future finite element models of the human infant head by providing insight into injury prediction thresholds for extra-axial hemorrhage.

Methods

Rapid Nonimpact Rotational Neonatal Porcine Brain Injury Studies

In order to correlate finite element model simulation of bridging vein elongations with the presence of EAH in the neonatal pig, we compiled a retrospective group of 3-5 day old porcine...
TBI studies from our lab in which animals experienced single or cyclic rapid nonimpact head rotations in the sagittal plane (single) or sagittal and axial planes (cyclic). Single rotational injuries were conducted at one of two load levels, with low loads averaging 40.2±5.9 rad/s and high loads averaging 149.8±5.3 rad/s (Tables 4.4 and 4.5). Piglets with post-injury survival times of 3-8 hours and 24 hours were included as the occurrence and extent of EAH did not differ in these groups [4, 6]. Axial plane rotations were included in the cyclic group due to low sample size of sagittal plane cyclic rotations. Due to excessive computational expense, only 10 of the 17 available cyclic porcine studies were chosen for further analysis. First, all cyclic porcine studies with EAH scores >0% (see Quantification of EAH) were included for analysis. Next studies that did not complete a full 30 seconds of cycling, or those that had other abnormalities (e.g. animal came loose on the injury device) were eliminated. Of the remaining available cyclic studies, the two with the highest average peak-to-peak velocities in each of the following groups were selected for analysis: 3-8 hour survival – sagittal, 3-8 hour survival – axial, 24 hour – axial, in addition to those with EAH>0%. Table 4.1 provides details about group sample sizes.

Table 4.1 Group sample sizes. For single rotations, which were all in the sagittal plane, \( n_{\text{dev}} \) indicates the number of studies in the EAH prediction criterion development group and \( n_{\text{val}} \) indicates the number of studies in the EAH prediction criterion validation group. Cyclic simulations were evaluated using the EAH prediction criterion (\( N_{\text{crit}} \)) validated in single rotation studies. No sagittal plane cyclic studies were performed in the 24 hour survival group.

<table>
<thead>
<tr>
<th>Rotation Type</th>
<th>Survival Time</th>
<th>3-8 hour</th>
<th>24 hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Low</td>
<td></td>
<td>( n=5 ) ( (n_{\text{dev}}=3, n_{\text{val}}=2) )</td>
<td>( n=6 ) ( (n_{\text{dev}}=4, n_{\text{val}}=2) )</td>
</tr>
<tr>
<td>Single High</td>
<td></td>
<td>( n=11 ) ( (n_{\text{dev}}=8, n_{\text{val}}=3) )</td>
<td>( n=14 ) ( (n_{\text{dev}}=9, n_{\text{val}}=5) )</td>
</tr>
<tr>
<td>Cyclic Sagittal</td>
<td></td>
<td>( n=4 )</td>
<td>-</td>
</tr>
<tr>
<td>Cyclic Axial</td>
<td></td>
<td>( n=2 )</td>
<td>( n=4 )</td>
</tr>
</tbody>
</table>

All protocols from the retrospective studies were approved by the University of Pennsylvania Institutional Animal Care and Use Committee. Neonatal piglets were anesthetized with 4% isoflurane inhaled via snout mask. After proper depth of anesthesia was established as determined by absence of a pinch reflex, the piglets were intubated and mechanically ventilated, while anesthesia was maintained with 1-4% isoflurane. Heart rate, respiratory rate, rectal
temperature, oxygen saturation, and end tidal CO2 were continuously recorded during anesthesia. Animals were mounted to either the single or cyclic head rotation device by securing the piglet head to the device bite plate via padded snout straps. For single rotations, our previously described head rotational acceleration device [8] was use to impart a rapid ventral-to-dorsal 60 degree rotation in the sagittal plane with the center of rotation at the piglet mid-cervical spine. Cyclic rotations were performed using our cyclic head rotation device, described previously [4]. Sagittal plane cyclic rotations were applied through a ±30 degree arc, while axial plane cyclic rotations were applied through a ±50 degree arc, to span the full range of motion of the porcine cervical spine in each direction without hyperextension. The neutral axis position of the neck was defined as 0 degrees. Cyclic head rotations were applied at a frequency of 2-3 Hz for 30 seconds, similar to the cyclic bridging vein extension tests in Chapter 2. For all rotational head injuries, angular velocity was recorded by an angular rate sensor (Model ARS-06, Applied Technology Associates, Albuquerque, NM), sampled at 3,000-10,000 Hz, depending on intended load level (cyclic, single low, or single high), with one cyclic study sampled at 250 Hz accidentally, though this was sufficient to capture the signal.

After injury, animals in the 3-8 hour survival group were continuously anesthetized until euthanasia, while those surviving 24 hours were weaned from anesthesia and upon demonstration of ability to ambulate to food and water, returned to the animal housing facility. At the designated survival time point, animals were anesthetized and sacrificed by overdose of sodium pentobarbital. Brains were perfusion fixed, as described previously [5], and carefully removed from the cranium making sure to preserve any blood in the subdural space. Brains were weighed, and digital color photographs were taken of the superior, inferior, left, and right surfaces.

Quantification of EAH

Extra-axial hemorrhage was quantified using a previously established protocol [2, 4, 6]. The digital photographs of the inferior, superior, left, and right brain surfaces were assessed for
surface blood as classified by pixel color in Adobe Photoshop (Adobe Systems, San Jose, CA). To account for regions of the brain appearing in more than one view, the surface of the brain was divided regionally using anatomic markers. The total pixel area of the cerebrum containing blood was summed and divided by the total pixel area of the cerebrum to determine the EAH percentage score. Blood on the surface of the cerebellum was not included in EAH analysis.

**Finite Element Model Development and Simulation**

A finite element model of the neonatal porcine head previously developed in our lab [2, 7] was modified to include bridging vein elements with properties and behavior as determined in Chapter 2. This full head model (Figure 4.1) included 13,018 linear hexahedral brain elements, 1,891 linear wedge falx elements, and 17,722 rigid shell skull elements. Material properties for brain and falx were consistent with those described in Chapter 3 for the sagittally transected newborn porcine head finite element model, as shown in Table 4.2. Again, the skull was represented as a rigid body.

![Finite element model of the neonatal porcine head](image)

**Figure 4.1** Finite element model of the neonatal porcine head, with brain (blue), skull (green), and falx (pink).
Table 4.2 Material properties used in the neonatal porcine whole head finite element model. Detailed parameter descriptions are presented in Chapter 3.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Material Property</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Brain</strong></td>
<td>$\mu_0 = 553$ Pa</td>
<td>Sullivan et al., 2015 [7]</td>
</tr>
<tr>
<td></td>
<td>$\alpha = 0.01$</td>
<td>Prange and Margulies, 2002 [9]</td>
</tr>
<tr>
<td></td>
<td>$C_1 = 0.3322$</td>
<td>Prange and Margulies, 2002 [9]</td>
</tr>
<tr>
<td></td>
<td>$C_2 = 0.3890$</td>
<td>Prange and Margulies, 2002 [9]</td>
</tr>
<tr>
<td></td>
<td>$\tau_1 = 2.9572$ s</td>
<td>Prange and Margulies, 2002 [9]</td>
</tr>
<tr>
<td></td>
<td>$\tau_2 = 0.1813$ s</td>
<td>Prange and Margulies, 2002 [9]</td>
</tr>
<tr>
<td></td>
<td>$\rho = 1.04$ g/cm$^3$</td>
<td>Sullivan et al, 2015 [7]</td>
</tr>
<tr>
<td></td>
<td>$\nu = 0.49999$</td>
<td>Sullivan et al, 2015 [7]</td>
</tr>
<tr>
<td><strong>Falx</strong></td>
<td>$\rho = 1.13$ g/cm$^3$</td>
<td>Coats et al, 2012 [2]</td>
</tr>
<tr>
<td></td>
<td>$\nu = 0.45$</td>
<td>Coats et al, 2012 [2]</td>
</tr>
<tr>
<td><strong>General Brain-</strong></td>
<td><strong>Skull Connectors</strong></td>
<td>$k_{sag} = 46.133$ N/m</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$k_{ax} = 3460$ N/m</td>
</tr>
</tbody>
</table>

The brain-skull boundary condition was defined once again using linear elastic connector elements linking each brain surface node to the nearest node on the inner surface of the skull or falx. The whole head model included 2,680 nodes on the outer surface of the brain, 22 of which were reserved for representation of the bridging veins, yielding 2,658 linear elastic connector elements defining the boundary condition between the brain and skull. For finite element model simulations of sagittal plane rotations, general connector stiffness was defined at 46.133 N/m as determined in Chapter 3, while axial plane rotations included a general connector stiffness of 3,460 N/m as determined previously [2].

The eleven bridging vein elements used in the sagittally transected porcine finite element model in Chapter 3 were retained in the whole head model we describe here. As the sagittal transection model included only the right side of the head, the additional eleven bridging vein elements in this whole head model were chosen from corresponding left side brain node locations, and connection points along the falx remained in the appropriate tributary segments, as described in Chapter 3. All together, 22 bridging vein elements were included in this whole head model to match the average number and distribution of bridging veins observed in the human adult [10]. Bridging vein elements were once again represented by nonlinear elastic connectors,
with force-displacement curves prescribed according to averaged porcine newborn bridging vein stress-stretch curves, obtained in Chapter 2 and processed as described in Chapter 3.

In both low and high level single rotation simulations, high rate bridging vein force-displacement relationships were used to describe bridging vein element behavior. This choice was made based on benchmark calculations of bridging vein stretch rate in the sagittal transection finite element model, presented in Chapter 3. The maximum stretch rates experienced by each bridging vein element in the sagittal transection finite element model were calculated from simulations with the optimized brain-skull boundary condition for both high rate and post-cyclic bridging vein behaviors. Using bridging vein element initial lengths, we found maximal bridging vein stretch rates were an order of magnitude higher than the high stretch rate at which neonatal porcine bridging veins were tested in Chapter 2 (Table 4.3). Next, stretch rates from these sagittal transection experiments were recalculated assuming an initial bridging vein length of 25mm, which is the maximum length recorded in a previous study of human adult bridging veins [11], and the maximum stretch rates were very similar to the high rates tested in Chapter 2 (Table 4.3). Finally to confirm this finding, the simulation with the highest average maximum stretch rate, was re-simulated with averaged high rate and post-cyclic bridging vein force-displacement curves calculated assuming an initial bridging vein length of 25mm, and again, the finite element bridging vein stretch rates were very similar to those used in the high rate tests in Chapter 2 (Table 4.3), solidifying our choice of high rate bridging vein behavior in single whole head rotation finite element models. Conversely, because post-cyclic bridging vein stress-stretch behavior was significantly altered compared to the previously unloaded low and high stretch rate behavior in Chapter 2, we chose to use post-cyclic bridging vein behavior in cyclic whole head finite element simulations, despite its lower average failure test stretch rate.
Table 4.3 True and hypothetical finite element model bridging vein stretch rates for comparison with high rate porcine newborn bridging vein stretch rates from Chapter 2, 12.75±2.05 s⁻¹.

<table>
<thead>
<tr>
<th>Bridging Vein Initial Length</th>
<th>Bridging Vein Behavior</th>
<th>Average Maximum Bridging Vein Stretch Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Determined by FE Bridging Vein Elements (n=6 simulations)</td>
<td>Post-Cyclic</td>
<td>409±253 s⁻¹</td>
</tr>
<tr>
<td></td>
<td>High Rate</td>
<td>384±220 s⁻¹</td>
</tr>
<tr>
<td>Estimated 25mm Initial Length from FE Model True Length Outputs (n=6 simulations)</td>
<td>Post-Cyclic</td>
<td>11.5±3.5 s⁻¹</td>
</tr>
<tr>
<td></td>
<td>High Rate</td>
<td>11.2±3.5 s⁻¹</td>
</tr>
<tr>
<td>Re-simulated with 25mm Initial Length Force-Displacement Curve (n=1 simulation)</td>
<td>Post-Cyclic</td>
<td>13.4±5.8 s⁻¹</td>
</tr>
<tr>
<td></td>
<td>High Rate</td>
<td>12.7±5.0 s⁻¹</td>
</tr>
</tbody>
</table>

Across all whole head finite element simulations, the bridging vein element failure criterion was indicated as the average high rate porcine newborn ultimate stretch from Chapter 2. Although ultimate stretch was found not to vary by failure test type or age in Chapter 2, the average high rate neonatal porcine ultimate stretch (1.261±0.036) was similar to and within one standard deviation of the overall average for neonatal porcine bridging veins (1.293±0.118), and porcine bridging veins from all ages (1.274±0.103). Furthermore, because this rate and age matched ultimate stretch metric was slightly lower than the age matched and overall average ultimate stretches it provides a worst-case scenario for bridging vein rupture.

Cyclic experimental velocity-time histories were too computationally expensive to achieve reasonable simulation run times. Thus, we chose to crop each velocity-time trace such that it only included the three middle-most cycles (Figure 4.2). Three cycles were chosen so that enough changes in direction were incorporated to identify whether this repetitive motion caused increasing tissue deformations, while minimizing run time.

For each animal study, the finite element model brain, skull, and falx were uniformly scaled based on the individual animal brain mass. Velocity-time histories from the single rotation animal experiments were filtered at CFC (channel frequency class) 200 for simulation. As CFC 200 filtering did not remove sufficient high frequency artifacts from cyclic velocity-time histories, the three-cycle abbreviated cyclic velocity-time histories were filtered, based on power spectral
density analysis, at 30 Hz with a low-pass second-order Butterworth filter for use in finite element model simulation. The filtered angular velocity-time histories from each animal experiment were used as load inputs for the corresponding finite element model simulations, with the center of rotation at the approximate location of experimental center of rotation. All simulations were conducted in ABAQUS Explicit version 6.11 (Simulia, Providence, RI) with double precision. To eliminate excessive brain deformation and negative element volumes, distortion and enhanced hourglass control were activated for brain elements.
Figure 4.2 Example whole injury angular velocity-time and angular acceleration-time plots with dashed lines indicating cycles selected for finite element analysis. Corresponding cropped angular velocity and angular acceleration time-history plots are enlarged beneath the whole injury traces.
Data Analysis

Porcine Rotational Brain Injury Kinematics

For porcine single head rotational brain injury studies performed in our lab, we typically report peak angular velocity extracted from the raw angular velocity-time history. Then the angular velocity trace is processed through a 10-point Gaussian smoothing algorithm and differentiated via central difference method to find angular acceleration. Finally, peak angular accelerations and decelerations are extracted. For comparison, peak angular velocity values from signals filtered for finite element simulation were extracted, and the finite element simulation filtered angular velocity traces were differentiated via forward difference method to find acceleration. Peak angular accelerations and decelerations calculated from velocity signals prepared for finite element simulation were then extracted. Peak angular velocities, accelerations, and decelerations in single rotations were compared between analysis methods using paired t-tests for each kinematic metric.

For porcine cyclic head rotational injuries, kinematics extracted from the three cycles for finite element simulation were also compared with those over the entire injury. First, the entire cyclic velocity-time history was filtered at 30Hz, to match the filtering of the finite element input trace. Both the full and cropped filtered angular velocity-time histories were differentiated using the forward difference method to find angular acceleration. The cycling frequency, peak-to-peak angular velocities, and peak acceleration magnitudes were extracted from both the full and finite element simulation cropped kinematic histories, as follows. Average cycling frequency was calculated by finding angular velocity zero-crossing time points immediately preceding points of peak angular velocity, finding the difference between consecutive zero-crossing time points, taking the reciprocal of these differences, and averaging all frequencies calculated over the length of the velocity-time history. Peak-to-peak velocity was extracted from each cycle in both a forward and backward sense by subtracting “valley” velocities from both the peak velocities immediately following and immediately preceding the particular valley velocity, respectively. Then the maximum peak-to-peak velocity over the angular-velocity time history was extracted, and
finally the average peak-to-peak velocity was found by averaging all peak-to-peak velocity values over the length of the velocity-time history. Peak acceleration magnitudes were extracted by cycle (two per cycle, one near each point of direction change) over the angular acceleration-time histories, maximum values were extracted and average values were found by averaging all peaks over the length of the acceleration-time history. Average cycling frequency, average peak-to-peak angular velocity, and average peak angular acceleration magnitude were compared between full porcine cyclic study time histories and the cycles cropped for finite element analysis using paired t-tests for each metric, to ensure the cycles selected for finite element analysis were representative, or worst-case scenarios compared to the entire cyclic rotational injury.

Relationship between Injury Parameters and Finite Element Model Predictions of Bridging Vein Rupture

For all simulations, scatterplots comparing the number of failed bridging vein elements ($N_{fail}$) with the associated simulated peak angular velocity (or maximum peak-to-peak angular velocity for cyclic simulations), simulated peak acceleration or deceleration (whichever was greater for single rotations; maximum peak acceleration magnitude for cyclic simulations), and total cerebrum EAH (%) after injury were generated to investigate relationships between injury parameters and finite element model simulation results. An additional graphical analysis investigating the cumulative number of bridging vein ruptures over simulation time as compared to the prescribed angular velocity and acceleration time histories was performed in an effort to understand the relationship between bridging vein rupture and injury kinematics.

Development and Validation of EAH Prediction

The presence of EAH was considered a binary variable. Based on prior analysis, where brain regions were considered positive for EAH if $\geq$25% blood surface coverage was detected [2], the smallest region analyzed by surface area was the midline, and retrospective analysis of twelve 3-5 day old piglet brains scored for hemorrhage revealed that 25% of the midline region
surface corresponds to 2.01±0.40% of the whole cerebrum surface [2]. As it is possible that brain surface blood may not have originated in a region, instead having traveled from a nearby region, we chose to designate brains with whole cerebrum blood surface coverage ≥2% as positive for EAH in this analysis (~1 cm diameter [2]), which is also a reasonable detection level for in vivo imaging (personal communication, Dr. Cindy Christian, Children’s Hospital of Philadelphia). Any brains with <2% of the cerebrum covered in blood were designated as negative for hemorrhage.

Of the 36 single sagittal rotation animal studies simulated, 24 were used to develop the EAH prediction criterion (N_{crit}) by determining the number of failed bridging vein elements (N_{fail}) associated with the presence of EAH having the highest sensitivity and specificity. The 24 studies chosen for the development group spanned both 3-8 hour and 24 hour survival time points (Tables 4.4 and 4.5) with low level peak raw signal angular velocities averaging 40.6±5.4 rad/s (Table 4.4), and high level raw signal peak angular velocities averaging 149.5±5.7 rad/s (Table 4.5), both near the overall average peak angular velocities for the low and high level single rotation groups (40.2±5.9 and 149.8±5.3 rad/s, respectively, Tables 4.4 and 4.5). Once N_{crit} was selected, the remaining 12 studies were analyzed to confirm the ability of N_{crit} to predict the occurrence of EAH, evaluated by positive and negative predictive values. Finally, N_{crit}, as determined from single sagittal head rotations, was evaluated as an EAH predictor with results from the cyclic porcine studies and finite element simulations, and its accuracy was assessed by positive and negative predictive values.

**Results**

*Neonatal Porcine Rotational TBI Study Kinematics*

Kinematic measurements for low load level single head rotations are reported Table 4.4, and high load level kinematics are reported in Table 4.5 for signals used in finite element model analysis (CFC 200 filtered). Kinematic measurements for single head rotations as determined from raw velocity-time histories and Gaussian smoothed accelerations are presented in Appendix F. The peak angular velocities, peak angular accelerations, and peak angular decelerations in the
traces filtered for finite element model analysis were significantly smaller than their raw (velocity) and Gaussian smoothed (acceleration, deceleration) counterparts. While differences in peak velocity between the two measurement methods were modest, peak accelerations and decelerations were quite a bit larger, especially deceleration measurements in the high load level group, as can be expected when filtering signals to eliminate high frequency noise. In addition, filtering of the angular velocity-time histories was required prior to finite element analysis for computational stability and efficiency.

Cyclic injury kinematic metrics are reported Table 4.6 for the cropped traces used as inputs for finite element model analysis. Values obtained over the entire injury trace are presented in Appendix F. Average frequency, average peak-to-peak angular velocity, and average peak angular acceleration magnitude were all significantly larger in the cropped trace for finite element model simulation than over the whole cyclic injury history. The increased frequency, average peak-to-peak angular velocity, and average peak acceleration magnitudes observed in the cropped traces are likely due to the fact that they were taken from the middle of the injury time history, and did not include device ramp up and ramp down time at the beginning and end of the injury (Figure 4.2). While maximum overall peak to peak angular velocity and peak angular acceleration magnitude over the entire injury often did not occur during the cropped trace, the modestly higher average frequency, average peak-to-peak angular velocity, and average peak acceleration magnitudes in the cropped traces indicate that they may be considered between average and worst case cycles compared with the entire injury time history.
Table 4.4 Single low level sagittal rotational injury kinematics as determined from velocity and acceleration traces filtered for finite element analysis, and resulting hemorrhage scores and number of failed bridging vein elements from finite element simulations ($N_{\text{fail}}$). All values are averaged across $N_{\text{crit}}$ determination groups and all studies.

<table>
<thead>
<tr>
<th>Subject</th>
<th>$N_{\text{crit}}$ Determination Group</th>
<th>Survival Time</th>
<th>Peak Angular Velocity (rad/s)</th>
<th>Peak Angular Acceleration (krad/s$^2$)</th>
<th>Peak Angular Deceleration (krad/s$^2$)</th>
<th>Total Cerebrum EAH (%)</th>
<th>$N_{\text{fail}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>090924; 1L</td>
<td>Development</td>
<td>3-8 hour</td>
<td>34.6</td>
<td>3.2</td>
<td>4.5</td>
<td>0.0%</td>
<td>0</td>
</tr>
<tr>
<td>090929; 2L</td>
<td>Development</td>
<td>3-8 hour</td>
<td>39.6</td>
<td>3.8</td>
<td>5.1</td>
<td>0.0%</td>
<td>0</td>
</tr>
<tr>
<td>091112; 3L</td>
<td>Development</td>
<td>3-8 hour</td>
<td>39.7</td>
<td>3.7</td>
<td>5.7</td>
<td>0.0%</td>
<td>0</td>
</tr>
<tr>
<td>110504B-2; 4L</td>
<td>Development</td>
<td>24 hour</td>
<td>51.1</td>
<td>4.9</td>
<td>13.1</td>
<td>0.0%</td>
<td>0</td>
</tr>
<tr>
<td>110511B-1; 5L</td>
<td>Development</td>
<td>24 hour</td>
<td>42.8</td>
<td>4.4</td>
<td>9.9</td>
<td>0.0%</td>
<td>0</td>
</tr>
<tr>
<td>110511B-2; 6L</td>
<td>Development</td>
<td>24 hour</td>
<td>40.5</td>
<td>3.1</td>
<td>8.8</td>
<td>0.0%</td>
<td>0</td>
</tr>
<tr>
<td>110520-2;7L</td>
<td>Development</td>
<td>24 hour</td>
<td>36.1</td>
<td>3.1</td>
<td>7.4</td>
<td>0.0%</td>
<td>0</td>
</tr>
<tr>
<td>091110; 8L</td>
<td>Validation</td>
<td>3-8 hour</td>
<td>33.3</td>
<td>2.8</td>
<td>4.2</td>
<td>0.0%</td>
<td>0</td>
</tr>
<tr>
<td>091124; 9L</td>
<td>Validation</td>
<td>3-8 hour</td>
<td>37.5</td>
<td>3.0</td>
<td>6.3</td>
<td>0.0%</td>
<td>0</td>
</tr>
<tr>
<td>110504B-1; 10L</td>
<td>Validation</td>
<td>24 hour</td>
<td>50.4</td>
<td>4.8</td>
<td>12.2</td>
<td>0.0%</td>
<td>0</td>
</tr>
<tr>
<td>110520-1; 11L</td>
<td>Validation</td>
<td>24 hour</td>
<td>36.4</td>
<td>3.7</td>
<td>7.0</td>
<td>0.0%</td>
<td>0</td>
</tr>
</tbody>
</table>

**Development Group Average**

$40.6\pm 5.4$  $3.7\pm 0.7$  $7.8\pm 3.1$  $0.0\%$  $0$

**Validation Group Average**

$39.4\pm 7.5$  $3.6\pm 0.9$  $7.4\pm 3.4$  $0.0\%$  $0$

**Overall Average**

$40.2\pm 5.9$  $3.7\pm 0.7$  $7.7\pm 3.0$  $0.0\%$  $0$
Table 4.5 Single high level sagittal rotational injury kinematics as determined from velocity and acceleration traces filtered for finite element analysis, and resulting hemorrhage scores and number of failed bridging vein elements from finite element simulations ($N_{\text{fail}}$). All values are averaged across $N_{\text{crit}}$ determination groups and all studies.

<table>
<thead>
<tr>
<th>Subject</th>
<th>$N_{\text{crit}}$ Determination Group</th>
<th>Survival Time</th>
<th>Peak Angular Velocity (rad/s)</th>
<th>Peak Angular Acceleration (krad/s$^2$)</th>
<th>Peak Angular Deceleration (krad/s$^2$)</th>
<th>Total Cerebrum EAH (%)</th>
<th>$N_{\text{fail}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>060517; 1H</td>
<td>Development</td>
<td>3-8 hour</td>
<td>157.3</td>
<td>44.1</td>
<td>58.3</td>
<td>36.3%</td>
<td>8</td>
</tr>
<tr>
<td>060811; 2H</td>
<td>Development</td>
<td>3-8 hour</td>
<td>159.3</td>
<td>43.5</td>
<td>57.1</td>
<td>51.6%</td>
<td>8</td>
</tr>
<tr>
<td>070830; 3H</td>
<td>Development</td>
<td>3-8 hour</td>
<td>158.3</td>
<td>44.3</td>
<td>40.0</td>
<td>37.0%</td>
<td>16</td>
</tr>
<tr>
<td>090721-1; 4H</td>
<td>Development</td>
<td>3-8 hour</td>
<td>149.3</td>
<td>36.4</td>
<td>54.4</td>
<td>33.7%</td>
<td>12</td>
</tr>
<tr>
<td>100408-1; 5H</td>
<td>Development</td>
<td>3-8 hour</td>
<td>152.3</td>
<td>38.3</td>
<td>58.7</td>
<td>30.3%</td>
<td>9</td>
</tr>
<tr>
<td>100422D-2; 6H</td>
<td>Development</td>
<td>3-8 hour</td>
<td>138.7</td>
<td>32.0</td>
<td>61.0</td>
<td>29.2%</td>
<td>19</td>
</tr>
<tr>
<td>110224; 7H</td>
<td>Development</td>
<td>3-8 hour</td>
<td>148.6</td>
<td>31.7</td>
<td>59.9</td>
<td>54.8%</td>
<td>10</td>
</tr>
<tr>
<td>110520-4; 8H</td>
<td>Development</td>
<td>3-8 hour</td>
<td>147.0</td>
<td>29.9</td>
<td>70.1</td>
<td>33.0%</td>
<td>8</td>
</tr>
<tr>
<td>080604; 9H</td>
<td>Development</td>
<td>24 hour</td>
<td>137.9</td>
<td>34.9</td>
<td>40.5</td>
<td>3.4%</td>
<td>6</td>
</tr>
<tr>
<td>090729-1; 10H</td>
<td>Development</td>
<td>24 hour</td>
<td>150.7</td>
<td>36.1</td>
<td>58.1</td>
<td>46.0%</td>
<td>8</td>
</tr>
<tr>
<td>090729-3; 11H</td>
<td>Development</td>
<td>24 hour</td>
<td>149.9</td>
<td>36.0</td>
<td>55.7</td>
<td>35.8%</td>
<td>8</td>
</tr>
<tr>
<td>110202-1; 12H</td>
<td>Development</td>
<td>24 hour</td>
<td>148.9</td>
<td>32.7</td>
<td>63.3</td>
<td>23.8%</td>
<td>12</td>
</tr>
<tr>
<td>110202-2; 13H</td>
<td>Development</td>
<td>24 hour</td>
<td>149.2</td>
<td>33.3</td>
<td>62.1</td>
<td>37.8%</td>
<td>8</td>
</tr>
<tr>
<td>110217; 14H</td>
<td>Development</td>
<td>24 hour</td>
<td>148.6</td>
<td>33.4</td>
<td>62.6</td>
<td>17.8%</td>
<td>11</td>
</tr>
<tr>
<td>110304-1; 15H</td>
<td>Development</td>
<td>24 hour</td>
<td>145.8</td>
<td>29.4</td>
<td>63.0</td>
<td>9.8%</td>
<td>12</td>
</tr>
<tr>
<td>110615-1; 16H</td>
<td>Development</td>
<td>24 hour</td>
<td>152.2</td>
<td>28.5</td>
<td>77.5</td>
<td>25.8%</td>
<td>16</td>
</tr>
<tr>
<td>110615-2; 17H</td>
<td>Development</td>
<td>24 hour</td>
<td>147.6</td>
<td>29.8</td>
<td>72.3</td>
<td>41.8%</td>
<td>10</td>
</tr>
<tr>
<td>060914; 18H</td>
<td>Validation</td>
<td>3-8 hour</td>
<td>155.0</td>
<td>43.3</td>
<td>48.5</td>
<td>43.5%</td>
<td>6</td>
</tr>
<tr>
<td>110128; 19H</td>
<td>Validation</td>
<td>3-8 hour</td>
<td>154.5</td>
<td>34.6</td>
<td>65.3</td>
<td>32.8%</td>
<td>8</td>
</tr>
<tr>
<td>110520-3; 20H</td>
<td>Validation</td>
<td>3-8 hour</td>
<td>145.4</td>
<td>29.3</td>
<td>66.8</td>
<td>48.0%</td>
<td>8</td>
</tr>
<tr>
<td>080606-1; 21H</td>
<td>Validation</td>
<td>24 hour</td>
<td>143.1</td>
<td>34.9</td>
<td>45.8</td>
<td>6.5%</td>
<td>8</td>
</tr>
<tr>
<td>090722-1; 22H</td>
<td>Validation</td>
<td>24 hour</td>
<td>150.6</td>
<td>36.1</td>
<td>55.6</td>
<td>16.4%</td>
<td>12</td>
</tr>
<tr>
<td>090722-2; 23H</td>
<td>Validation</td>
<td>24 hour</td>
<td>149.6</td>
<td>36.4</td>
<td>54.8</td>
<td>34.1%</td>
<td>13</td>
</tr>
<tr>
<td>090729-2; 24H</td>
<td>Validation</td>
<td>24 hour</td>
<td>150.2</td>
<td>36.1</td>
<td>57.2</td>
<td>38.8%</td>
<td>8</td>
</tr>
<tr>
<td>110304-2; 25H</td>
<td>Validation</td>
<td>24 hour</td>
<td>156.0</td>
<td>31.1</td>
<td>67.4</td>
<td>30.8%</td>
<td>12</td>
</tr>
<tr>
<td><strong>Development Group Average</strong></td>
<td></td>
<td></td>
<td><strong>149.5±5.7</strong></td>
<td><strong>35.0±5.1</strong></td>
<td><strong>59.7±9.5</strong></td>
<td><strong>32.2±13.5%</strong></td>
<td><strong>10.6±3.5</strong></td>
</tr>
<tr>
<td><strong>Validation Group Average</strong></td>
<td></td>
<td></td>
<td><strong>150.6±4.6</strong></td>
<td><strong>35.2±4.2</strong></td>
<td><strong>57.7±8.2</strong></td>
<td><strong>31.4±13.8%</strong></td>
<td><strong>9.4±2.6</strong></td>
</tr>
<tr>
<td><strong>Overall Average</strong></td>
<td></td>
<td></td>
<td><strong>149.8±5.3</strong></td>
<td><strong>35.0±4.7</strong></td>
<td><strong>59.0±9.0</strong></td>
<td><strong>32.0±13.3%</strong></td>
<td><strong>10.2±3.3</strong></td>
</tr>
<tr>
<td>Subject</td>
<td>Rotational Plane</td>
<td>Survival Time</td>
<td>Average Frequency (Hz)</td>
<td>Average Peak Peak Angular Velocity (rad/s)</td>
<td>Maximum Peak Peak Angular Velocity (rad/s)</td>
<td>Average Peak Acceleration Magnitude (rad/s^2)</td>
<td>Maximum Peak Acceleration Magnitude (rad/s^2)</td>
</tr>
<tr>
<td>------------------</td>
<td>------------------</td>
<td>---------------</td>
<td>-------------------------</td>
<td>-------------------------------------------</td>
<td>-------------------------------------------</td>
<td>-----------------------------------------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>090407; 1C</td>
<td>Sagittal</td>
<td>3-8 hr</td>
<td>2.27±0.004</td>
<td>20.3</td>
<td>573</td>
<td>0.0%</td>
<td>0</td>
</tr>
<tr>
<td>090618; 2C</td>
<td>Sagittal</td>
<td>3-8 hr</td>
<td>2.61±0.02</td>
<td>24.2</td>
<td>889±109</td>
<td>980</td>
<td>0.0%</td>
</tr>
<tr>
<td>091015; 3C</td>
<td>Sagittal</td>
<td>3-8 hr</td>
<td>2.62±0.005</td>
<td>24.7±0.3</td>
<td>571±18</td>
<td>595</td>
<td>0.7%</td>
</tr>
<tr>
<td>091015; 4C</td>
<td>Sagittal</td>
<td>3-8 hr</td>
<td>2.91±0.02</td>
<td>28.2±0.1</td>
<td>913±289</td>
<td>1147</td>
<td>0.3%</td>
</tr>
<tr>
<td>091105; 5C</td>
<td>Sagittal</td>
<td>3-8 hr</td>
<td>2.87±0.03</td>
<td>29.2±0.3</td>
<td>940±268</td>
<td>1202</td>
<td>0.0%</td>
</tr>
<tr>
<td>091218; 6C</td>
<td>Sagittal</td>
<td>3-8 hr</td>
<td>2.87±0.005</td>
<td>29.2±0.3</td>
<td>763±124</td>
<td>858</td>
<td>0.0%</td>
</tr>
<tr>
<td>100106; 7C</td>
<td>Sagittal</td>
<td>24 hr</td>
<td>2.84±0.02</td>
<td>30.0±0.3</td>
<td>1097±377</td>
<td>1401</td>
<td>1.8%</td>
</tr>
<tr>
<td>100121; 8C</td>
<td>Sagittal</td>
<td>24 hr</td>
<td>2.89±0.001</td>
<td>27.5±0.1</td>
<td>961±260</td>
<td>1153</td>
<td>2.7%</td>
</tr>
<tr>
<td>100610; 9C</td>
<td>Axial</td>
<td>24 hr</td>
<td>2.87±0.008</td>
<td>32.4±2.2</td>
<td>732±331</td>
<td>1024</td>
<td>0.0%</td>
</tr>
<tr>
<td>100630; 10C</td>
<td>Axial</td>
<td>24 hr</td>
<td>2.87±0.07</td>
<td>28.7±3.8</td>
<td>792±201</td>
<td>1024</td>
<td>0.0%</td>
</tr>
</tbody>
</table>

Table 4.6: Cyclic rotational injury kinematics as determined from velocity and acceleration traces cropped for finite element analysis, and resulting hemorrhage scores and number of failed bridging vein elements from finite element simulations (N fail). If a metric was averaged across all cycles in a single trace (e.g. frequency), the overall average value represents the mean of the average values for each trace.
Relationship between Injury Kinematics and $N_{\text{fail}}$

$N_{\text{fail}}$ for each simulation are listed in Tables 4.4, 4.5, and 4.6. For both single low level and cyclic rotation finite element simulations, no bridging vein element failures occurred. On the other hand, for single high level rotation simulations, varying numbers of bridging vein failures occurred. Investigating $N_{\text{fail}}$ as a function of peak angular velocity (Figure 4.3) yielded no distinct pattern, but there does appear to be a peak angular velocity at which bridging vein element failure begins to occur, between the peak angular velocities observed in high and low level single rotation injuries. This cutoff was also observed when inspecting the relationship between $N_{\text{fail}}$ and peak angular acceleration magnitude (Figure 4.4). Furthermore, $N_{\text{fail}}$ appears to increase linearly with peak accelerative load. Finally, $N_{\text{fail}}$ increases with increasing EAH until approximately 10% EAH cerebrum surface coverage, at which point there is no longer a distinct pattern (Figure 4.5).

![Figure 4.3 $N_{\text{fail}}$ versus peak angular velocity (or maximum peak-to-peak velocity for cyclic simulations) across all studies. The $N_{\text{crit}}$ development group is represented by blue circles, the $N_{\text{crit}}$ validation group is indicated by red triangles, and the cyclic simulations are shown with black 'x's. The discontinuity in peak angular velocities and $N_{\text{fail}}$ indicates there may be a threshold level of angular velocity between those observed in the low and high single rotation load levels at which bridging vein failure is initiated.](image-url)
Figure 4.4 $N_{fail}$ versus the larger of maximum angular acceleration or deceleration for single rotations, or maximum peak angular acceleration magnitude for cyclic simulations. Again, blue circles represent the $N_{crit}$ development group, red triangles represent the $N_{crit}$ validation group, and cyclic simulations are indicated by black ‘x’s. A similar discontinuity is seen as that observed in Figure 4.3 with angular velocity, but increasing accelerations also appear to correlate with increasing $N_{fail}$, suggesting accelerative kinematics may be the cause of bridging vein rupture.

Figure 4.5 $N_{fail}$ versus EAH% across all studies. Once again blue circles indicate studies in the $N_{crit}$ development group, red triangles represent the $N_{crit}$ validation group, and black ‘x’s show cyclic simulations. Increasing amounts of EAH appear to correlate with increasing $N_{fail}$ until ~10% EAH brain surface coverage. Beyond 10% EAH, no discernable pattern is apparent.
Bridging vein element failures occur at two distinct time points during single high level rotations (Figure 4.6, representative example): first, after initial accelerations are sustained where ruptures occur in the posterior cluster of bridging veins (with exception of one case which had a mixture of anterior and posterior failures at this time) as the moving skull initially pulls away due to inertia from the stationary posterior-superior brain surface during the neck extensional rotation, and then again as the skull stops suddenly with peak head decelerations, at which point bridging vein failures occur in the anterior cluster because the brain continues to rotate backward, again due to inertia. In addition, bridging vein failure exhibits a local cascading phenomenon such that not all failures are simultaneous at these two distinct time points in the angular velocity pulse. We note that some simulations only exhibit bridging failures in the posterior cluster during the finite element simulated rotation, which may have inadvertently truncated the latter portion of this event. All bridging vein element failure time histories are presented in Appendix G.

![Graph](image)

**Figure 4.6** Representative example of a high load level finite element simulation angular velocity-time history, angular acceleration-time history, and resulting $N_{fail}$ over time. Bridging vein failures occur at two time points: once initial acceleration is sustained, and then just after peak decelerations occur.
Development of EAH Prediction Criterion

When examining simulations in the \( N_{\text{crit}} \) development group, with a detection level of EAH≥2%, sensitivity and specificity for values of \( N_{\text{crit}} \) between 1 and 6 were both 100%. As there were no development group simulations resulting in \( N_{\text{fail}} \) between 1 and 5, an \( N_{\text{crit}} \) of 6 was chosen as the number of failed bridging vein elements associated with the presence of EAH.

Validation of EAH Prediction Criterion

Again, with a detection level of EAH≥2% and \( N_{\text{crit}} \) of 6, evaluation of the validation group yielded positive and negative predictive values of 100%, indicating that an \( N_{\text{crit}} \) of 6 predicted the occurrence of EAH perfectly in the validation group, as it did in the development group.

Extension of EAH Prediction Criterion to Cyclic Rotational Injuries

Cyclic rotational injuries produced very small amounts of EAH, with only one above the 2% whole cerebrum threshold. On the other hand, no bridging vein element failures occurred in any cyclic injury finite element simulations. Thus, using the \( N_{\text{crit}} \) of 6 validated in single head rotations produced a negative predictive value of 90% for cyclic simulations, indicating that this predictor is 90% accurate in hemorrhage predictive capabilities for cyclic rotational head injuries.

Discussion

In this study, we incorporated bridging veins into a finite element model of the neonatal porcine head, and investigated the effect of an array of rotational kinematic loads on the occurrence of bridging vein element failures. From this, we developed and independently validated a threshold for the number of failed bridging veins associated with the presence of extra-axial hemorrhage from newborn porcine in vivo rotational brain injury study pathology. We find that there are likely kinematic thresholds for angular velocity and angular acceleration that result in the failure of bridging veins between the load levels investigated in this study, and that the number of bridging vein failures detected in a finite element analysis increases with increasing
peak rotational accelerations. The extent of EAH detected post-injury also begins to increase with a modest number of finite element model predicted bridging vein failures, but as many bridging vein elements begin to fail, there is no distinct pattern of increasing EAH with increasing numbers of bridging vein failures across studies. We determined a critical value of six bridging vein element failures is associated with 100% sensitivity and specificity for detection of EAH in our newborn porcine rapid nonimpact rotation TBI model, but applying this criterion to a newborn porcine cyclic rotational injury model only yielded 90% accuracy. The prediction criterion determined in this study for the porcine newborn will be employed in future finite element models of the human infant head to estimate the likelihood of extra-axial hemorrhage resulting from both accidental and abusive trauma.

The loading condition at which bridging vein failures begin to occur in this finite element model is between the low and high level rotational loading groups simulated in this study. Future in vivo studies may be conducted in this realm, with peak angular velocities between 50 and 140 rad/s to determine a more definitive threshold level for extra-axial hemorrhage. However, our finding of increasing numbers of finite element model bridging vein failures with higher rotational acceleration magnitudes draws a parallel with classic studies indicating that rotational accelerative trauma causes diffuse brain injury that is more severe at the surface of the brain, thought to be due to differences in complex multi-component non-homogeneous tissue interfaces [12]. Ommaya and Gennarelli go on to suggest that smooth surfaces of the brain would suffer the least amount of damage while regions where surfaces and interfaces are rough would incur the most damage [12], which may agree with our observation in Chapter 3 of decreased brain-skull displacements and possibility of increased shear damage to the brain tissue near the porcine frontal pole/olfactory bulbs, though this needs to be analyzed more rigorously. If increased brain damage at the periphery is indeed caused by the obstruction of motion and contact with surrounding tissue, the converse assumption that increased brain-skull displacements occurring in regions of smoother cortical surfaces would lead to the hypothesis that increased displacement of the brain relative to the skull at the superior margin would cause increased stretch to the
parasagittal bridging veins at this interface. Rotational injury experiments in rhesus monkeys found that increasing levels of angular acceleration caused acute subdural hematoma via rupture of the parasagittal bridging veins, but this observation was also dependent on the impulse duration and rise time [13]. Thus, it may also be important to investigate bridging vein rupture extra-axial hemorrhage thresholds as a function of rotational acceleration, rise time, and impulse duration, rather than velocity.

We found that increasing levels of EAH were associated with increasing amounts of bridging vein failures up until 10% EAH, after which the distribution of EAH was insensitive to an increasing number of bridging vein element failures. In this study EAH was measured in terms of two-dimensional surface areas, from photographs taken after the brains had been immersed in 10% formalin for at least five days [2]. Comparison of the extent of EAH with peak angular velocities and peak angular acceleration magnitudes (Figure 4.7) reveals increasing EAH with increasing velocity and acceleration, but these patterns are not strong for either kinematic metric. A different trend may emerge if EAH was measured volumetrically. Future investigations may quantify EAH via imaging techniques such as magnetic resonance imaging or computed tomography to obtain a more descriptive volumetric measure. In addition, as some of the subdural surface blood may have washed off in the formalin, pictures of superior surface of the brains before formalin immersion, with skull removed and dura intact, just after sacrifice and perfusion fixation were investigated for all but one porcine rotational injury for which photographs were unavailable. Brains were designated positive for subdural hemorrhage when a large, dark, consolidated mass of blood was visible over the sagittal midline. Neither any of the single low level nor any cyclic rotation studies were designated as positive for subdural hemorrhage using this analysis. Of note, the single cyclic study that was designated as positive for EAH (>2% brain surface coverage) had a small cortical focal bleed lateral to the midline, consistent with the hypothesis that this may have been caused by insertion of a thermal diffusion probe after injury (see below). While all high level single rotation studies were designated in Results as positive for EAH, exceeding our brain surface coverage threshold of >2%, 75% (18 of 24, one subject’s
photos were unavailable) of these studies had diffuse subdural hemorrhages just after injury in this secondary analysis, often spilling away from the sagittal midline into surrounding regions, while the remaining 25% (6 of 24) of high level single rotations did not have these large coagulated masses, instead displaying more thin diffuse hemorrhage. This secondary analysis indicates that subdural hemorrhage often occurred together with our measured extra-axial hemorrhage in these high level injuries. Furthermore, the presence of these large subdural bleeds centered on the sagittal midline is consistent with our previous analysis of regional EAH [2], and also leads to the hypothesis that the bridging veins, draining into the superior sagittal sinus along the midline, are indeed the cause of these hemorrhages.

**Figure 4.7** EAH versus Peak Angular Velocity (left) and EAH vs Peak Angular Acceleration Magnitudes (right) across all studies. The N\textsuperscript{crit} development group is represented by blue circles, the validation group is shown with red triangles and the cyclic injuries are represented by black ‘x’s. While EAH appears to increase with both increasing peak angular velocity and increasing peak angular acceleration magnitudes, these patterns are fairly non-specific and could be improved by volumetric measurement of EAH using in vivo imaging methods.

We determined that six failed bridging vein elements best correlated with the presence of EAH detected after in vivo rotational brain injuries in the neonatal piglet, with both a sensitivity and specificity of 100% for single rapid sagittal rotations, and a 90% predictive accuracy for cyclic sagittal rotations. The single false negative produced in cyclic injury finite element simulations may be confounded by cerebral blood flow measurements conducted on cyclically injured piglets surviving 24 hours or longer, for which a 2mm hole was created in the skull near the sagittal suture for insertion of a thermal diffusion probe [4]. It is possible that creation of the hole in the skull or insertion of the measurement probe may have caused hemorrhage not associated with
the cyclic rotational injury. Indeed, we previously calculated EAH scores for four sham animals that had cerebral blood flow measurements taken without a history of rotational head injury and found that whole cerebrum EAH percentage scores ranged from 0-5% in these animals [6].

Nonetheless, our critical value of six failed bridging veins performed very well over a myriad of experimental simulations, and is an improvement over a previous study which used peak strains experienced by boundary condition connectors, finding 80% sensitivity and 85% specificity for similar hemorrhage detection thresholds [2]. It is also an improvement, in terms of predictive power, over another previous study employing a representative pia-arachnoid complex element boundary condition, which found that cortical principal stresses predicted the occurrence of EAH with 94% sensitivity and 100% specificity [3]. However, this model was more sensitive to hemorrhage predictions in the sense that only 1% of a given region was required to be covered in blood to detect EAH (translating to ~0.08% of the whole brain at minimum), though this small amount of blood may not be detectable with conventional medical imaging. It is important to note, however, that we cannot say whether detectable EAH may be associated with an amount of bridging vein failures between one and five in the presented finite element model, as no simulations produced these amounts of failed bridging vein elements. Again, we suggest future investigations of in vivo injuries at peak velocities between the low and high level groups investigated in this study to further refine the model presented in this study.

Limitations

The presented study includes some limitations. First, finite element model bridging vein element length was not matched to in vivo lengths, and it is likely that in vivo neonatal porcine bridging vein lengths are much larger than the initial lengths determined by our choices for bridging vein elements. While porcine newborn bridging vein test gage lengths in Chapter 2 ranged between approximately 2 and 6mm, porcine newborn finite element model bridging vein lengths ranged from about 0.4 to 1.4mm. Because the optimized finite element brain-skull boundary condition determined in Chapter 3 is displacement based, it is possible that longer
bridging vein elements subjected to the same displacements as simulated in these models would not reach their critical failure stretch. In addition, we assume that the bridging veins are taut at the onset of rotation, while in vivo, there may be a considerable amount of slack in the vessel between its brain and sinus attachment points, or pull out of the soft brain tissue during an insult. However, it is also possible that the bridging veins may snag on or be entangled with other structures between the brain and skull, namely the dura and arachnoid membranes and arachnoid trabeculae [14], causing smaller gage lengths that require less displacement to cause rupture. Thus we conclude that while the simulated bridging vein lengths are likely artificially low, they represent a worst case scenario for rupture incidence. Importantly, this caveat simply indicates that we may overestimate the total number of failed bridging veins, but does not impact the validity of our EAH predictions. We assigned our EAH prediction criterion, $N_{\text{crit}}$, based on the number of bridging vein failures predicted by this model, and as such, the critical number of six is specifically a product of this model. It follows that models employing bridging vein lengths closer to those observed in vivo may obtain a different $N_{\text{crit}}$ threshold.

A second limitation is that the finite element model brain-skull boundary condition was optimized in Chapter 3, at peak angular velocities similar to those in the single low level rotations in this study, and it is unknown whether brain-skull displacements are rate dependent. As the material properties of the pia-arachnoid complex have been characterized as rate-dependent in tension [15], normal traction [16], and shear [17], future sagittal transection studies should investigate brain-skull displacement at higher loads to ascertain whether this boundary condition is also rate-dependent, and if so, appropriate rate-dependent boundary conditions should be employed in future finite element analyses.

A third limitation is that all single rotation studies were performed with neck extension head rotations, which lead to bridging vein element failures in the posterior cluster at the onset of accelerative load as the movement of the skull preceded the motion of the posterior-superior brain surface, and in the anterior cluster just after peak decelerations as the brain continued to rotate backward after the skull stopped moving. Future studies may investigate neck flexion head
rotations to determine how inertial effects may influence the motion of the brain and, in turn, the location and timing of bridging vein ruptures in this rotational direction within the sagittal plane.

Finally, although secondary analysis of subdural hemorrhage just after sacrifice showed severe hemorrhage spanning the sagittal midline, it is possible that the observed hemorrhage may originate from tears or damage in blood vessels other than the bridging veins. Future animal studies may investigate the presence of ruptured bridging veins after high level loading, perhaps by injecting dye into the superior sagittal sinus after sacrifice.

Conclusions

In the present study we found that peak angular acceleration magnitudes correlate with the number of predicted bridging vein failures in a finite element model of neonatal porcine rotational brain injury, and that a critical number of six bridging vein element failures provide 100% sensitivity and specificity for hemorrhage prediction in single rapid nonimpact head rotations. This is an improvement over past models that yielded overall sensitivities of 80-94% and specificities of 85-100% for single rapid nonimpact rotations. In addition, this predictor yielded 90% accuracy for hemorrhage prediction in cyclic head rotations. The findings and threshold criteria determined in this study will be used in future finite element models of the human infant head to ascertain the likelihood of EAH given varying injury scenarios. We conclude that finite element models should include biofidelic representations of the parasagittal bridging veins to better predict the occurrence of EAH.

References


CHAPTER 5: FINITE ELEMENT MODEL PREDICTIONS OF BRIDGING VEIN RUPTURE RESULTING FROM VIGOROUS SHAKING IN THE HUMAN INFANT

Abstract

For 45 years, there has been substantial scientific and legal debate about whether or not traumatic brain injury in the infant can result from episodes of vigorous shaking without impact. Clinically, extra-axial hemorrhage secondary to bridging vein rupture, together with ischemic injury, and retinal hemorrhages are present in diagnosed cases of abusive head trauma. In this study, we sought to determine whether rupture of the parasagittal bridging veins may occur as a result of vigorous shaking without head impact. We measured head kinematics associated with shaking an instrumented human infant surrogate and included human infant bridging vein mechanical properties in a computational model of the human infant head to simulate episodes of vigorous shaking, and predict that, in some instances of shaking, the number of bridging vein failures exceeded a threshold associated with extra-axial hemorrhage from a porcine model of traumatic brain injury. Higher angular accelerations increased bridging vein element failures, and the addition of higher angular velocities further exacerbated bridging vein failures. The data presented in this study will help inform clinical differential diagnoses of abusive head trauma in infants.

Introduction

Whether or not vigorous shaking without impact can cause traumatic brain injuries in the infant is subject to substantial scientific and legal debate [1-8], though the presence of extra-axial hemorrhage (EAH) is often reported in cases of suspected and diagnosed abusive head trauma in young children [9-11]. One mechanism of EAH is thought to be the rupture or tearing of the parasagittal bridging veins, which drain blood from the brain into the superior sagittal sinus, housed in the falx cerebri, rigidly attached to the inner surface of the skull [12-14]. The hypothesized mechanism relating bridging vein failure to abusive head trauma is that during rapid
head rotations, the movement of the brain lags that of the skull, causing the bridging veins to rapidly elongate and possibly tear, creating hemorrhage within the subdural space. Because of the paucity of pediatric bridging vein mechanical properties reported in literature, biofidelic measurements of head kinematics during shaking episodes, and accurate computational model portrayals of the infant head, it has been difficult to determine objectively whether or not the head kinematics associated with vigorous shaking in the infant may be sufficient to produce bridging vein tears and subsequent EAH.

In this study, we measured head kinematics recorded during shaking of an instrumented human infant surrogate and included human infant bridging vein mechanical properties in a computational model of the human infant head to simulate episodes of vigorous shaking without impact and predict whether the number of bridging vein failures exceeded a threshold associated with EAH in a porcine model of traumatic brain injury. Our lab has developed increasingly biofidelic versions of instrumented human infant surrogates to gain insight into the kinematics of the head during accidental falls and abusive trauma [15-18]. In the present study, we asked ten volunteers to shake the latest version of our infant surrogate [18] with maximal effort to measure the kinematics of the human infant head under shaking. These shaking episodes were then used as loading inputs for a finite element model (FEM) of the human infant head previously developed in our lab [19]. We modified that FEM to include elements representative of human infant bridging veins with age and species matched mechanical properties measured in Chapter 2, and as the human infant brain-skull boundary condition is unknown, we simulated shakes using both axial and sagittal plane equivalent boundary condition stiffnesses as determined in our porcine model (Chapter 3, Coats et al [20]) to provide an injury possibility corridor in the human infant. The number of bridging vein element failures resulting from the finite element model simulations of shaking was then compared with the threshold of bridging vein element failures that best correlated with the presence of EAH in pathology investigations after in vivo newborn porcine brain injuries, determined in Chapter 4, to predict whether bridging veins rupture in sufficient numbers to produce EAH during vigorous shaking without impact in the human infant. These
studies will provide objective biomechanical data to inform clinical diagnoses of abusive head trauma.

**Methods**

*Construction of an Instrumented Human Infant Anthropomorphic Surrogate*

In order to estimate the kinematics of the infant head under shaking type insults, volunteers were asked to shake an instrumented 1.5 month old human infant surrogate (Figure 5.1), previously developed in our lab [15-18]. The most recent edition of our surrogate was used [18], with a total head and body mass of 4.4 kg, and head mass of 1 kg, yielding a head-to-body mass ratio of 0.23, comparable to ratios based on measurements reported by Duhaime et al for one month old infants [15].

The surrogate also included biofidelic skull, suture, and neck properties. Specifically, the tensile and bending properties of the surrogate neck [18] were matched on corresponding property measurements of infant cadaveric osteoligamentous cervical spine by Luck et al (tension) [21] and Luck (bending) [22]. The surrogate neck was constructed from a 2.54 cm diameter cylindrical mold of Ecoflex 00-30 super soft silicone rubber (SmoothOn) with a piece of Chemical Resistant clear Tygon tubing (McMaster-Carr, 1.6mm inner diameter, 4.8mm outer diameter, 75A durometer rating) embedded along the central axis. The rubber cylinder was then potted into two 3.8cm diameter plastic pipe fittings with plaster, and three sets of two neoprene rubber bands (McMaster-Carr, 0.8mm thick, 8mm wide, 50A durometer rating) were attached on the ventrolateral surface of the neck to provide increased resistance to bending in extension. The flexible length of the neck was approximately 3-4 cm, and the Tygon tubing governed tensile response while the silicone rubber allowed bending in all three rotational directions.

A metal plate extended from the top of the neck to the center of the surrogate head for rigid fixation of a tri-axial angular velocity transducer (ARS-06 Triaxial unit, Applied Technology Associates, Albuquerque, NM). The tri-axial angular velocity transducer allowed for measurements of sagittal, axial and coronal plane angular velocities. The surrounding head
volume was filled with a linear viscoelastic dielectric gel (Sylgard 527 A&B Silicone Dielectric Gel, Dow Corning, 1:1 A to B mix ratio), to approximate the response of human infant brain tissue. The gel has a shear modulus of 765±44Pa [23, 24], while human infant brain tissue is estimated to have a shear modulus of 559 Pa, approximated by scaling the properties of human adult brain by the adult-to-infant shear modulus ratio reported for porcine tissue previously [19, 25]. Additional details on surrogate anthropometry and material properties are presented in Sullivan et al [18] and Coats and Margulies [17].

Figure 5.1 Instrumented human surrogate (left), and surrogate neck and angular rate sensor mounting plate assembly (right)

Measurement and Analysis of Head Shaking Kinematics

Ten adult volunteers were asked to shake the 1.5 month old infant surrogate. The volunteer pool was made up of five males and five females, ranging in age from 17 to 40 years.
old (averaging 26.5±7.7 years), and ranging in weight from 56 to 93 kg (averaging 73.9±12.1 kg).

In order to estimate naïve and worst-case kinematics resulting from episodes of vigorous shaking, each volunteer shook the surrogate twice, each with a different set of instructions. First, volunteers were simply told to shake the surrogate with maximal effort and without impact. In a second trial, volunteers were asked to shake the surrogate with maximal effort and without impact again, but with an additional goal of ensuring complete flexion and extension of the neck during shaking. In all cases, angular velocity was measured at a sampling rate of 7,500 Hz, and 10 shakes were recorded and analyzed in each trial.

While the rotation of the surrogate neck was not constrained to a single plane, only the angular velocity associated with sagittal plane rotations was extracted for further analysis, allowing direct comparison with previous studies [15, 16, 26], which only investigated sagittal plane motion during shaking. Based on power spectral density analysis, angular velocity-time histories were filtered at 40 Hz, with a low-pass second-order Butterworth filter. Angular velocity-time histories were differentiated using the forward difference method to find angular acceleration. The cycling frequency, peak-to-peak angular velocities, and peak acceleration magnitudes, were extracted from each shaking trace using the same methods as presented for porcine cyclic studies in Chapter 4. For each shaking sequence, average frequency, average peak-to-peak angular velocity, maximum peak-to-peak angular velocity, average peak angular acceleration magnitude, and maximum peak angular acceleration magnitude, were recorded and compared between the first and second shaking trials using a series of paired t-tests.

**Human Infant Finite Element Model Development**

A finite element model of the human infant head previously developed in our lab [19] was modified to include bridging vein elements with human infant properties and behavior measured in Chapter 2, and a linear elastic connector brain-skull boundary condition, approximated with both the optimized newborn porcine sagittal plane brain-skull boundary condition determined in Chapter 3, and the previously determined porcine newborn axial plane brain-skull boundary
condition, in separate simulations. This model (Figure 5.2) included 11,067 4-node linear tetrahedral brain elements, 12,726 6-node triangular or 8-node tetrahedral continuum shell skull elements, and 2,485 3-node triangle or 4-node quadrilateral membrane suture elements. The brain was defined as a nonlinear, homogeneous, isotropic hyperelastic material, using the first-order Ogden strain energy density function, as described in Chapter 3, with a shear modulus of 559 Pa as estimated by Coats et al [19] based on published adult values scaled using the ratio between adult and newborn porcine brain shear moduli reported by Prange and Margulies [25]. Viscoelastic response was defined using the two-term Prony series function, again described in Chapter 3, with relaxation moduli and time constants estimated as those reported for neonatal porcine tissue as measured by Prange and Margulies [25]. Poisson’s ratio for brain was defined as 0.4999 according to Coats et al [19]. Suture was defined as a linear elastic material with an elastic modulus of 8.1MPa as measured by Coats and Margulies [27], and density estimated as that of human adult dura [28]. While in past studies using this finite element model, measured material properties were used to define the human infant skull, because no impact was associated with these shaking simulations, we elected to define the skull as a rigid body. This simplification greatly reduced the long computational run time associated with the increased time course of these shaking simulations compared with past impact simulations. All material properties are listed in Table 5.1.
Figure 5.2 Human Infant Finite Element Model. The brain (blue) is pictured on the left, and the skull (red) and suture (yellow) are pictured on the right.

Table 5.1 Material Properties used in the Human Infant Finite Element Model

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Material Property</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Brain</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\mu_0 = 559 \text{ Pa}$</td>
<td>Coats et al., 2007 [19]</td>
</tr>
<tr>
<td></td>
<td>$\alpha = 0.01$</td>
<td>Prange and Margulies, 2002 [25]</td>
</tr>
<tr>
<td></td>
<td>$C_1 = 0.3322$</td>
<td>Prange and Margulies, 2002 [25]</td>
</tr>
<tr>
<td></td>
<td>$C_2 = 0.3890$</td>
<td>Prange and Margulies, 2002 [25]</td>
</tr>
<tr>
<td></td>
<td>$\tau_1 = 2.9572 \text{ s}$</td>
<td>Prange and Margulies, 2002 [25]</td>
</tr>
<tr>
<td></td>
<td>$\tau_2 = 0.1813 \text{ s}$</td>
<td>Prange and Margulies, 2002 [25]</td>
</tr>
<tr>
<td></td>
<td>$\rho = 1.04 \text{ g/cm}^3$</td>
<td>Coats et al., 2007 [19]</td>
</tr>
<tr>
<td></td>
<td>$\nu = 0.4999$</td>
<td>Coats et al, 2007 [19]</td>
</tr>
<tr>
<td><strong>Suture</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\rho = 1.13 \text{ g/cm}^3$</td>
<td>Galford and McElhaney, 1970 [28]</td>
</tr>
<tr>
<td></td>
<td>$E = 8.1 \text{ MPa}$</td>
<td>Coats and Margulies, 2006 [27]</td>
</tr>
<tr>
<td></td>
<td>$\nu = 0.49$</td>
<td>Coats et al, 2007 [19]</td>
</tr>
<tr>
<td><strong>General Brain-Skull Connectors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$k_{sag}=902.5 \text{ N/m}$</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>$k_{ax}=67,686 \text{ N/m}$</td>
<td>N/A</td>
</tr>
</tbody>
</table>

The brain-skull boundary condition was defined in a manner similar to those in Chapters 3 and 4 for porcine finite element models, using linear elastic connector elements linking each brain surface node to the nearest node on the inner surface of the skull. The human finite element model included 735 nodes on the outer surface of the brain, and again, 22 of these nodes were reserved for bridging vein elements, yielding 713 linear elastic connector elements to define the boundary condition between the brain and skull. To approximate the sagittal plane brain-skull...
displacement response measured in the newborn pig in Chapter 3 and the previously determined axial plane newborn pig brain-skull boundary condition, the general connectors were approximated as springs in parallel. The number of general connector nodes in the porcine model in Chapter 4 (2,658) was divided over the surface area of the modeled porcine brain (7,470 mm²), then multiplied by the surface area of the modeled human brain (39,198 mm²), to determine the number of connector elements required in the human model (13,948) to match the porcine model boundary condition. Because only 713 brain surface nodes were available, the general connector stiffness was scaled linearly such that the effective stiffness of all general connectors would match the boundary conditions determined for the newborn pig. Multiplying the number of nodes required to match the porcine boundary condition (13,948) by the sagittal plane optimized porcine general connector stiffness (46.133 N/m) and axial plane general connector stiffness (3,460 N/m) to obtain effective stiffness values, and then dividing by the available general connector nodes in the human model (713) yielded human model general connector stiffnesses of 902.5 N/m to match the porcine infant sagittal boundary condition and 67,686 N/m to match the axial boundary condition.

Bridging vein elements were assigned using similar methods as those described in Chapters 3 and 4, but because the human model does not include a falx, the anterior-posterior arc length of the brain on the superior surface was measured along the midline from the most anterior point to the most posterior point. The length was split into four segments, from anterior to posterior the first segments were 20% of the entire length and the last two were 30% of the entire length, and the first and third segments were designated as tributary for bridging veins according to the distribution of bridging veins observed in human adults [29]. Brain nodes just lateral to the midline within the anterior-posterior first and third segment lengths were identified as potential bridging vein attachment points, and chosen such that right and left side bridging veins were evenly distributed along the length of both segments. Again, five bridging veins per side were selected in the first segment, and six bridging veins per side were selected in the third segment, for a total of 22 bridging veins, according to the average number and distribution of bridging veins...
in the human adult [29]. The resulting length of the bridging vein elements ranged from 0.7-
1.9mm, which, similar to the porcine model in Chapter 4, are smaller than the experimental gage
lengths of the human infant bridging veins tested in Chapter 2 (2.7-6.5mm), and unknown
additional length was secured in the apparatus grips. Thus we conclude that the bridging vein
element lengths in this model are underestimations of in vivo length and as such this model will
likely overestimate the occurrence of bridging vein rupture. Bridging vein force-displacement
behavior was defined using an average human infant post-cyclic stress-stretch curve based on
measurements from Chapter 2 and processed according to the methods outlined in Chapter 3.
To match porcine finite element model conditions from Chapters 4, bridging vein failure criterion
was defined as the average human infant high rate ultimate stretch.

**Finite Element Model Simulations of Vigorous Shaking in the Human Infant**

Because increased amounts of bridging vein failure were associated with higher peak
accelerations in the porcine finite element model in Chapter 4, we sought to simulate an array of
peak accelerations in the human infant finite element model. Measured kinematics from the infant
surrogate shaking studies were examined to choose which shaking episodes to simulate in the
finite element model. Initially, two studies were selected, one with consistent low angular
accelerations and angular velocities, and a second with consistent high accelerations and
velocities. No infant surrogate shakes produced instances of mismatched low velocity and high
acceleration. Thus, to determine whether high angular accelerations without the presence of high
angular velocities were responsible for bridging vein failure, the low angular velocity-low angular
acceleration trace was modified such that peak accelerations were similar to those observed in
the high velocity-high acceleration trace, but peak velocities and angular excursion were
preserved. This was accomplished by reducing the time over which peak angular accelerations
were endured, and increasing the time spent at peak angular velocities to match angular
excursion between the original low velocity-low acceleration trace and this fabricated low velocity-
high acceleration trace. Because higher angular velocities and accelerations tended to occur in
shaking episodes in which the surrogate neck sustained considerable damage (Table 5.2), a fourth shaking episode was chosen for simulation in which angular accelerations and velocities were relatively high, but no neck damage was noted. Three consistent cycles along the time history of the chosen shaking events were simulated, with exception of the last shaking event (high velocity, high acceleration, no neck damage) in which four cycles were simulated in order to capture two occurrences of particularly high acceleration. Finally, the sagittal plane porcine cyclic injury trace with the largest peak-to-peak angular velocities and peak angular acceleration magnitudes (subject 091105; 4C, Chapter 4) was examined in the human infant finite element model to confirm the absence of hemorrhagic injury at this load level. Peak-to-peak angular velocities (averaging 28.2±0.1 rad/s) and peak angular acceleration magnitudes (averaging 913±289 rad/s²) were scaled from the porcine brain mass in the chosen cyclic study (42.06 g) to the approximate mass of the human infant brain (500 g) using conventional mass scaling methods [30], and an idealized trace was created employing these scaled values (peak-to-peak angular velocities of 12.4±0.1 rad/s, peak angular acceleration magnitudes of 175±56 rad/s²) for simulation in the human infant finite element model. Simulated angular velocity and angular acceleration cycles with respect to their entire shaking trace are shown in Figures 5.3-5.7 (with exception of the fabricated low angular velocity – high angular acceleration trace shown in Figure 5.5, and the trace scaled from the porcine in vivo shaking study in Figure 5.7, which have no respective full shaking histories), with all surrogate shakes presented in Appendix H. Each of the selected velocity loading traces were simulated twice: once with the effective sagittal plane boundary condition stiffness, and again with the effective axial plane boundary condition stiffness.
Figure 5.3 High angular velocity – high angular acceleration surrogate shaking episode kinematics. Entire angular velocity (A) and acceleration (C) traces, and corresponding cropped angular velocities (B) and angular accelerations (D) for finite element analysis.
Figure 5.4 Low angular velocity – low angular acceleration surrogate shaking episode kinematics. Entire angular velocity (A) and acceleration (C) traces, and corresponding cropped angular velocities (B) and angular accelerations (D) for finite element analysis.
Figure 5.5 Fabricated low angular velocity – high angular acceleration surrogate shaking episode finite element model simulated kinematics. Simulated angular velocity (A) and acceleration (B) traces. Velocity limits and excursion match the low angular velocity – low angular acceleration shake shown in Figure 5.4, while the time course of accelerative portions of the curve was shortened to produce angular accelerations similar to high acceleration episodes show in Figures 5.3 and 5.6.
Figure 5.6: High angular velocity – high angular acceleration surrogate shaking episode without neck damage kinematics. Entire angular velocity (A) and acceleration (C) traces, and corresponding cropped angular velocities (B) and angular accelerations (D) for finite element analysis.
Figure 5.7 Idealized curve mass-scaled from porcine cyclic injury (091105; 4C, Chapter 4) peak angular velocity and acceleration values to human infant. Simulated angular velocity-time history (A) and angular acceleration-time history (B).

The selected cropped angular velocity-time histories were used as inputs for finite element simulation, with a center of rotation 2.8 cm below the center of the foramen magnum, estimated by linearly interpolating between reported lengths of a 24 week old and 5 month old cervical spine to 1.5 months [22], and then approximating the location of C4-C5 by assuming equal height all cervical vertebrae and multiplying by 4/7. While several studies indicate that the center of rotation of the cervical spine is much higher in young children, near C2-C3 [31, 32], we chose to place the center of rotation in our model lower in the neck so as to create a longer moment arm, corresponding to a worst-case scenario for dynamic injury. All simulations were conducted in ABAQUS Explicit version 6.11 (Simulia, Providence, RI) with double precision.

The number of failed bridging veins occurring in each simulation were extracted. Previously, we determined a threshold ($N_{crit}$) for detectable EAH ($\geq 2\%$ of porcine cerebrum blood surface coverage) to be six failed bridging vein elements in Chapter 4. We designated any human infant finite element simulations in which at least six bridging vein elements had failed as predictive of extra-axial hemorrhage.
Results

*Head Shaking Kinematics Measured from a Human Infant Anthropomorphic Surrogate*

All kinematic measurements across subject and shaking trial are presented in Table 5.2. Average shake frequency was significantly lower in the second shaking trial compared to the first shaking trial, with the second trial average frequencies approximately half those of the first trial. In contrast, average peak to peak angular velocity, and maximum peak angular acceleration magnitude were significantly higher in the second shaking trials than the first shaking trials. While average peak angular acceleration magnitude was also higher in the second shaking trials than the first, this observation was not statistically significant, though it is a considerable trend (p=0.0798). Overall, the second trial resulted in approximately 22% higher average peak to peak angular velocities, 24% higher average peak acceleration magnitudes, and 20% higher maximum peak acceleration magnitudes than the first trial. Interestingly, maximum peak to peak angular velocity did not differ between trials.

Of note, all but one of the second trial shaking episodes produced damage to the surrogate neck, and seven of the ten first trial shaking episodes also produced damage to the surrogate neck, quantified by the number of broken ventrolateral rubber bands in each trial (Table 5.2).
Table 5.2 Surrogate Shaking Kinematics and Instances of Neck Failure. Averaged values are mean ± standard deviation. Y = yes, N = no. The surrogate neck had six ventrolateral bands (three sets of two). *Indicates significantly different from Trial 1.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Trial</th>
<th>Average Frequency (Hz)</th>
<th>Average Peak Angular Velocity (rad/s)</th>
<th>Maximum Peak Angular Velocity (rad/s)</th>
<th>Average Peak Acceleration (rad/s²)</th>
<th>Maximum Peak Acceleration (rad/s²)</th>
<th>Surrogate Neck Damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>4.29±0.63</td>
<td>41.9±11.6</td>
<td>61.2</td>
<td>1040±357</td>
<td>1950</td>
<td>Y - 1 band</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>3.39±0.39</td>
<td>41.5±13.9</td>
<td>62</td>
<td>1006±477</td>
<td>2389</td>
<td>Y - 2 bands</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>3.67±0.56</td>
<td>23.2±6.1</td>
<td>45.5</td>
<td>418±244</td>
<td>1398</td>
<td>N</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>1.37±0.34</td>
<td>39.5±8.8</td>
<td>54.3</td>
<td>867±298</td>
<td>1375</td>
<td>Y - 6 bands</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>3.02±0.91</td>
<td>38.9±11.9</td>
<td>54.1</td>
<td>835±355</td>
<td>1521</td>
<td>N</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>2.81±0.59</td>
<td>42.7±9.9</td>
<td>56.5</td>
<td>1018±314</td>
<td>1729</td>
<td>Y - 2 bands</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>3.58±0.67</td>
<td>35.2±9.4</td>
<td>55.5</td>
<td>764±276</td>
<td>1363</td>
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</tr>
<tr>
<td>8</td>
<td>1</td>
<td>3.49±0.79</td>
<td>44.1±13.8</td>
<td>70.1</td>
<td>1161±499</td>
<td>2320</td>
<td>Y - 6 bands</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>3.47±0.27</td>
<td>38.4±11.6</td>
<td>75.8</td>
<td>895±410</td>
<td>1783</td>
<td>N</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>1.61±0.31</td>
<td>50.4±9.5</td>
<td>59.3</td>
<td>1436±551</td>
<td>2165</td>
<td>Y - 6 bands</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>2.23±0.96</td>
<td>51.9±8.3</td>
<td>71.4</td>
<td>1405±508</td>
<td>2718</td>
<td>Y - 3 bands</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>1.15±0.19</td>
<td>46.0±6.6</td>
<td>63.2</td>
<td>895±397</td>
<td>2002</td>
<td>N</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>1.75±0.42</td>
<td>41.9±10.5</td>
<td>56.4</td>
<td>855±418</td>
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</tr>
<tr>
<td>4</td>
<td>2</td>
<td>0.98±0.07</td>
<td>39.7±4.1</td>
<td>47.2</td>
<td>651±225</td>
<td>1191</td>
<td>Y - 2 bands</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>1.61±0.17</td>
<td>49.9±3.8</td>
<td>57.1</td>
<td>1086±327</td>
<td>1638</td>
<td>Y - 3 bands</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>1.29±0.22</td>
<td>48.5±6.0</td>
<td>55.4</td>
<td>996±423</td>
<td>1817</td>
<td>Y - 3 bands</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>1.66±0.22</td>
<td>55.5±5.9</td>
<td>61.8</td>
<td>1694±486</td>
<td>2457</td>
<td>Y - 6 bands</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>1.75±0.42</td>
<td>52.5±9.6</td>
<td>67.1</td>
<td>1273±687</td>
<td>2799</td>
<td>Y - 6 bands</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>1.59±0.11</td>
<td>51.5±7.5</td>
<td>62.9</td>
<td>1472±665</td>
<td>2902</td>
<td>Y - 6 bands</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>1.47±0.37</td>
<td>47.3±8.5</td>
<td>60.6</td>
<td>1355±608</td>
<td>2462</td>
<td>Y - 6 bands</td>
</tr>
<tr>
<td>Trial 1 Average</td>
<td></td>
<td>3.07±0.92</td>
<td>39.6±7.0</td>
<td>59.4±8.6</td>
<td>944±266</td>
<td>1799±393</td>
<td></td>
</tr>
<tr>
<td>Trial 2 Average</td>
<td></td>
<td>1.55±0.35*</td>
<td>48.5±4.9*</td>
<td>60.3±6.7</td>
<td>1168±324</td>
<td>2163±584*</td>
<td></td>
</tr>
<tr>
<td>Overall Average</td>
<td></td>
<td>2.31±1.03</td>
<td>44.0±7.5</td>
<td>59.9±7.5</td>
<td>1056±311</td>
<td>1981±519</td>
<td></td>
</tr>
</tbody>
</table>

Finite Element Model Predictions of Bridging Vein Rupture

Simulated cropped angular velocity trace kinematics and the resulting number of bridging vein element failures for each brain-skull boundary condition are presented in Table 5.3. As expected, utilizing the stiffer axial plane brain-skull boundary condition consistently produced fewer bridging vein element failures than the sagittal plane brain-skull boundary effective stiffness. In addition, increasing peak angular acceleration magnitudes caused increasing...
numbers of bridging vein element failures. In context of the threshold of bridging vein element failures associated with the presence of EAH (≥ 2% coverage over the surface of the cerebrum) in our newborn porcine model presented in Chapter 4 ($N_{crl}=6$), we would expect EAH in the human infant for the high velocity-high acceleration, fabricated low velocity-high acceleration, and high velocity-high acceleration without neck damage shakes, regardless of the choice of boundary condition. As anticipated, we would not expect hemorrhage in the newborn porcine cyclic injury trace scaled to the mass of the human infant using either boundary condition. For the low velocity-low acceleration shaking simulation, 20 bridging vein element failures occurred with the sagittal boundary condition indicative of hemorrhage, while only 3 occurred when the axial boundary condition was employed suggesting an absence of hemorrhage, and thus, prediction of EAH is uncertain for this low velocity-low acceleration shaking condition.

When bridging vein failures occur, temporally, they do so near points of peak acceleration, which occur at points of rotational direction changes in these cyclic shaking angular velocity time histories (Figure 5.8, example; all simulations shown in Appendix I), either as the motion of the brain impacts the inside of the skull just before a change in direction, or as the motion of the skull begins to lead the brain again after a direction change.
Figure 5.8 Example finite element model simulated angular velocity, angular acceleration and resulting bridging vein element failures over time
**Table 5.3** Kinematics of cropped simulated shaking traces and resulting number of bridging vein failures

<table>
<thead>
<tr>
<th>Test Description</th>
<th>Subject</th>
<th>Trial</th>
<th>Number of Cycles Simulated</th>
<th>Average Frequency (Hz)</th>
<th>Average Peak-Peak Angular Velocity (rad/s)</th>
<th>Maximum Peak-Peak Angular Velocity (rad/s)</th>
<th>Average Peak Acceleration (rad/s²)</th>
<th>Maximum Peak Acceleration (rad/s²)</th>
<th>Brain-Skull Boundary Condition</th>
<th>N_fall</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Velocity - High Acceleration</td>
<td>7</td>
<td>2</td>
<td>3</td>
<td>1.64±0.04</td>
<td>58.7±2.7</td>
<td>61.8</td>
<td>2069±417</td>
<td>2456</td>
<td>Sagittal</td>
<td>22</td>
</tr>
<tr>
<td>Low Velocity - Low Acceleration</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>4.04±1.07</td>
<td>22.6±1.5</td>
<td>24.3</td>
<td>375±94</td>
<td>481</td>
<td>Sagittal</td>
<td>20</td>
</tr>
<tr>
<td>Fabricated Low Velocity - High Acceleration</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>6.18±1.72</td>
<td>22.6±1.5</td>
<td>24.3</td>
<td>2250±559</td>
<td>2875</td>
<td>Sagittal</td>
<td>22</td>
</tr>
<tr>
<td>High Velocity - High Acceleration, No Neck Damage</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>1.33±0.06</td>
<td>40.2±22.5</td>
<td>63.2</td>
<td>1178±438</td>
<td>2002</td>
<td>Sagittal</td>
<td>20</td>
</tr>
<tr>
<td>Idealized, Mass-Scaled from Porcine Cyclic</td>
<td>N/A</td>
<td>N/A</td>
<td>3</td>
<td>2.79±0.18</td>
<td>12.4±0.1</td>
<td>12.5</td>
<td>175±56</td>
<td>220</td>
<td>Sagittal</td>
<td>5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Brain-Skull Boundary Condition</th>
<th>N_fall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sagittal</td>
<td>22</td>
</tr>
<tr>
<td>Axial</td>
<td>21</td>
</tr>
<tr>
<td>Sagittal</td>
<td>20</td>
</tr>
<tr>
<td>Axial</td>
<td>3</td>
</tr>
<tr>
<td>Sagittal</td>
<td>22</td>
</tr>
<tr>
<td>Axial</td>
<td>21</td>
</tr>
<tr>
<td>Sagittal</td>
<td>20</td>
</tr>
<tr>
<td>Axial</td>
<td>17</td>
</tr>
<tr>
<td>Sagittal</td>
<td>5</td>
</tr>
<tr>
<td>Axial</td>
<td>0</td>
</tr>
</tbody>
</table>
Discussion

In this study, we measured kinematics of the infant head during vigorous shaking with an instrumented surrogate, and simulated episodes of vigorous shaking in a finite element model of the human infant head. We found that kinematic metrics differed between shakes in which volunteers were asked to shake with maximal effort and shakes in which volunteers were instructed to shake with maximal effort while also ensuring complete flexion and extension of the neck. In addition, regardless of which instructions were given, damage to the surrogate neck was often produced during shaking. When episodes of shaking were simulated in the finite element model, high angular accelerations produced multiple bridging vein failures, above a threshold associated with extra-axial hemorrhage in a porcine finite element model validated with in vivo injury pathology. These data will deepen our mechanistic understanding of brain injuries in infants resulting from abusive trauma.

Our finding of significantly decreased average frequency when maximum range of motion was used in instrumented surrogate shakes, is likely attributable to the longer amount of time required to achieve maximum flexion and extension. The modestly higher average peak angular acceleration magnitudes and significantly higher maximum peak angular acceleration magnitudes in maximal range of motion shakes may be due to the increased effort required to produce complete flexion and extension of the flexible surrogate neck, which could not support the weight of the surrogate head. The higher average peak to peak angular velocities observed when maximal range of motion was achieved are likely a consequence of the higher angular accelerations and lower shaking frequencies (yielding a longer average duration of each shake). These findings may be confounded by the fact that the maximal range of motion shaking trials were always conducted after the shaking trials where maximal range of motion was not specified, but it is unlikely that volunteers were acclimated to shaking the surrogate and shook more efficiently after a single trial.

The average peak to peak angular velocities, average peak angular acceleration magnitudes, and average shaking frequencies observed in our study (44.0±7.5 rad/s, 1056±311 rad/s^2, 4.7±1.6 Hz) are consistent with the magnitude of injuries observed experimentally in infant brain injury models, and predicted by simulations of infant head impacts.
rad/s², and 2.31±1.03 Hz, respectively) are similar to those observed in a few anecdotal trials used to benchmark metrics for the cyclic porcine in vivo brain injuries (47.12±13.96 rad/s, 1512±1295 rad/s², and 2.19±0.49 Hz, respectively) [33], simulated in Chapter 4. The same measures are lower than the respective kinematics measured in a previous version of the surrogate employing a hinge neck (61±9.6 rad/s, 3480±2467 rad/s², and 3.08±1.00 Hz, respectively) [16, 33]. The finding of lower average peak to peak angular velocities, average peak angular acceleration magnitudes, and average shaking frequencies with the flexible neck in this study, compared to the previous hinge neck correlates well with two studies investigating hinge and flexible rubber necks [15, 26]. Indeed, our latest edition instrumented infant surrogate with a more biofidelic neck has yielded significantly different kinematic responses in simulated falls than previous versions, with decreased impact force and peak angular accelerations, lowering estimated injury risk [18]. While we cannot conclude that the observation of surrogate neck damage in the majority of all shaking trials (Table 5.2) is indicative of neck injury, recent clinical investigations of abusive head trauma are increasingly reporting the presence of neck injury in addition to the classic head injury presentations, even in the absence of other obvious external injuries [34-37]. While the surrogate neck is state of the art with properties matched to those measured in mechanical testing of the infant cervical spine, there are several shortcomings, such that the observed damage may have confounded head kinematic measurements, as discussed below in the Limitations section.

Because the properties of the brain-skull interface in the human infant are unknown, we hypothesized that they are likely stronger than those in the newborn pig, as we found that human infant bridging veins are significantly stronger than porcine in Chapter 2. Thus, we tested both the sagittal plane boundary condition and the much stiffer axial plane boundary condition determined for the newborn pig in our human infant finite element model, in an attempt to define an injury corridor. The number of bridging vein element failures in simulations employing the sagittal plane boundary condition was consistently higher than those using the axial plane boundary condition, but these differences only affected EAH prediction in the low velocity-low
acceleration surrogate shaking trace (sagittal $N_{\text{fail}}=20$, axial $N_{\text{fail}}=3$). We found that shaking episodes involving high angular accelerations produced multiple bridging vein element failures, greater than our threshold for the number of bridging vein element failures best correlating with the presence of extra-axial hemorrhage ($N_{\text{crit}}=6$) determined in the newborn pig in Chapter 4, regardless of which brain-skull boundary condition was used. Indeed our observation of bridging vein element failures associated with simulations of vigorous shaking agrees with a two previous studies that found finite element model brain-skull displacement during a representative shaking simulation was sufficient to cause bridging vein rupture [38, 39]. As expected, simulating an idealized porcine cyclic injury trace with angular velocity and acceleration mass-scaled to the human infant brain did not produce sufficient bridging vein failures for association with EAH (sagittal boundary condition $N_{\text{fail}}=5$, axial boundary condition $N_{\text{fail}}=0$), which matches the results of the porcine in vivo injury and finite element model in Chapter 4. Accurate brain-skull interface properties may improve these measurements, but the availability of cadavers for measurement of the mechanical properties of the human infant brain-skull interface and ethics surrounding such studies likely prohibit collection of these data. Regardless, taken together, the presented data indicate that, under certain circumstances, vigorous inflicted shaking without impact may produce bridging vein ruptures and associated extra-axial hemorrhage in the human infant.

**Limitations**

Although comprehensive compared to the current literature, this study presents several limitations. First, while the instrumented infant surrogate used in this study has improved neck biofidelity compared to previous versions, the mechanical properties of the neck were validated quasistatically [18], and may not be representative of human properties at higher rates. The surrogate neck also lacks a hard bony structure representative of the cervical vertebrae. Coupled together, these two idealizations may have affected shaking kinematics as the head moves back and forth rapidly, allowing motion outside of what can be expected anatomically. Future iterations...
of the infant surrogate may investigate the inclusion of a hard skeletal structure representative of
the vertebral column, or a neck with dynamic tension and bending properties, as available.

A second limitation of this study is that during surrogate shaking studies, only the
rotational motion of the head was captured, but volunteers likely also rotated the torso of the
surrogate in order to produce the back-and-forth motion of the head in the sagittal plane. As the
rotational motion of the torso was not accounted for, it may have confounded the sagittal
rotational head kinematics, and the angular velocity time histories used as inputs for finite
element model analyses may have over- or underestimated the actual velocity of the head
relative to the torso, but probably overestimated the angular excursion of the head relative to the
torso. Thus, using the global rotational motion of the head likely errs on the side of worst-case
scenario measurements, consistent with this study. Future examinations should include motion
capture of the surrogate torso to determine more precisely the relative angular motion of the head
relative to the body.

A third limitation is that no mesh convergence study was performed on the human finite
element model brain. As we specified the brain-skull boundary condition according to measured
displacements from Chapter 3, we believe any differences in finite element model bridging vein
element elongation between the current brain and a brain with optimized mesh would be minimal.
Nonetheless, as the motion of the brain plays an important role in dictating the elongation of the
bridging veins, future studies should ensure that an appropriate brain mesh resolution is used.

A fourth limitation is that the human infant finite element model does not include
geometry and elements representative of the falx. While the motion of the brain in purely sagittal
plane rotations, like those simulated in these studies, was unlikely to be affected by the absence
of a falx, this model may be improper for rotations in other directions or off-axis loading, in which
the falx may restrict the motion of one or both hemispheres. Incorporation of geometry and
elements representative of the falx and other membranous structures (e.g. tentorium) is
suggested for future simulations investigating hemorrhage and other brain injuries including
motion that is not purely sagittal.
As an important caveat, we are likely overestimating bridging vein element failures due to several assumptions and limitations. First, we assumed that the bridging veins are taut between the brain and skull, while instead there may some slack length between their brain and sinus attachment points. In addition, as explained previously, the human infant bridging vein element lengths were shorter than documented excised lengths. As the brain-skull boundary condition adapted from that measured in Chapter 3 for the newborn piglet was a displacement based metric, it is possible that an underestimation of bridging vein element lengths caused an overestimation of bridging vein failures. On the other hand, the interaction of the bridging veins with surrounding tissue in the subdural space is not well documented. It is possible that the bridging veins may snag or become entangled with surrounding tissue, namely the arachnoid trabeculae and the dura and arachnoid membranes [14], resulting in shorter gage lengths that require less relative displacement between the brain and skull to cause rupture. In addition, we did not control bridging vein orientation for angle of entry into the superior sagittal sinus (attachment to the skull in this model). In the human adult, an imaging study found that nearly all of the bridging veins entering the sinus in the posterior cluster attached at acute angles against the direction of blood flow in the sinus, while attachment angles in the anterior cluster were more variable, with about 20% attaching in the direction of blood flow in the sinus, 40% at right angles, and the remaining 40% at angles against the direction of blood flow in the sinus *cite Han*. The angle of bridging vein attachment to the superior sagittal sinus may influence occurrence of and time point at which bridging veins may fail due to the inertial motion of the brain relative to the skull. Future modeling efforts should correct for entrance angle in addition to bridging vein length for more accurate predictions of bridging vein rupture. Finally, we modeled bridging veins assuming rigid connection points at the brain and skull, and the interaction of the bridging veins with the dura and brain is unknown. We hypothesize that, especially with the soft brain tissue, the bridging veins may pull out at their attachment points, without immediately incurring mechanical loads, as the brain moves relative to the skull. This may yield an increased gage length between the brain and dura, which could in turn protect against stretch-related rupture. Regardless, our
estimations rely on assumptions of worst-case scenarios for bridging vein rupture, and the likelihood of an observation of extra-axial hemorrhage is based on the number of failed bridging veins that best predicted hemorrhage in our porcine model in Chapter 4, which included similar limitations.

Conclusions

In summary, we measured head kinematics during episodes of vigorous shaking using an instrumented 1.5 month old infant surrogate, and found that employing the entire range of motion of the neck yielded higher angular velocities and angular accelerations compared with unspecified excursions. We simulated five shaking episodes with a range of angular velocity and acceleration combinations in a finite element model of the human infant head with two brain-skull boundary conditions, and found that high angular accelerations yielded multiple bridging vein element failures, above a threshold level associated with extra-axial hemorrhage in a newborn porcine finite element model validated with in vivo injury extra-axial hemorrhage pathology in Chapter 4. Thus, we conclude, that under certain circumstances, it may be possible to produce bridging vein ruptures and subsequent extra-axial hemorrhage in the human infant from a shaking insult without impact. This data may be used to inform clinical differential diagnoses of abusive head trauma in infants.

References

1. Rorke-Adams, L.B., The triad of retinal haemorrhage, subdural haemorrhage and encephalopathy in an infant unassociated with evidence of physical injury is not the result of shaking, but is most likely to have been caused by a natural disease: No. J Prim Health Care, 2011. 3(2): p. 161-3.

2. Squier, W., The triad of retinal haemorrhage, subdural haemorrhage and encephalopathy in an infant unassociated with evidence of physical injury is not the result of shaking, but
is most likely to have been caused by a natural disease: Yes. J Prim Health Care, 2011.


CHAPTER 6: CONCLUSIONS AND FUTURE DIRECTIONS

Introduction

The overarching goal of this dissertation research was to determine whether bridging vein rupture and subsequent extra-axial hemorrhage may result from vigorous shaking without impact in the human infant. To achieve this goal, we measured the longitudinal mechanical properties of bridging veins, quantified in situ sagittal plane brain-skull displacements in the newborn pig, developed a threshold number of bridging vein element failures in a finite element model of the newborn porcine head that best correlated with extra-axial hemorrhage observed after in vivo rapid rotational head injuries, and applied this threshold to finite element model simulations of the human infant head during shaking to predict whether or not extra-axial hemorrhage may be observed following shaking insults without impact in the human infant. Based on our findings, under some conditions, it may be possible to generate a combination of sagittal head rotational kinematics that could produce extra-axial hemorrhage in the human infant. A summary of key findings, limitations, and areas for future investigation are outlined over the following topics:

Bridging Vein Tissue Mechanical Properties and Mechanical Disruption, The Brain-Skull Boundary During Rapid Head Rotations, Predictions of Bridging Vein Rupture and Subsequent Extra-Axial Hemorrhage, and Additional Areas for Future Investigation.

Bridging Vein Tissue Mechanical Properties and Mechanical Disruption

We found that longitudinal mechanical properties of bridging vein tissue did not display any age dependency, but, with the exception of elastic modulus, did differ between human and porcine species, with human tissue producing higher values than porcine. Load-related mechanical properties (σy, σu, E) are rate-dependent, with values increasing with rate, but parameters that are purely displacement-related (λy, λu) do not differ with changes in stretch rate. A history of cyclic loading prior to elongation to failure had a significant effect on bridging vein behavior, resulting in longer stress-stretch toe regions and an increase in yield stretch, 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. Finally, during cyclic loading to a common level of displacement, peak stress achieved by the bridging veins decreased exponentially with successive cycles.

We conclude that because bridging vein mechanical properties did not vary with age, any predisposition to bridging vein 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.). Based on our results, computational modeling efforts to determine the likelihood of bridging vein rupture during traumatic events should incorporate: 1) species dependencies to accurately communicate findings from animal models of traumatic brain injury, 2) rate-dependencies of load-related mechanical parameters, and 3) differences observed in bridging vein mechanical behavior when modeling repeated-loading events. Finally, because no differences were detected in bridging vein ultimate stretch with changes in stretch rate and prior loading history, we recommend the use of ultimate stretch as a bridging vein rupture criterion in computational modeling efforts.

We did not investigate changes to the bridging vein tissue structure during or after cyclic loading, but our findings of longer toe region, increased yield stretch, and elastic moduli similar to both high and low rate failure elongations in elongations to failure after a cyclic loading protocol suggest that subcatastrophic cyclic loading may have conferred mechanical damage or alterations to the bridging vein tissue structure, as collagen fibers are pulled apart from one another transverse to their axes, as individual longitudinally-oriented fibers are damaged, and/or as collagen fiber orientation progressively aligns with the direction of loading [1-3]. In addition, although similar, post-cyclic failure elongation tests were conducted at stretch rates roughly double those of the naïve low rate failure elongation group, and while these rates were still
comparatively lower than the high rate group, it is unknown whether rate-matched failure elongation tests after cyclic loading would yield mechanical property measurements different than those observed in naïve failure elongations, due to tissue structural alterations. Future longitudinal tension testing of bridging vein tissue may include rate-matched failure elongation tests with and without prior cyclic loading, observation of collagen fiber orientation with successive cycling and comparative histological investigations of the unloaded, cyclically loaded, and failure loaded vessel wall to determine ultrastructural changes to bridging vein tissue caused by repeated loading.

We also did not fully examine whether bridging veins exhibit viscoelastic behavior. Studies of multiple bridging vein loading protocols with prescribed periods of relaxation [4], and load or stress-controlled studies to evaluate creep response are required to assess the presence of viscoelastic recovery and mechanical fatigue. A confounding factor of these proposed ex vivo studies is the inability to measure the biomechanical influence of potential in vivo biological remodeling between bridging vein elongations. Nonetheless, together with the previously recommended rate-matched naïve and post-cyclic failure elongation studies and ultrastructural investigations with various loading protocols, these tests will give a complete profile of bridging vein fatigue behavior.

The Brain-Skull Boundary During Rapid Head Rotations

Measurements of in situ sagittal plane brain-skull displacements of the neonatal pig head, transected at the sagittal midline, were significantly lower than loading condition-matched brain-skull displacements in axial plane transections, indicating that the response of the brain-skull boundary is dependent on the direction of rotation and/or region of the brain. However, a brain-skull boundary condition prescribed to match sagittal plane displacement measurements in a finite element model of the neonatal porcine head was much less stiff than its axial plane counterpart, and as such, the observed differences in brain-skull boundary response may be attributable to the irregular shape of the sagittal plane head cross-section compared to the
relatively symmetric axial plane, regional dependencies within each cross-section, or direction dependent properties of other structures in the pia-arachnoid complex (PAC). Furthermore, it is unlikely that bridging veins dominate the mechanical tethering between the brain and skull in the sagittal plane, as varying bridging vein properties according to observed rate dependencies did not affect finite element model brain-skull displacements. Finally, a second rotation of the sagittally transected newborn porcine head yielded significantly larger brain-skull displacements than those observed in the initial rotation, but this difference was below our measurement error.

Based on our findings, we encourage the use of appropriate brain-skull boundary conditions in finite element models of the head, dependent on the direction of simulated rotation or brain region. To improve upon brain-skull boundary conditions prescribed in finite element models of the head, the use of macroscale elements representative of regional and inter-individual variability observed in the structure of the PAC [5, 6] and employing measured PAC material properties [7-9] should be compared with observations of direction-dependent brain-skull displacements during rapid head rotations. Additional material property investigations of the PAC are also required to determine whether the PAC is isotropic in transverse shear.

Our observation of significantly higher brain-skull displacements in a second rotation of the transected porcine newborn head, may indicate alterations or damage to the brain-skull boundary condition had occurred as a result of the first rotation. However, this observation holds little value as the difference between the brain-skull displacements observed in the first and second rotations was smaller than our measurement error. To determine if multiple rapid rotations alter the brain-skull boundary such that brain-skull displacements progressively increase with multiple rotational loadings above measurement error, future studies may examine more than two consecutive rotations of physical transections at the same load level. Findings from these proposed studies would provide valuable information for modeling the brain-skull boundary condition in incidences of shaking or repeated insults.

It is very important to note that in our investigation brain-skull displacements were measured in sagittal plane rotations with peak angular velocities of approximately 52 rad/s. It is
unknown whether the brain-skull displacements and prescribed finite element boundary condition would be similar at higher loading levels. As material properties of the PAC have been characterized as rate-dependent [7-9], future physical transection studies at different levels of peak angular velocity and acceleration are critical to fully characterizing the brain-skull boundary. In addition, the density of PAC structural elements has been shown to vary across the surface of the brain in the neonatal pig [5], and our sagittal plane transections suggest that the brain-skull displacement may vary around the periphery of the brain in this plane as well. Future higher resolution physical transection studies may attempt to determine regional variation in brain-skull displacements to more accurately define computational model boundary conditions.

In summary, while we have determined that brain-skull displacements during rapid rotations of the head are dependent on the direction of rotation and this finding necessitates that finite element model investigations use appropriate representations of the brain-skull boundary dependent on simulated rotational direction or region of the brain, substantial additional work is required to fully characterize the brain-skull boundary, and results may have significant effects on computational model predictions of traumatic brain injury.

Predictions of Bridging Vein Rupture and Subsequent Extra-Axial Hemorrhage

Using in vivo injury pathology from newborn porcine models of single and cyclic rapid rotational traumatic brain injury, we developed and independently validated a threshold for the number of failed bridging vein elements in a finite element model of the neonatal porcine head associated with the presence of extra-axial hemorrhage. We determined a critical value of six bridging vein element failures is associated with 100% sensitivity and specificity for detection of EAH in our newborn porcine rapid nonimpact rotation TBI model, but applying this criterion to a newborn porcine cyclic rotational injury model only yielded 90% accuracy. From these porcine simulations, we also observed a trend of increasing angular accelerations producing larger numbers of bridging vein element failures. We conclude that finite element models should include
biofidelic representations of the parasagittal bridging veins to better predict the occurrence of extra-axial hemorrhage.

We also measured kinematics of the infant head during vigorous shaking using an instrumented human infant surrogate, finding that shakes employing the entire range of motion of the surrogate neck resulted in higher angular velocities and accelerations than shaking with maximal effort without specifying range of motion instructions. We simulated a range of angular velocity and acceleration combinations produced by the surrogate shaking tests in a finite element model of the human infant head and found that shakes with high angular accelerations produced multiple bridging vein element failures, above our threshold of six corresponding to the presence of extra-axial hemorrhage in the neonatal pig. Although no bridging vein element failures were predicted in our porcine model under cyclic head rotations, this may be expected, as conventional mass scaling approaches [10] dictate that for the maximum peak acceleration magnitudes observed in the simulated human infant shaking episodes (2,002-2,875 rad/s^2, Chapter 5, Table 5.3) applied to the ~500g human infant brain, equivalent cyclic rotations experienced by the ~40g newborn porcine brain would include cycles with peak acceleration magnitudes of 10,781-15,482 rad/s^2, levels well above those applied in the in vivo cyclic piglet rotational injury studies (Chapter 4, Table 4.6). As such, we simulated an idealized shaking velocity history of a porcine cyclic injury trace with both peak angular velocities and accelerations scaled to the mass of the human infant brain and confirmed a lack of predicted extra-axial hemorrhage. Taken together these data indicate that, under certain circumstances, vigorous inflicted shaking without impact may produce bridging vein ruptures and associated extra-axial hemorrhage in the human infant.

In the porcine newborn model of traumatic brain injury used to develop a finite element model threshold for the number of failed bridging vein elements associated with pathology observations of extra-axial hemorrhage, there appear to be kinematic thresholds of angular velocity and angular acceleration that result in the failure of bridging vein elements and measurable extra-axial hemorrhage. However, the point at which finite element model bridging
vein element failure and in vivo injury extra-axial hemorrhage begin to occur was not represented by our collection of retrospective studies, and in turn, we cannot say whether detectable extra-axial hemorrhage may be associated with an amount of bridging vein failures between one and five in the presented finite element model. Future in vivo studies of newborn porcine rapid head rotations in the sagittal plane may be conducted in this realm, with peak angular velocities between 50 and 140 rad/s, and more importantly, peak angular acceleration magnitudes between 15,000 and 30,000 rad/s² (as calculated with CFC 200 filtering of velocity), to determine a more definitive threshold level for extra-axial hemorrhage.

Both our neonatal porcine and human infant finite element models may have over-predicted the occurrence of bridging vein element failures. Because the finite element model brain-skull boundary conditions were matched to in situ brain-skull displacements and finite element model bridging vein element initial lengths were shorter than those used in mechanical property testing, it is possible that longer bridging vein elements subjected to the same displacements as simulated in these models would not reach their critical failure stretch. Future studies should examine average in vivo lengths of bridging veins and incorporate these in computational analyses.

Similarly, the interface between the bridging veins and tissue they attach to (dura and brain) is unknown. Specifically, it may be that the bridging veins pull out of the surrounding tissue at their attachment points, especially in the case of the soft brain, without incurring mechanical loads and effectively increasing the gage length of the bridging veins between the brain and skull. This possible increased gage length may protect against stretch-related rupture. Future animal investigations may attempt to determine the possibility of relative movement between the bridging veins and the brain post-mortem by cutting a superior rectangular section of the skull spanning the midline, and video recording the movement of the bridging veins as the cut skull section is pulled away from the brain.

Our measurements of angular velocities and accelerations experienced by the head of a 1.5 month old human infant surrogate during shaking may be confounded by the properties and
assembly of the surrogate neck, which were validated with quasistatic infant cervical spine properties, and lacks a hard structure representative of the cervical vertebrae, respectively. In addition, the motion of the surrogate torso was not recorded during surrogate shaking studies, and as such, the relative motion between the head and the body of the surrogate is unknown as is how this omission affects the measured head kinematics. Future iterations of the infant surrogate may include a hard skeletal structure representative of the vertebral column and/or a neck with dynamic tension and bending properties as available, and kinematic examinations should include motion capture of the surrogate torso to determine more precisely the relative angular motion of the head relative to the body.

In our human infant finite element model, no mesh convergence study was performed on the brain. As the motion of the brain plays an important role in dictating the elongation of the bridging veins, future finite element modeling efforts should ensure that an appropriate brain mesh is employed. The human infant finite element model also does not include geometry and elements representative of the falx. Although the superior attachment point of the bridging vein elements would have ideally been on an inner falx surface, because we employed purely sagittal plane motion in our shaking simulations, the influence of the falx on the motion of the brain relative to the skull is likely minimal. However, this model is not a good choice for any non-sagittal or off-axis motions, in which the falx may restrict brain motion. Future computational modeling efforts should include appropriate falx geometry and elements to more accurately describe the brain-skull interface, especially when estimating or predicting the incidence of hemorrhage or other peripheral brain injuries.

While there is certainly room for improvement in our predictions of bridging vein rupture leading to extra-axial hemorrhage following rapid sagittal plane head rotations, our use of measured pediatric bridging vein mechanical properties, and correlation of finite element model predictions of bridging vein failure with in vivo animal injury pathology are novel. Accordingly, a final step in validating finite element model predictions of bridging vein failure in the human infant should include simulations of well-witnessed cases of traumatic brain injury in the human infant
(e.g. accidental falls) and comparison of any resulting bridging vein element failures with corresponding case radiography. Taking this idea one step further, stronger validation this model may include correlations of finite element bridging vein failures with observations of torn bridging veins at autopsy in well-witnessed cases of trauma.

**Additional Areas for Future Investigation**

While we suggest several avenues for future studies closely related to the studies in this dissertation throughout this final chapter, an additional two questions also come to mind. First, measured extra-axial hemorrhage and subdural hemorrhage before immersion in formalin observed in porcine in vivo injuries presented in Chapter 4 is often diffuse and quite severe. It may be that increased pressure associated with this severe hemorrhage causes the hypoxic-ischemic injury, and the excess of blood may track from the brain down the optic nerve to the eye, resulting in the appearance of retinal hemorrhage. As hypoxic-ischemic injury and retinal hemorrhages in addition to subdural and subarachnoid hemorrhage constitute the “triad” of presenting injuries common to abusive head trauma, future physical and computational fluidic modeling in animals may provide insight into the hypothesis of hemorrhage traveling from the brain to the eye.

Second, during cyclic shaking type insults, frequency may have a significant effect on tissue deformation regardless of other kinematic measures. It is likely that the complex shape of the head causes the brain and other intracranial tissue to have multiple natural, or resonant, frequencies. Dependent on the shake frequency and natural tissue frequencies, constructive interference may amplify tissue deformations, or conversely destructive interference may diminish tissue deformations. Specifically, if the frequency of the shaking was near a resonant frequency, or harmonic, composite tissue deformations may be amplified beyond what is expected based on velocities and accelerations alone. Variation of shake frequency in cyclic porcine (or other animal) in vivo studies may produce different injuries or injury severity, regardless of velocity or acceleration levels. Similar parametric alteration of shake frequency in computational models of
the head may provide insight into the effect of harmonic amplification in brain and other soft tissue deformations during shaking. In any case, the complex kinematics of shaking type insults should be more thoroughly investigated to produce better understanding of potential injury modalities.

Conclusion

For many years, whether or not traumatic brain injury may result from vigorous shaking without impact in the infant has been a topic of heated scientific and legal debate. The detailed and comprehensive biomechanical studies presented in this dissertation suggest that high level rotational acceleration events predispose bridging vein ruptures, and under certain circumstances, it may be possible to cause bridging vein ruptures and subsequent extra-axial hemorrhage in the human infant from vigorous inflicted shaking insults without impact. This research should be considered in clinical differential diagnosis strategy for abusive head trauma in infants, and may also inform educational public health initiatives and legal judgements associated with improving the welfare of young children.

References


APPENDIX A: EFFECT OF CRYOPRESERVATION CONDITIONS ON ELASTIC MODULUS OF IMMATURE PORCINE SAPHENOUS VEINS

Presented at the Biomedical Engineering Society Annual Meeting, September 2013, Seattle, WA (Poster)

Introduction

Motivation

Because autopsies may be performed up to 72 hours post mortem, the effect of the time delay between death and human tissue acquisition on the vessel mechanical properties became a concern. In fact, a prior study suggests that using cadaveric arterial tissue for mechanical testing presents limitations due to this time delay as samples refrigerated for 24 of 48 hours before mechanical testing displayed decreased elastic moduli compared to fresh and frozen samples [1].

Objective

In a series of pilot studies to demonstrate feasibility and inform our studies of pediatric porcine parasagittal bridging veins, we sought to optimize sample harvest, preservation, and testing techniques by determining the effects of freeze/thaw rate, cryopreservation solution, strain rate, and time delay before cryopreservation (to simulate post-mortem time before autopsy) on the axial elastic modulus of pediatric (4 week old) porcine saphenous veins.
Experimental Design

Table A.1 shows a summary of the studies conducted.

Table A.1 Summary of pediatric porcine saphenous vein cryopreservation studies.

<table>
<thead>
<tr>
<th>Cryo-Method</th>
<th>Cryo-Solution</th>
<th>Strain Rate</th>
<th>Delay</th>
<th># Pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td>-20°C</td>
<td>Physiologic Saline</td>
<td>LOW</td>
<td>0 hrs</td>
<td>N=5</td>
</tr>
<tr>
<td>Gradual, -20°C→-80°C</td>
<td>10% DMSO-90% DMEM</td>
<td>HIGH</td>
<td>24 hrs</td>
<td>N=5</td>
</tr>
<tr>
<td>Rapid, -80°C</td>
<td>10% DMSO-90% DMEM</td>
<td>LOW</td>
<td>72 hrs</td>
<td>N=5</td>
</tr>
<tr>
<td></td>
<td>10% DMSO-90% DMEM</td>
<td>HIGH</td>
<td>Fresh</td>
<td>N=5</td>
</tr>
</tbody>
</table>

Realizing the loss of paired analysis and that in some cases more than one variable was changed between sets of experiments, some groups were combined after initial analyses showed no significant effect of a specific variable on the elastic modulus of saphenous veins. This aided in better answering more questions than originally intended by the experimental design.

**Effect of Freeze/Thaw Rate**

To investigate the effect of freeze and thaw rate on vessel elastic modulus, saphenous veins were harvested from N=5 piglets and cut into 4 pieces and frozen immediately (0-hour delay) in a 10% dimethylsulfoxide (DMSO) - 90% Dulbecco’s Modified Eagle Media (DMEM) cryopreserving solution. Two segments from each pig were frozen and thawed rapidly, while the two others were frozen and thawed gradually. One vessel from each freeze/thaw rate group was pulled to failure at a LOW rate, while the other was pulled to failure at a HIGH rate. Data was analyzed using a two-way repeated measures ANOVA with significance defined as p<0.05 to determine freeze/thaw method and strain rate effects and their interactions.

**Effect of Cryopreservation Solution**

Immediately frozen (0-hour delay) samples were pooled across experiments (frozen in physiologic saline at -20°C, frozen in 10% DMSO - 90% DMEM gradually and rapidly) and compared using a two-way ANOVA, with significance defined as p<0.05, to determine cryopreservation solution (physiologic saline or 10% DMSO - 90% DMEM) and strain rate (LOW or HIGH) effects on elastic modulus and their interactions.
Effect of Strain Rate

To determine if the elastic modulus of veins was altered by strain rate, saphenous veins that were frozen immediately (0-hour delay) were again pooled across experiments. Those pulled at LOW strain rates were compared to those pulled at HIGH strain rates using an unpaired t-test, with significance defined as p<0.05.

Effect of Post-Mortem Delay

Saphenous veins from N=5 piglets were cut into four samples and frozen to -20°C in physiologic saline, two of which were frozen immediately (0-hour delay), one of which was frozen after a 24-hour refrigerated delay, and the fourth of which was frozen after a 72-hour refrigerated delay. One of the immediately frozen samples was pulled to failure at a HIGH strain rate, while all other samples (the other 0-hour delay, 24-hour delay, and 72-hour delay) were pulled to failure at a LOW strain rate.

Saphenous veins from an additional two groups of N=5 pigs were each cut into three samples. In each of these groups of N=5 pigs, one vein segment per pig was tested fresh, without freezing, and another was immediately frozen. In N=5 of these pigs, the remaining segment was frozen with a 6-hour refrigeration delay, and in the other N=5 pigs, the remaining segment was frozen with an 18-hour refrigeration delay. For these groups all vessels were frozen in 10% DMSO - 90% DMEM using the rapid freeze/thaw method.

To examine the effect of delay to specimen acquisition caused by post-mortem time before autopsy, saphenous vein specimens frozen at different delay time points after harvest: 0 hours, 6 hours, 18 hours, 24 hours, and 72 hours were compared to each other as well as specimens tested freshly (without freezing) using a one-way ANOVA with significance defined as p<0.05.
Methods

Cryopreservation

For experiments in which vessel segments were frozen in physiologic saline, specimens were simply placed in 0.5ml Eppendorf tubes filled with physiologic saline and frozen in a -20°C freezer. Before testing, these specimens were removed from the -20°C freezer and allowed to thaw at room temperature.

Saphenous vein segments frozen in the 10% DMSO - 90% DMEM cryoprotective solution were placed in increasing concentrations of DMSO in DMEM (2.5%, 5%, and 7.5%, v/v) for ten minutes each and allowed to equilibrate at the final concentration (10% v/v) for twenty minutes before freezing. Specimens were placed in 0.5ml Eppendorf tubes filled with 10% DMSO - 90% DMEM solution and then those in the gradual freeze/thaw group were frozen in a -20°C freezer for 1 hour before storage in a -80°C freezer, while specimens in the rapid freeze/thaw group were frozen by direct placement in a -80°C freezer. Prior to testing, specimens in the gradual freeze/thaw group were thawed in a -20°C freezer for 1 hour and removed and thawed to room temperature. Those in the rapid freeze/thaw group were thawed to room temperature by removal from the -80°C freezer. Specimens were rinsed of DMSO by placing them in decreasing concentrations of DMSO in DMEM (7.5%, 5%, 2.5%, and 0%, v/v) for ten minutes each and then placed in fresh DMEM (0% DMSO) before testing. This cryopreservation procedure and slight variations are widely practiced [2-8].

Post-Mortem Delay

Samples not in the fresh (never frozen) or immediate freeze (0-hour delay) groups were refrigerated during their respective delay times. For delay samples frozen in 10% DMSO - 90% DMEM solution, DMSO concentration began after refrigeration and before freezing.
Mechanical Testing

After rinsing specimens of DMSO, vessels were cleaned of any remaining exterior fascia and fixed to two pieces of fine grit sandpaper with a small amount of cyanoacrylate on each end. The ends were then gripped in flat-plate style grips and loaded into the testing apparatus. A custom-designed drop test apparatus, previously used to test high rate material properties of infant suture and skull [9] was used in this study, different from the apparatus used in the bridging vein tests in the bridging vein mechanical tests in Chapter 2. The drop test apparatus consists of a metal plate that slides down three shafts and hits a lever arm. The top specimen grip is attached to the opposite end of the lever arm than that which is struck by the plate. To ensure a fully linear extension, the top specimen grip is attached to the lever arm with a universal joint. The bottom grip is attached to a load cell which sits on the lever arm platform. A laser displacement sensor measures the global elongation of the vessel by tracking the height of the lever arm near where the top grip is attached, calibrated to account for the non-linearity of this motion. Strain rates in the LOW strain rate group were 85.87±3.1 s\(^{-1}\) and strain rates in the HIGH strain rate group were 196.98±8.55 s\(^{-1}\) (mean ± SEM). Elastic modulus was calculated from strains of 0.2 to 0.6 or vessel failure, whichever occurred first.
Results

For all plots, N is the number of specimens, not the number of pigs. All plots show mean ± SEM.

**Figure A.1** Effect of Freeze/Thaw Rate on Pediatric Porcine Saphenous Vein Elastic Modulus

*Effect of Freeze/Thaw Rate*

Freeze/thaw rate did not affect elastic modulus (p=0.9805, Figure A.1) and no significant interaction was found between freeze/thaw rate and strain rate (p=0.5299).
Because freeze/thaw rate and cryopreservation solution did not significantly affect elastic modulus, the effect of strain rate (high: 196.98±8.55 s$^{-1}$, or low: 85.87±3.1 s$^{-1}$, mean ± SEM) was assessed pooling freeze/thaw rates and cryopreservation solutions. Strain rate did not significantly affect elastic modulus (p=0.8159, Figure A.2).

**Figure A.2** Effect of Strain Rate on Pediatric Porcine Saphenous Vein Elastic Modulus
Because freeze/thaw rate did not significantly affect elastic modulus, the effect of cryopreservation solution was analyzed, pooling freeze/thaw rates. Choice of cryopreservation solution had no significant effect on elastic modulus. (p=0.7665, Figure A.3), and there was no significant interaction between cryopreservation solution and strain rate (p=0.1591, Figure A.3).
Post-mortem delay did not significantly affect elastic modulus ($p=0.0935$, Figure A.4), but was trending to decreased modulus with longer chilled storage prior to freezing. Small sample sizes may limit our statistical power. Regardless we conclude that longer storage times should be avoided.

Conclusions

Freeze/thaw rate, cryopreservation solution, and strain rate do not affect the elastic modulus of pediatric porcine saphenous veins. Post-mortem delays before cryopreservation of up to 72 hours reduced elastic modulus, but did not reach significance. Regardless, we would advise against utilizing cadaveric venous tissue obtained more than 24 hours post-mortem.

Acknowledgements

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References


APPENDIX B: BRIDGING VEIN MECHANICAL TEST CURVES

B.1 Porcine Newborn Bridging Vein Cyclic Loading Peak Stress Decay and Stress-Stretch Curves

Porcine Newborn Subject 1

Porcine Newborn Subject 2
B.2 Porcine Adult Bridging Vein Cyclic Loading Peak Stress Decay and Stress-Stretch Curves

Porcine Adult Subject 1

Porcine Adult Subject 2
Porcine Adult Subject 9

Porcine Adult Subject 10
B.3 Human Infant Bridging Vein Cyclic Loading Peak Stress Decay and Stress-Stretch Curves

Human Infant Subject 1

Human Infant Subject 2
Human Infant Subject 3

Human Infant Subject 4
B.4 Porcine Newborn Bridging Vein Failure Elongation Stress-Stretch Curves

Porcine Newborn Subject 1

Porcine Newborn Subject 2
Porcine Newborn Subject 9

Stress (MPa) vs. Stretch

- Low Rate
- High Rate
- Post-Cyclic
B.5 Porcine Adult Bridging Vein Failure Elongation Stress-Stretch Curves

Porcine Adult Subject 1

Porcine Adult Subject 2
Porcine Adult Subject 9

Stress (MPa) vs. Stretch

- Low Rate
- High Rate
- Post-Cyclic

Porcine Adult Subject 10

Stress (MPa) vs. Stretch

- Low Rate
- High Rate
- Post-Cyclic
Porcine Adult Subject 11

Porcine Adult Subject 12
B.6 Human Infant Bridging Vein Failure Elongation Stress-Stretch Curves

Human Infant Subject 1

Human Infant Subject 2
APPENDIX C: REPEATED LOADING BEHAVIOR OF PORCINE COMMON CAROTID ARTERIES

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Repeated Loading Behavior of Pediatric Porcine Common Carotid Arteries

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Abstract

Rapid flexion and extension of the neck may occur during scenarios associated with traumatic brain injury (TBI), and understanding the mechanical response of the common carotid artery (CCA) to longitudinal stretch may enhance understanding of contributing factors that may influence CCA vasospasm and exacerbate ischemic injury associated with TBI. Immature (4 week old) porcine CCAs were tested under subcatastrophic (1.5 peak stretch ratio) cyclic loading at 3Hz for 30s. Under subcatastrophic cyclic longitudinal extension, the immature porcine CCA displays softening behavior. This softening can be represented by decreasing peak stress and increasing corner stretch values with an increasing number of loading cycles. This investigation is an important first step in the exploration of fatigue-like behavior in arterial tissue that may be subjected to repeated longitudinal loads.

Keywords: vessel, stiffness, mechanical properties, vascular, longitudinal, whiplash
Introduction

With over 250,000 emergency department visits, 15,000 hospitalizations, and nearly 1,000 deaths annually in the U.S. [1], traumatic brain injury (TBI) in children aged 0-4 years is a major public health concern. Some TBIs are caused by rapid rotations of the head about the neck (whiplash, cyclic shaking-type injuries), and there is evidence that mechanical stretch of the cerebral arteries is a trigger for vasoconstrictive responses [2-4]. Most studies have focused on intracranial vasculature vasospasm in adult TBI patients, with a paucity examining vasospasm in pediatric TBI [5]. Recently, we reported that the CCA displays prolonged constriction after a single rapid sagittal head rotation in the immature pig [6]. It is possible that this CCA constriction and reduction in blood flow may mediate decreased global cerebral blood flow [7], decreased brain tissue oxygenation [8], and increased lactate/pyruvate ratio [8] observed following similar injuries in this animal model.

Our goal was to elucidate the longitudinal mechanical behavior of the immature porcine CCA during nondestructive cyclic extension. Mechanical properties of carotid arteries are known to vary with age [9-14], and previous studies typically compare adult and advanced age. Most studies report the pressure-diameter and circumferential compliance relationship of the adult CCA [15-17]. Few have measured longitudinal mechanical properties [18-24], and only three have evaluated adult behavior during cyclic loading [25-27].

Methods

Specimen Preparation

All euthanasia and procurement procedures were approved by the University of Pennsylvania Institutional Animal Care and Use Committee. Right CCAs were harvested from 4-week old female farm pigs, chosen to match subjects used in previous rapid non-impact rotation studies [6, 8], immediately after sacrifice (n=8). Following removal of exterior fascia, each specimen was cut perpendicular to the longitudinal axis into three sections of near equal length, and placed in serial dilutions of 2.5, 5, and 7.5% dimethyl sulfoxide (DMSO) in Dulbecco’s
Modified Eagle Media (DMEM) for ten minutes each, followed by a final concentration of 10% DMSO-90% DMEM for 20 minutes prior to freezing in Eppendorf tubes at -80°C. The location of each sample along the carotid axis (proximal, middle, and distal) was not recorded so that no systematic bias was introduced. Previous studies have shown that cryopreservation does not significantly alter the material properties of blood vessels [28-35].

On the day of testing, samples were removed from -80°C storage, thawed at room temperature, and rinsed of DMSO by placement in serial dilutions of 7.5, 5, 2.5, and 0% DMSO in DMEM for ten minutes each. After rinsing, specimens were placed in fresh DMEM prior to testing. Cross-sectional area varied along specimen length. To determine average specimen cross-sectional area, the entire specimen was placed between two glass slides and the thickness and width of the specimen was measured in triplicate using calipers to find outer circumference (C, C=2πr_0) and wall thickness (h, h=r_0−r_i). Artery cross-sectional area (A) was approximated as that of a hollow cylinder:

\[ A = \pi (r_0^2 - r_i^2) \quad [1] \]

where \( r_o \) is the outer radius and \( r_i \) is the inner radius.
Test Apparatus

Figure C.1 Vessel testing device including (A) lab jack, (B) load cell, (C) saline inlet, (D) pressure sensors, (E) 14G blunt-tipped needle grips, (F) gear-chain system, (G) rotary motor, (H) threaded shafts, (I) translating plate, and (J) laser displacement sensor.

In a setup similar to previous investigations [36, 37], CCA test specimens were cannulated with 14G blunt-tipped needles and secured via silk suture (6-0) tied around the vessel circumference at the level of a groove machined in the needle. A small amount of cyanoacrylate was applied at both ends of the specimen distal to the suture fixation to secure the vessel on the needles. Each of the blunt tipped needles was fastened to a Delrin block via a luer connection with a rotating lock ring to prevent specimen twisting. The blocks had channels machined through them to allow for fluid pressurization of the vessels and pressure sensor attachment (26PCBFM6G, Honeywell, Columbus, OH). Physiologic saline was inserted through a syringe into the channel in the first block, needle, vessel, opposite needle, and the second block, where the channel ended.
A custom device was constructed to pull vessels cyclically (Figure C.1). Briefly, a computer-controlled closed loop stepper motor (Vexta #AS46AAP, Oriental motor, Braintree, MA) coupled to a gear-chain system was used to rotate threaded shafts to move a plate up and down at a specified frequency. The bottom grip was attached to the moving plate, while the top grip was attached to a 1000g capacity load cell (Model 31, Honeywell-Sensotec, Columbus, OH). The load cell was fastened to a lab jack mounted on the ceiling of a cage surrounding the entire device, which allowed for gage length adjustment of the specimen. A laser displacement sensor (LC 1607-50, Micro-Epsilon, Ortenburg, Germany) measured the global elongation of the vessel by tracking the position of the translating plate. The lab jack was adjusted until the artery specimen was taut and the load cell just began to register a load. The specimen gage length was measured from suture tie to suture tie with calipers, as the in vivo length was not recorded at excision.

Test Protocol

Specimens were pressurized with physiologic saline to a target physiological pressure, ~100 mmHg [38-40], a stopcock on the saline inlet was closed, and stretch tests were conducted at peak stretch ratios of 1.5 and a cycling frequency of 3Hz (yielding average stretch rates of 3s⁻¹) for 30 seconds (~90 cycles). For real-world scenarios with sustained cyclic loads we used previous measurements of vigorous shaking (3Hz, 30 seconds) seen in child abuse [41, 42]. The cycling stretch limit was chosen as a comparative benchmark for future studies where, at extremes, vessels may reach stretch ratios of 1.5. Load and displacement values (sampled at 1 kHz) were recorded on a computer.

Data Analysis

Recorded load and displacement signals were low-pass filtered (second order Butterworth) at 35 Hz and 20 Hz, respectively. Stretch ratio and First Piola-Kirchhoff stress were then calculated from the displacement and load traces for each cycle from displacement minimum to load maximum, as follows:
\[
\lambda(t) = \frac{l(t)}{l_0} \quad [2]
\]

\[
\sigma(t) = \frac{F(t)}{A} \quad [3]
\]

where \( \lambda \) is stretch ratio, \( t \) is time, \( l \) is the instantaneous length of the specimen, \( l_0 \) is the gage length, \( \sigma \) is stress, \( F \) is instantaneous load, and \( A \) is cross-sectional area as defined in Eq. 1.

Stretch rate was defined as the slope of the stretch-time relationship over the loading portion of each cycle.

The stress-stretch behavior was a nonlinear and biphasic, with stiffer behavior at higher stretch magnitudes (Figure C.2). We calculated modulus in the toe (low stretch) and linear (high stretch) regions as well as the approximate corner stretch where the transition to stiffer behavior occurred (Figure C.2, inset). The toe region was defined in two steps. First, a linear regression was calculated between 20-40% of the length of the loading portion of each cycle, omitting the unloading segment (Figure C.2). Next, that regression was applied to the entire loading portion of the cycle, and the magnitude of the residuals (or absolute value of the difference) between the measured stress values and the linear fit were extracted. The toe region was defined as the segment where residual magnitudes were not continuously above 0.05 MPa. Finally, the modulus of the toe region, \( E_{\text{toe}} \), was defined as the slope of the stress-stretch relationship in the toe region.

A similar two-step process was used to find the linear region for a given cycle, except this time a linear regression was taken between 75% of the average peak stress of the last ten cycles and the peak stress of the cycle being analyzed. After evaluating residual magnitudes over the entire loading portion of the cycle, the slope of a second linear regression of linear region stress-stretch was defined as the linear region modulus, \( E_{\text{linear}} \). The corner stretch, \( \lambda_{\text{corner}} \), was defined as the stretch at which the toe region line of best fit and the linear region line of best fit were equal. Peak stresses (\( \sigma_{\text{peak}} \)) were also extracted for each cycle. Hysteresis was defined by the area encompassed by each cycle's loading and unloading curves. Because in some experiments (4 out of 8) we observed yield-like behavior during the first loading cycle occurring before reaching a
stretch ratio of 1.5, we report $E_{\text{toe}}$, $E_{\text{linear}}$, $\lambda_{\text{corner}}$, and $\sigma_{\text{peak}}$ for the second cycle through the last cycle (88 or 89).

A series of one-way ANOVAs were performed to examine differences between the second cycle ($E_{\text{toe},2}$, $E_{\text{linear},2}$, $\lambda_{\text{corner},2}$, $\sigma_{\text{peak},2}$), sixth cycle ($E_{\text{toe},6}$, $E_{\text{linear},6}$, $\lambda_{\text{corner},6}$, $\sigma_{\text{peak},6}$; after preconditioning), and corresponding fatigued parameters, defined as the average value of the last ten cycles ($E_{\text{toe},\infty}$, $E_{\text{linear},\infty}$, $\lambda_{\text{corner},\infty}$, $\sigma_{\text{peak},\infty}$). For all tests, significance was defined as $p \leq 0.05$. If significance was achieved, post hoc Tukey-Kramer analysis was performed to determine group-wise differences.

Results

Figure C.2 Representative plot of cyclic stress vs stretch ratio and a single cycle loading portion (inset) defining the toe and linear regions. Peak stress decreases and corner stretch increases with successive cycles. A large amount of hysteresis is observed in the first cycle compared with subsequent cycles.

Peak stretch magnitude and cycle frequency were maintained for the duration of the cyclic tests. With successive cycles, peak stress and hysteresis decreased and corner stretch increased, moving rightward (Figure C.2). Typically, when testing the mechanical properties of soft tissues a few (~5) preconditioning cycles are performed at experimental or sub-experimental
load levels to obtain repeatable results. We observe continued changes in peak stress and corner stretch beyond the typical preconditioning realm.

We find that $E_{\text{toe},2}$ is significantly higher than $E_{\text{toe},6}$ and $E_{\text{toe},\infty}$, while $\sigma_{\text{peak},2}$ is significantly higher than $\sigma_{\text{peak},6}$, which in turn is significantly higher $\sigma_{\text{peak},\infty}$. In addition, $\lambda_{\text{corner},2}$ and $\lambda_{\text{corner},6}$ are significantly less than $\lambda_{\text{corner},\infty}$. On the other hand, no difference was observed between $E_{\text{linear},2}$, $E_{\text{linear},6}$, and $E_{\text{linear},\infty}$ (Table C.1, means and standard deviations). Taken together, these data support evidence of classic fatigue behavior in the stress-stretch curve, with softening sustained past the traditional preconditioning regime.

<table>
<thead>
<tr>
<th></th>
<th>$E_{\text{toe}}$ (MPa)</th>
<th>$E_{\text{linear}}$ (MPa)</th>
<th>$\lambda_{\text{corner}}$</th>
<th>$\sigma_{\text{peak}}$ (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Second Cycle (2)</td>
<td>0.069±0.025 [A]</td>
<td>1.72±0.96 [A]</td>
<td>1.37±0.04 [A]</td>
<td>0.21±0.07 [A]</td>
</tr>
<tr>
<td>Sixth Cycle (6)</td>
<td>0.037±0.022 [B]</td>
<td>1.68±0.92 [A]</td>
<td>1.38±0.04 [A]</td>
<td>0.17±0.06 [B]</td>
</tr>
<tr>
<td>Fatigued ($\infty$)</td>
<td>0.041±0.011 [B]</td>
<td>1.67±0.91 [A]</td>
<td>1.42±0.03 [B]</td>
<td>0.14±0.05 [C]</td>
</tr>
</tbody>
</table>

**Discussion**

We investigated the longitudinal behavior of CCAs from “toddler-like” 4 week old pigs [43] under cyclic extension. Previous studies have reported axial displacement of the common carotid artery wall in vivo during the normal cardiac cycle [44-53], with longitudinal strains between 1 and 8% (stretch ratios of 1.01-1.08) in the human CCA [46, 47] and between 1 and 6% (stretch ratios of 1.01-1.06) in the adult pig [45]. Strain rate associated with this axial motion was only reported in human CCAs, on the order of 0.2-0.4 s$^{-1}$ [46]. Furthermore, the CCA is under axial tension in vivo, and one study found axial pre-stretches of approximately 1.347 in adult porcine CCAs [54]. This pre-stretch (defined as the elongation from the traction-free length to in situ length) has also been shown to increase with age during postnatal development in dogs [55]. Finally, in a study of ferret CCAs the longitudinal ultimate stretch ratio as calculated from the in vitro relaxed length...
varied between 2.53 for low rate (0.2 s\(^{-1}\)) and 2.60 for high rate (~200 s\(^{-1}\)) tests [18]. Thus, our prescribed maximum stretch ratio (1.5, relative to the traction-free state) is slightly larger than in vivo pulsatile extension, and our stretch rate (3 s\(^{-1}\)) is about one order of magnitude higher than pulsatile rates, anchoring our experiments as a nondestructive traumatic event rather than a typical physiological condition.

Qualitatively similar to previous investigations [18, 19, 24, 27], we observed a biphasic response, with a low modulus in the toe region at small stretches where 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 artery yielding much stiffer behavior. With continuous cycling, the biphasic behavior persisted, with a significant decrease in toe region modulus and peak stress, and an increase in corner stretch. The limited published data regarding the longitudinal properties of the porcine CCA under cyclic loading [25-27] show that the majority of stress softening occurs in the initial loading cycle [25, 27], similar to our observations regarding first cycle hysteresis (Figure C.2). The same phenomenon was reported in human aorta [56]. We speculate that our observations of large amounts of softening occurring in the initial cycle and softer response in the low-stretch toe region with successive cycling may be attributed to irreversible (plastic) changes to matrix elements as collagen fibers are pulled apart from one another transverse to their axes, or due to reorientation or damage to collagen fibers. Indeed, several studies of ligament and tendon preconditioning have documented changes in collagen fiber alignment towards the direction of loading with successive cycling [57, 58]. However, the levels of stretch examined in this study were not high enough to show any appreciable change in the high-stretch linear region response with increasing numbers of loading cycles. Future studies should investigate the role of fiber reorientation in cyclic deformation of CCAs, and higher levels of peak stretch. To determine whether the observed softening may be attributed to transient changes associated with viscoelasticity rather than permanent alterations due to tissue fatigue, periods of relaxation may be prescribed between multiple loading protocols for comparison across the same sample. Finally, future studies may use histological sections to
investigate if the prolonged softening, similar to the large amount of softening in the initial cycle, is due to structural alterations in the vessel wall: either to the matrix as collagen fibers are pulled apart, or to collagen fibers themselves by comparing unloaded samples, samples undergoing a single cycle, and those undergoing many cycles.

Preconditioning is a procedure often used in mechanical testing of soft tissues in which a specified loading and unloading protocol is recommended to stabilize the mechanical response of the tissue. However, preconditioning is not a standardized procedure and the number of cycles, peak loading parameter, and the inclusion and length of viscoelastic recovery rest periods between cycles vary widely between studies [59]. Furthermore, it is generally accepted that a preconditioning loading protocol should be conducted under conditions as similar as possible to the test itself [59-65]. However, in the case of large deformation or failure testing, preconditioning tissue to test levels may damage the tissue prior to testing [66]. No preconditioning was performed on the samples tested in this study, as the aim of the experiments presented was to investigate the effect of prolonged cycling on tissue response. Nonetheless, future studies should determine the influence of a preconditioning protocol at sub-damage loading levels, perhaps to in situ longitudinal stretches, on CCA behavior under cyclic loading.

**Limitations**

There are several limitations to our findings. First, vessels were moistened and tested at room temperature, as in other studies [18, 21, 25, 26]. Future studies may evaluate properties at body temperature. Second, we measured needle-to-needle displacement to capture average vessel response. Investigators interested in edge effects near the grips [67] may use speckle patterning to capture local variations in vessel stretch. Third, we stretched our CCA specimens from their traction-free state. To capture in vivo conditions, future studies should characterize hysteresis and fatigue in vessels stretched from their in situ length. Finally, we used a displacement-controlled study design, and observed softening beyond the sixth cycle, as evidenced by the increasing corner stretch point (where stress begins to increase dramatically...
with each cycle) and decreasing peak stress. Future stress or force-controlled studies should be conducted to confirm fatigue by an increase in creep response beyond the first few cycles.

**Conclusions**

To our knowledge, this study is the first to investigate cyclic loading of the CCA beyond 5 cycles [27]. We find that under subcatastrophic cyclic longitudinal extension, the immature porcine CCA displays softening behavior. This softening can be represented by decreasing toe region modulus and peak stress as well as increasing corner stretch with an increasing number of loading cycles. This investigation is an important first step in modeling fatigue-like behavior in arterial tissue that may be subjected to repeated longitudinal loads.

**Acknowledgements**

We would like to thank Amy Clevenger and Jill Ralston for their technical assistance. We are grateful for the support of the American Heart Association (12PRE12040315), the National Institutes of Health (U01 NS069545 and R21 HD078842), and the University of Pennsylvania Rachleff Scholars Program. Study sponsors had no role in study design, data collection, interpretation, manuscript writing, or submission. The authors (Pasquesi, Liu, and Margulies) have no relationships or obligations with individuals or organizations that could bias our findings.

**References**


APPENDIX D: AVERAGED BRIDGING VEIN STRESS-STRETCH CURVES

Porcine Newborn High Rate Averaged Bridging Vein Stress-Stretch Curve. Black ‘X’ represents average high rate ultimate stretch failure criterion.

Porcine Newborn Post-Cyclic Averaged Bridging Vein Stress-Stretch Curve. Black ‘X’ represents average high rate ultimate stretch failure criterion.
Human Infant High Rate Averaged Bridging Vein Stress-Stretch Curve. Black ‘X’ represents average high rate ultimate stretch failure criterion.

Human Infant Post-Cyclic Averaged Bridging Vein Stress-Stretch Curve. Black ‘X’ represents average high rate ultimate stretch failure criterion.
APPENDIX E: SAGITTAL PLANE PHYSICAL TRANSECTION BRAIN-SKULL

DISPLACEMENT TIME-HISTORIES

Angular Velocity (rad/s)

Angular Acceleration (rad/s²)

Displacement (mm)

Pre-Motion
Animal 1, Rotation 1; 120906
Post-Motion

0 0.01 0.02 0.03 0.04 0.05 0.06 0.07 0.08
0 0.01 0.02 0.03 0.04 0.05 0.06 0.07 0.08
0 0.01 0.02 0.03 0.04 0.05 0.06 0.07 0.08

Time (s)
APPENDIX F: ALTERNATE MEASURES OF NEONATAL PORCINE ROTATIONAL INJURY KINEMATICS

F.1 Single Low Level Rotational Injury Kinematics Determined from Raw Angular Velocity and Gaussian Smoothed Angular Acceleration Traces

Table F.1 Single low level sagittal rotational injury kinematics as determined from raw velocity and Gaussian smoothed acceleration traces. All values are averaged across N_{crit} determination groups and all studies.

<table>
<thead>
<tr>
<th>Subject</th>
<th>N_{crit} Determination Group</th>
<th>Survival Time</th>
<th>Peak Angular Velocity (rad/s)</th>
<th>Peak Angular Acceleration (krad/s^2)</th>
<th>Peak Angular Deceleration (krad/s^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>090924; 1L</td>
<td>Development</td>
<td>3-8 hour</td>
<td>34.7</td>
<td>3.1</td>
<td>4.3</td>
</tr>
<tr>
<td>090929; 2L</td>
<td>Development</td>
<td>3-8 hour</td>
<td>41.0</td>
<td>3.1</td>
<td>3.9</td>
</tr>
<tr>
<td>091112; 3L</td>
<td>Development</td>
<td>3-8 hour</td>
<td>39.5</td>
<td>3.6</td>
<td>5.7</td>
</tr>
<tr>
<td>110504B-2; 4L</td>
<td>Development</td>
<td>24 hour</td>
<td>51.3</td>
<td>5.8</td>
<td>17.0</td>
</tr>
<tr>
<td>110511B-1; 5L</td>
<td>Development</td>
<td>24 hour</td>
<td>43.3</td>
<td>5.6</td>
<td>16.6</td>
</tr>
<tr>
<td>110511B-2; 6L</td>
<td>Development</td>
<td>24 hour</td>
<td>41.3</td>
<td>3.8</td>
<td>11.8</td>
</tr>
<tr>
<td>110520-2;7L</td>
<td>Development</td>
<td>24 hour</td>
<td>34.7</td>
<td>4.7</td>
<td>10.3</td>
</tr>
<tr>
<td>091110; 8L</td>
<td>Validation</td>
<td>3-8 hour</td>
<td>33.3</td>
<td>2.7</td>
<td>4.1</td>
</tr>
<tr>
<td>091124; 9L</td>
<td>Validation</td>
<td>3-8 hour</td>
<td>37.4</td>
<td>2.9</td>
<td>6.1</td>
</tr>
<tr>
<td>110504B-1; 10L</td>
<td>Validation</td>
<td>24 hour</td>
<td>51.1</td>
<td>5.5</td>
<td>14.5</td>
</tr>
<tr>
<td>110520-1; 11L</td>
<td>Validation</td>
<td>24 hour</td>
<td>37.1</td>
<td>5.0</td>
<td>10.4</td>
</tr>
</tbody>
</table>

**Development Group Average**
40.8±5.7  | 4.2±1.1  | 9.9±5.5

**Validation Group Average**
39.7±7.8  | 4.0±1.4  | 8.8±4.6

**Overall Average**
40.4±6.2  | 4.2±1.2  | 9.5±5.0
### F.2 Single High Level Rotational Injury Kinematics Determined from Raw Angular Velocity and Gaussian Smoothed Angular Acceleration Traces

**Table F.2** Single high level sagittal rotational injury kinematics as determined from raw velocity and Gaussian smoothed acceleration traces. All values are averaged across $N_{crit}$ determination groups and all studies.

<table>
<thead>
<tr>
<th>Subject</th>
<th>$N_{crit}$ Determination Group</th>
<th>Survival Time</th>
<th>Peak Angular Velocity (rad/s)</th>
<th>Peak Angular Acceleration (krad/s$^2$)</th>
<th>Peak Angular Deceleration (krad/s$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>060517; 1H</td>
<td>Development</td>
<td>3-8 hour</td>
<td>166.7</td>
<td>65.8</td>
<td>111.0</td>
</tr>
<tr>
<td>060811; 2H</td>
<td>Development</td>
<td>3-8 hour</td>
<td>169.7</td>
<td>61.4</td>
<td>78.1</td>
</tr>
<tr>
<td>070830; 3H</td>
<td>Development</td>
<td>3-8 hour</td>
<td>160.5</td>
<td>43.6</td>
<td>54.2</td>
</tr>
<tr>
<td>090721-1; 4H</td>
<td>Development</td>
<td>3-8 hour</td>
<td>150.2</td>
<td>38.2</td>
<td>68.6</td>
</tr>
<tr>
<td>100408-1; 5H</td>
<td>Development</td>
<td>3-8 hour</td>
<td>157.5</td>
<td>41.7</td>
<td>61.4</td>
</tr>
<tr>
<td>100422D-2; 6H</td>
<td>Development</td>
<td>3-8 hour</td>
<td>139.2</td>
<td>37.4</td>
<td>139.5</td>
</tr>
<tr>
<td>110224; 7H</td>
<td>Development</td>
<td>3-8 hour</td>
<td>151.4</td>
<td>32.2</td>
<td>90.5</td>
</tr>
<tr>
<td>110520-4; 8H</td>
<td>Development</td>
<td>3-8 hour</td>
<td>148.8</td>
<td>32.7</td>
<td>90.5</td>
</tr>
<tr>
<td>080604; 9H</td>
<td>Development</td>
<td>24 hour</td>
<td>145.0</td>
<td>34.9</td>
<td>53.3</td>
</tr>
<tr>
<td>090729-1; 10H</td>
<td>Development</td>
<td>24 hour</td>
<td>151.8</td>
<td>35.7</td>
<td>78.8</td>
</tr>
<tr>
<td>090729-3; 11H</td>
<td>Development</td>
<td>24 hour</td>
<td>152.6</td>
<td>35.6</td>
<td>70.7</td>
</tr>
<tr>
<td>110202-1; 12H</td>
<td>Development</td>
<td>24 hour</td>
<td>150.7</td>
<td>33.1</td>
<td>80.4</td>
</tr>
<tr>
<td>110202-2; 13H</td>
<td>Development</td>
<td>24 hour</td>
<td>150.8</td>
<td>32.8</td>
<td>77.2</td>
</tr>
<tr>
<td>110217; 14H</td>
<td>Development</td>
<td>24 hour</td>
<td>149.8</td>
<td>33.7</td>
<td>80.9</td>
</tr>
<tr>
<td>110304-1; 15H</td>
<td>Development</td>
<td>24 hour</td>
<td>147.4</td>
<td>29.9</td>
<td>72.6</td>
</tr>
<tr>
<td>110615-1; 16H</td>
<td>Development</td>
<td>24 hour</td>
<td>155.9</td>
<td>33.4</td>
<td>106.9</td>
</tr>
<tr>
<td>110615-2; 17H</td>
<td>Development</td>
<td>24 hour</td>
<td>153.0</td>
<td>29.4</td>
<td>91.1</td>
</tr>
<tr>
<td>060914; 18H</td>
<td>Validation</td>
<td>3-8 hour</td>
<td>169.6</td>
<td>58.6</td>
<td>59.3</td>
</tr>
<tr>
<td>110128; 19H</td>
<td>Validation</td>
<td>3-8 hour</td>
<td>156.6</td>
<td>40.9</td>
<td>87.4</td>
</tr>
<tr>
<td>110520-3; 20H</td>
<td>Validation</td>
<td>3-8 hour</td>
<td>148.3</td>
<td>28.7</td>
<td>83.9</td>
</tr>
<tr>
<td>080606-1; 21H</td>
<td>Validation</td>
<td>24 hour</td>
<td>149.9</td>
<td>34.1</td>
<td>55.8</td>
</tr>
<tr>
<td>090722-1; 22H</td>
<td>Validation</td>
<td>24 hour</td>
<td>151.8</td>
<td>39.1</td>
<td>70.7</td>
</tr>
<tr>
<td>090722-2; 23H</td>
<td>Validation</td>
<td>24 hour</td>
<td>151.1</td>
<td>39.4</td>
<td>69.3</td>
</tr>
<tr>
<td>090729-2; 24H</td>
<td>Validation</td>
<td>24 hour</td>
<td>150.6</td>
<td>35.7</td>
<td>77.1</td>
</tr>
<tr>
<td>110304-2; 25H</td>
<td>Validation</td>
<td>24 hour</td>
<td>147.3</td>
<td>32.5</td>
<td>86.5</td>
</tr>
<tr>
<td><strong>Development Group Average</strong></td>
<td></td>
<td></td>
<td>153.0±7.4</td>
<td>38.3±10.2</td>
<td>82.7±21.6</td>
</tr>
<tr>
<td><strong>Validation Group Average</strong></td>
<td></td>
<td></td>
<td>153.2±7.2</td>
<td>38.6±9.0</td>
<td>73.8±12.1</td>
</tr>
<tr>
<td><strong>Overall Average</strong></td>
<td></td>
<td></td>
<td>153.0±7.2</td>
<td>38.4±9.7</td>
<td>79.8±19.3</td>
</tr>
</tbody>
</table>
Table F.3 Cyclic rotational injury kinematics as determined from full velocity and acceleration traces, and resulting hemorrhage scores and number of failed bridging vein elements from finite element simulations ($N_{fail}$). If a metric was averaged across all cycles in a single trace (e.g. frequency), the overall average value represents the mean of the average values for each trace.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Rotational Plane</th>
<th>Survival Time</th>
<th>Average Frequency (Hz)</th>
<th>Average Peak- Peak Angular Velocity (rad/s)</th>
<th>Maximum Peak- Peak Angular Velocity (rad/s)</th>
<th>Average Peak Acceleration Magnitude (rad/s²)</th>
<th>Maximum Peak Acceleration Magnitude (rad/s²)</th>
<th>Total Cerebrum EAH (%)</th>
<th>$N_{fail}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>090407; 1C</td>
<td>Sagittal</td>
<td>3-8 hr</td>
<td>2.18±0.30</td>
<td>19.3±1.8</td>
<td>20.6</td>
<td>493±81</td>
<td>634</td>
<td>0.0%</td>
<td>0</td>
</tr>
<tr>
<td>090618; 2C</td>
<td>Sagittal</td>
<td>3-8 hr</td>
<td>2.50±0.39</td>
<td>23.4±2.3</td>
<td>24.7</td>
<td>818±174</td>
<td>1307</td>
<td>0.0%</td>
<td>0</td>
</tr>
<tr>
<td>091015; 3C</td>
<td>Sagittal</td>
<td>3-8 hr</td>
<td>2.48±0.40</td>
<td>24.0±2.7</td>
<td>26.2</td>
<td>536±97</td>
<td>671</td>
<td>0.7%</td>
<td>0</td>
</tr>
<tr>
<td>091105; 4C</td>
<td>Sagittal</td>
<td>3-8 hr</td>
<td>2.73±0.48</td>
<td>26.4±3.4</td>
<td>28.6</td>
<td>736±270</td>
<td>1161</td>
<td>0.3%</td>
<td>0</td>
</tr>
<tr>
<td>091218; 5C</td>
<td>Axial</td>
<td>3-8 hr</td>
<td>2.73±0.44</td>
<td>28.0±3.8</td>
<td>32.1</td>
<td>814±296</td>
<td>1424</td>
<td>0.0%</td>
<td>0</td>
</tr>
<tr>
<td>100526; 6C</td>
<td>Axial</td>
<td>3-8 hr</td>
<td>2.89±0.27</td>
<td>30.7±2.4</td>
<td>35.2</td>
<td>691±205</td>
<td>1166</td>
<td>0.0%</td>
<td>0</td>
</tr>
<tr>
<td>100106; 7C</td>
<td>Axial</td>
<td>24 hr</td>
<td>2.73±0.36</td>
<td>29.0±3.2</td>
<td>31.2</td>
<td>1001±403</td>
<td>1719</td>
<td>1.8%</td>
<td>0</td>
</tr>
<tr>
<td>100213; 8C</td>
<td>Axial</td>
<td>24 hr</td>
<td>2.77±0.42</td>
<td>26.8±3.5</td>
<td>33.7</td>
<td>869±293</td>
<td>1643</td>
<td>2.7%</td>
<td>0</td>
</tr>
<tr>
<td>100610; 9C</td>
<td>Axial</td>
<td>24 hr</td>
<td>2.84±0.27</td>
<td>31.4±2.3</td>
<td>38.5</td>
<td>711±273</td>
<td>1440</td>
<td>0.0%</td>
<td>0</td>
</tr>
<tr>
<td>100630; 10C</td>
<td>Axial</td>
<td>24 hr</td>
<td>2.85±0.29</td>
<td>28.5±1.3</td>
<td>30.8</td>
<td>733±317</td>
<td>1248</td>
<td>0.0%</td>
<td>0</td>
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<tr>
<td><strong>Overall Average</strong></td>
<td></td>
<td></td>
<td><strong>2.70±0.22</strong></td>
<td><strong>26.8±3.7</strong></td>
<td><strong>30.2±5.3</strong></td>
<td><strong>740±150</strong></td>
<td><strong>1241±361</strong></td>
<td><strong>0.5±0.9%</strong></td>
<td><strong>0</strong></td>
</tr>
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</table>
APPENDIX G: PORCINE FINITE ELEMENT MODEL SIMULATED ANGULAR VELOCITY, SIMULATED ANGULAR ACCELERATION, AND BRIDGING VEIN FAILURE - TIME HISTORIES

G.1 Single Low Level Sagittal Finite Element Simulated Kinematics and Resulting Bridging Vein Element Failures Across Time
G.2 Single High Level Sagittal Finite Element Simulated Kinematics and Resulting Bridging Vein Element Failures Across Time
225
G.1 Cyclic Finite Element Simulated Kinematics and Resulting Bridging Vein Element Failures Across Time
APPENDIX H: INSTRUMENTED SURROGATE SHAKING TRACES

H.1 Trial 1 Shakes, without Instruction to use Maximum Neck Range of Motion

Trial 1, Subject 1

![Angular Velocity and Acceleration Graphs](Image)
Trial 1, Subject 9

Angular Velocity (rad/s)

Time (s)

Angular Acceleration (rad/s^2)
H.2 Trial 2 Shakes, with Instruction to use Maximum Neck Range of Motion

![Graph showing angular velocity and acceleration over time for Trial 2, Subject 1.](image)
Trial 2, Subject 10

Angular Velocity (rad/s)

Angular Acceleration (rad/s²)

Time (s)
APPENDIX I: HUMAN FINITE ELEMENT MODEL SIMULATED VELOCITY,
SIMULATED ACCELERATION, AND BRIDGING VEIN FAILURE - TIME HISTORIES

High Velocity - High Acceleration, Sagittal Boundary Condition

Angular Velocity (rad/s)

Angular Acceleration (rad/s²)

N (N)

Time (s)
Fabricated Low Velocity - High Acceleration, Sagittal Boundary Condition
Fabricated Low Velocity - High Acceleration,
Axial Boundary Condition

Angular Velocity (rad/s)

Angular Acceleration (rad/s²)

N_{oil}

Time (s)
High Velocity - High Acceleration, No Neck Damage, Sagittal Boundary Condition

Angular Velocity (rad/s)

Angular Acceleration (rad/s²)

N_{roll}

Time (s)