Age-dependent material properties of the porcine cerebrum: effect on pediatric inertial head injury criteria

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Abstract

During growth and development, the immature central nervous system undergoes rapid alterations in constituents and structure. We hypothesize that these alterations are accompanied by changes in the mechanical properties of brain tissue which, in turn, influence the response of the brain to traumatic inertial loads. Samples of frontal cerebrum from neonatal (2—3 days) and adult pigs were harvested and tested within 3 h post-mortem. The complex shear modulus of the samples was measured in a custom-designed oscillatory shear testing device at engineering shear strain amplitudes of 2.5% or 5% from 20—200 Hz, at 25 °C and 100% humidity. In this range, the elastic and viscous components of the complex shear modulus increased significantly with the development of the cerebral region of the brain. Using an idealized model of the developing head, the age-dependent material properties of brain tissue were shown to affect the mechanical response of the brain to inertial loading. This study is a first step toward developing head injury tolerance criteria specifically for the pediatric population. © 1998 Elsevier Science Ltd. All rights reserved.

Keywords: Scaling; Brain injury; Infant; Constitutive properties; Viscoelastic

1. Introduction

Prior to four years of age, the human central nervous system (CNS) undergoes a period of accelerated growth and development (Calder, 1984). During this period, the majority of traumatic pediatric head injuries are due to motor vehicle accidents and falls (Bruce, 1990; Pascucci, 1988). When infants and young children suffer head trauma, they exhibit a unique pattern of neural tissue injuries, and they have secondary sequelae, such as diffuse cerebral swelling, which occur with a frequency and follow a time course that are distinct from adults (Bruce, 1990; Shapiro, 1985).

During development, there is an increase in dendritic and axonal branching in the cerebrum accompanied by increases in DNA-P content (cellularity), increases in lipid content as axonal segments are myelinated and decreases in water content (Dobbing, 1964). No previous studies have investigated the changes in the mechanical properties of the cerebrum associated with development. Thus, the infant and young child have been treated traditionally as miniature adults when determining pediatric head injury thresholds. Researchers have postulated that head injury thresholds for concussion, subdural hematoma and diffuse axonal injury associated with inertial loading in the adult may be scaled to the infant as a function of brain mass alone (Duhaime et al., 1987; Margulies and Thibault, 1992). These studies concluded that the lower brain mass of the one month old infant allows the pediatric brain to sustain higher rotational accelerations than its adult counterpart before the onset of injury.

No human experimental studies have been performed to determine directly inertial head injury tolerances for the pediatric population. The approach taken in previous studies (Dejeammes et al., 1984; Duhaime et al., 1987; Mohan et al., 1979; Stürtz, 1980) utilized brain mass scaling relations (Holbourn, 1956; Ommaya et al., 1967) to establish pediatric inertial head injury tolerance levels from experimental primate tolerance data. Recently, Melvin (1995) recognized the important role of material properties in defining age-specific injury tolerance criteria and emphasized the current lack of material property data within the literature.
Based on age-dependent changes of the biological constituents of brain tissue, we hypothesize that there exist corresponding age-dependent changes in material properties. Furthermore, we hypothesize that these tissue properties affect the existing relations used to predict the current thresholds for purely inertial traumatic brain injury in the infant. Understanding the unique mechanical response of the developing CNS is critical to predicting the functional and structural tolerance of the infant brain to purely inertial head injury, and to developing protective measures for the pediatric population.

2. Methods

In this study, we report mechanical properties of tissue obtained from the pig. Dobbing and colleagues (Dickerson and Dobbing, 1966; Dobbing, 1964) proposed matching CNS development between mammalian species by correlating the timing of pre- and post-natal component growth of each species, assuming that interspecies differences in fundamental compositional units, like myelin, are minor. Using the rate of growth of brain mass, Dobbing and coworkers determined that during the first decade of human life, months of life in a human were roughly comparable to weeks in a pig. This interspecies developmental scaling rule was further supported by our analysis and comparison of Dobbing’s DNA-P, cholesterol and water content data for pig and human (Figs. 1–3). To examine the variation in the mechanical properties of the developing brain we compared the viscoelastic properties of tissue from fully developed, one-year-old pigs (similar to a greater than four-year-old human child) with those from 2–3 day old pigs (less than one-month-old human newborn).

Fresh whole porcine brains were obtained from 2–3 day old (N = 12) and one year old (N = 12) domestic pigs according to a protocol approved by the Animal Care and Use Committee of the University of Pennsylvania (IACUC Protocol #100) and the United States Department of Agriculture. The excised cerebrum and thalamus were stored in refrigerated artificial cerebrospinal fluid, and tests were completed within three hours postmortem.

All specimens were removed from the same location in the frontal cerebrum with the same neuroanatomical orientation to minimize the possible influences of inhomogeneity or anisotropy. A cylindrical sample of tissue was removed from the cerebrum and was sliced perpendicular to its long axis approximately 5–7 mm from the medial end of the core to remove a disc-shaped tissue specimen approximately 1–2 mm thick and 10–12 mm in diameter (Fig. 4), free of any penetrating sulci. One sample per animal was tested. The stress-free sample diameter and thickness (h) were measured with dial calipers (± 0.1 mm) while the sample was floating in CSF solution, and cross-sectional area (A_0) was computed. No swelling was observed during specimen preparation.

Brain tissue has a high bulk modulus to shear modulus ratio (approximately 10^5), and is therefore most likely to fail in shear (Holbourn, 1943; McElhaney et al., 1976). Tissue samples were mounted in a custom-designed testing apparatus (Arbogast et al., 1997), enclosed within
a humidity chamber (\( \sim 25^\circ C \) and 100\% humidity) to prevent dehydration during testing, and equilibrated for 5 min. Although testing was not performed at body temperature, 37\(^\circ C\), previous isothermal tests performed on porcine brain tissue at four temperatures between 5\(^\circ C\) and 25\(^\circ C\) failed to demonstrate temperature-dependent changes in the shear modulus of the tissue (Arbogast et al., 1997).

Each sample was subjected to an oscillatory simple shear strain protocol with an amplitude of 2.5\% or 5\% (engineering strain) in a sequence over the frequency range of 20–200 Hz in 10 Hz increments, randomized with respect to increasing or decreasing frequency. The shear strain was always applied in the same plane of each tissue specimen, but the orientation of each specimen within the device was randomized, implicitly
incorporating an assumption of in-plane isotropy. The amplitude of the time-varying voltage signals corresponding to input displacement and resultant shear force, and the phase difference between the two signals at their zero-crossings were measured at each frequency with a digitizing oscilloscope (Model 54602B, Hewlett Packard, Loveland, CO) at 100 ksamples sec \(^{-1}\).

### 3. Data analysis

Shear stress, \(\tau_0\), and engineering shear strain, \(\gamma_0\), were computed from the amplitude of the sinusoidal force \((F)\) and displacement \((D)\) signals at each test frequency using the following relationships (Ferry, 1980):

\[
\tau_0 = \frac{F}{A_0} \quad \text{and} \quad \gamma_0 = \frac{D}{h},
\]

where \(A_0\) is the shear area of the sample and \(h\) is the thickness of the sample.

We analyzed brain tissue as a linear viscoelastic material that, subjected to a periodic shear strain \(\gamma(t) = \gamma_0 \cos(\omega t)\), responded with a periodic shear stress that led the strain by a phase angle, \(\delta\). The complex shear modulus, \(G'(\omega) = G''(\omega) + iG''(\omega)\), of the tissue was determined from periodic stress and strain measurements according to Ferry (Ferry, 1980):

\[
G'(\omega) = \left(\frac{\tau_0}{\gamma_0}\right) \cos(\delta),
\]

\[
G''(\omega) = \left(\frac{\tau_0}{\gamma_0}\right) \sin(\delta),
\]

where \(G'(\omega)\) and \(G''(\omega)\) represent the elastic (storage modulus) and viscous (loss modulus) components of the complex shear modulus at the frequency \(\omega\), \(\tau_0\) is the amplitude of the shear stress, \(\gamma_0\) is the amplitude of the shear strain, and \(\delta\) is the phase angle between the sinusoidally varying responses. This formulation neglected any inertial effects due to sample mass. The significance of the material properties’ \((G'^*, G' \text{ and } G'')\) dependence on age and frequency was evaluated using analysis of variance (ANOVA \(p < 0.05\)).

### 4. Results

The shear modulus of porcine brain tissue was found to have significant age dependence, supporting our hypothesis that constituent alterations are accompanied by changes in mechanical properties. First, regardless of age and strain amplitude, \(G'\) and \(G''\) increased significantly as a function of frequency (Fig. 5a and b). Second, except for \(G'(\omega)\) at 5% strain amplitude, the components of the complex shear modulus demonstrated a significant increase with age. At 5% strain amplitude, \(G'(\omega)\) of the adult tissue was not significantly different than the corresponding pediatric \(G'(\omega)\) across all frequencies. Finally, the cross-correlation between age and frequency revealed that (1) the slope of \(G''(\omega)\) increased significantly with age, and (2) \(G'(\omega)\) only shifted in magnitude with no significant age-related change in frequency dependence.

### 5. Discussion

Our central hypothesis is that the shear properties of porcine brain tissue in vitro are age dependent, and will affect the biomechanical response of the infant cerebrum to rotational accelerations produced by non-centroidal rotations of the head associated with traumatic brain injury. The major findings of this study may be summarized as follows: first, based on our analysis of studies performed by Dobbing and colleagues, the porcine brain was comparable to the human brain in growth and the development of its constituents (DNA-P, myelin and water content) such that our 2–3 day-old infant porcine data is representative of that for a one-month old infant human. Second, the elastic and viscous behavior of

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Fig. 4. Harvest location for porcine brain samples, indicated by the shaded circular area, and schematic diagram of disc-shaped tissue specimen. A cylindrical plug of cerebral tissue, whose long axis was perpendicular to the sagittal plane of the brain, was removed from the cerebrum just superior to the cingulate gyrus. Disc-shaped specimens 10–12 mm in diameter and 1–2 mm thick were cut from the middle of the cylindrical plug for testing.
porcine cerebral tissue was generally increased with age, within the range of strains tested in our experiments. Third, the complex shear modulus of infant and adult cerebral tissue increased with increased strain rate. Our experimental studies were conducted at strain rates between 2 and 40 s⁻¹, encompassing the intracranial strain rates associated with traumatic inertial brain injury reported in the literature (Margulies et al., 1990). Fourth, the frequency-dependent complex shear modulus of the infant cerebrum was insensitive to shear strain magnitude in our experimental range, while the complex shear modulus of the adult cerebrum decreased with increased shear strain magnitude.

The results of our study are in general agreement with the published literature regarding animal and human brain tissue properties. A review of the mechanical properties of adult human and animal brain (Fallenstein et al., 1969; Koeneman, 1966; McElhaney et al., 1976; Metz, 1970; Ommaya, 1968; Shuck and Advani, 1972) demonstrated that the reported properties of brain tissue from different species and testing conditions varied by as much as an order of magnitude (Table 1). The frequency-dependent behavior reported by Shuck and Advani for adult human cerebrum bears remarkable resemblance to our findings in adult porcine cerebrum, including a larger slope in $G''(\omega)$ than $G'(\omega)$, which resulted in an intersection of the two curves between 100 and 150 Hz. However, the magnitudes reported by Shuck and Advani for $G'$ and $G''$ are an order of magnitude higher than our values for adult porcine tissue across our entire experimental frequency range. Regardless of the differences in the relative magnitudes of the experimentally determined modulus for porcine and human cerebrum, it is important to note that the general frequency-dependent behavior of adult porcine tissue matched that of adult human tissue.

This discrepancy may be due to interspecies or testing protocol differences. Shuck and Advani tested tissue within hours after autopsy, which may have occurred hours or days after death. We tested porcine brain tissue within 3 h after death. In a study of the viscoelastic properties of articular cartilage, Fitzgerald and Freeland (1970) demonstrated an abrupt increase of approximately 50% in the storage modulus of the tissue at five hours post-mortem, with the storage modulus remaining at this increased level for time thereafter. Fitzgerald and Freeland attributed this stiffening to the completion of rigor mortis, but Hayes and Bodine (1978) proposed an alternative explanation in which the cleavage of macromolecules during tissue degeneration caused an increased swelling pressure and thus an increased stiffness in the tissue. Presently, it is unclear what effect post-mortem time might have on the mechanical response of brain tissue.

Our study has two major limitations. First, brain tissue properties were determined in one location and one orientation only and second, the samples were loaded in vitro in simple shear rather than pure shear. The first limitation of our study was the narrow regional and directional focus of our mechanical property measurements. Our goal was to measure the age-related properties of brain tissue that are independent of the possible anisotropic and inhomogeneous nature of brain tissue, and therefore we tested samples oriented in the same direction and harvested from the same location. Although samples were composed of a gray/white matter mixture we have assumed, for purposes of mechanical characterization, that the cerebrum of the
Table 1
Previously determined values of storage modulus ($G'$) and loss modulus ($G''$) of brain tissue from the literature. Our data are included for comparison.

<table>
<thead>
<tr>
<th>Testing technique</th>
<th>Species</th>
<th>Frequency (Hz)</th>
<th>$G'$ (Pa)</th>
<th>$G''$ (Pa)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harmonic shear 2.5%</td>
<td>Infant domestic pig</td>
<td>20–200:</td>
<td></td>
<td></td>
<td>Our data</td>
</tr>
<tr>
<td>engineering shear strain</td>
<td></td>
<td>20</td>
<td>771</td>
<td>222</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>50</td>
<td>798</td>
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<td></td>
<td></td>
<td>100</td>
<td>925</td>
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<td></td>
<td></td>
<td>150</td>
<td>1092</td>
<td>484</td>
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<td></td>
<td></td>
<td>200</td>
<td>1257</td>
<td>678</td>
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<tr>
<td>Harmonic shear 2.5%</td>
<td>Adult domestic pig</td>
<td>20–200:</td>
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<td></td>
<td>Our data</td>
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<tr>
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<td>200</td>
<td>1756</td>
<td>2139</td>
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<td>Free vibration</td>
<td>Rhesus monkey</td>
<td>31</td>
<td>30300</td>
<td>18000</td>
<td>Galford and McElhaney (1970)</td>
</tr>
<tr>
<td>Free vibration</td>
<td>Human</td>
<td>34</td>
<td>22300</td>
<td>8700</td>
<td>Galford and McElhaney (1970)</td>
</tr>
<tr>
<td>Harmonic shear</td>
<td>Human white matter</td>
<td>9–10</td>
<td>750–1410</td>
<td>300–600</td>
<td>Fallenstein et al. (1969)</td>
</tr>
<tr>
<td>Driving point impedance</td>
<td>Rhesus monkey (in vivo)</td>
<td>80</td>
<td>19600</td>
<td>11200</td>
<td>Fallenstein et al. (1969); Wang and Wineman (1972)</td>
</tr>
<tr>
<td>Harmonic shear</td>
<td>Human</td>
<td>5–350:</td>
<td></td>
<td></td>
<td>Shuck and Advani (1972)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>7600</td>
<td>2800</td>
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<td></td>
<td></td>
<td>15</td>
<td>8400</td>
<td>3500</td>
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<td></td>
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<td>35</td>
<td>11700</td>
<td>5200</td>
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<td>105</td>
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<td>350</td>
<td>33900</td>
<td>81400</td>
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</table>

Porcine brain is homogeneous. In a viscoelastic characterization of adult human brain tissue, Shuck and Advani averaged together gray and white matter results when reporting dynamic (10–350 Hz) viscoelastic properties and considered brain tissue to be homogeneous and isotropic. Other investigators have also assumed homogeneous and isotropic properties for the brain to simplify experimental design and data reduction (Koeneman, 1966; Mendis et al., 1995; Pamidi and Advani, 1978).

The second limitation of our study was the in vitro simple shear loading condition applied by our experimental device. Our analysis assumed a state of pure shear attained when shear stress was maximum and normal stresses were zero. For the maximum simple shear strain of 5% used in our study, $\tau_{\text{measured}}$ is 99.9% of $\tau_{\text{pure}}$ and thus, we neglected the effect of normal stresses on our computations of shear modulus (Arbogast, 1997).

Furthermore, the effects of in vitro versus in vivo mechanical behavior of the brain have not been clearly elucidated and may be influenced substantially by the presence of the extensive pressurized cerebral vasculature (Ommaya, 1968). It is presently unknown what effects the in vivo developing vasculature has on the biomechanical response of the pediatric brain.

Our second hypothesis is that the variation of mechanical properties in the developing brain has profound effects on the response of the brain to traumatic loads. Holbourn (1956), and later Ommaya et al. (1967), developed scaling relationships to correlate brain injury thresholds between a “model” and a “prototype” (primate and human adult, respectively) under purely inertial loads. However, the brain mass scaling relationship assumes that the material properties of the brain, such as density and elastic shear modulus, are identical. Likewise, the scaling relationship assumes that the cerebral shear strain thresholds for brain injury are identical for the model and prototype, and brain tissue behaves as a linear elastic material (Ommaya et al., 1967). Rotational acceleration injury thresholds determined experimentally for primates were scaled to adult human levels through the relation:

$$\hat{\theta}_p = \hat{\theta}_m \left(\frac{M_m}{M_p}\right)^{2/3},$$

where $\hat{\theta}_p$ and $\hat{\theta}_m$ are the rotational accelerations of the prototype and the model species, respectively, and $M_m$ and $M_p$ are the corresponding brain masses. Researchers
have used this relationship to scale rotational acceleration injury thresholds between species such as primate and man, but also between the human adult and infant.

Using the elastic component of our findings, we can create a new form of the scaling relationship that incorporates the age-dependent changes in the elastic properties of cerebral brain tissue into Eq. (4). Assigning the “model” to the adult rotational acceleration tolerance and the “prototype” to the corresponding infant brain tolerance, the new form of the scaling relation becomes (Thibault, 1997):

\[
\hat{\theta}_{\text{Infant}} = \hat{\theta}_{\text{Adult}} \left( \frac{M_{\text{adult}}}{M_{\text{Infant}}} \right)^{2/3} \left( \frac{G_{\text{Infant}}}{G_{\text{Adult}}} \right).
\]

(5)

Substituting our porcine brain at 2.5% shear strain \((G'_{\text{Infant}}/G'_{\text{Adult}} = 0.667\) across the entire frequency range tested) into this new relation attenuates the influence of brain mass, predicting a lower scaled rotational acceleration threshold for children than that based on brain mass alone. Of note, our porcine brain data at 5% strain show no difference in the storage moduli of the infant and adult brain, implying that at this higher strain level, brain mass alone may influence the scaling relationship. Numerous studies have demonstrated the non-linear viscoelastic behavior of adult brain tissue over a wide range of strains and strain rates (Donnelly and Medige, 1997; McElhaney et al., 1976; Mendis et al., 1995; Miller and Chunzei, 1997; Shuck and Advani, 1972). To predict tolerance criteria for non-linear, large deformation inertial brain injury in the immature brain, it is necessary to characterize the brain tissue properties in the infant at strains larger than 5%. Likewise, characterization of the age-specific functional and structural tolerance of the individual neural and vascular elements within the brain is essential to developing more comprehensive age-dependent head injury criteria.

Duhaime and colleagues (1987) quantified the non-centroidal rotational head accelerations produced during inertial loading (shaking) of a one-month-old anthropomorphic infant dummy. Based on the peak values of rotational acceleration produced during shaking, they determined that shaking alone could not produce concussion, subdural hematoma or diffuse axonal injury in the infant. They also concluded that impact loading must occur to produce significant rotational accelerations. The threshold for each injury was based on brain mass scaling. According to our analysis, the rotational accelerations measured by Duhaime and colleagues during shaking alone were still well below the injury threshold for concussion, subdural hematoma and diffuse axonal injury, even after adjustment with our material property data at 2.5% strain. However, even though the rotational accelerations measured by Duhaime during impact loading exceeded the scaled traumatic brain injury thresholds, the impact load was applied to the rigid braincase of the infant dummy and did not account for the compliance of the infant cranial vault. In actuality, the developing pediatric cranial vault is a compliant structure capable of dynamic shape changes during impact loading. Diffuse brain injuries, often associated with rotational accelerations applied to the adult brain, may occur in the infant brain during impact without rotational acceleration (Thibault, 1997). Thus age-specific and loading-specific head injury thresholds must be developed to reflect the unique biomechanical responses of the developing pediatric head. This study is an essential first step in understanding the complex, age-dependent biomechanical response of the developing pediatric brain to inertial loading.

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