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Umbilical cord artery mechanical properties at various gestational ages

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Abstract:

Objective: Umbilical cord tissue is naturally available after birth and may provide insight into the health of a newborn. Intraventricular hemorrhage (IVH) is a common complication of prematurity that is suspected to be associated with structural deficiency of the vasculature. We are interested in determining whether umbilical vessel properties could be used to indicate increased risk for IVH. As a first step toward this, we investigated umbilical artery properties as a function of gestational age.

Study Design: Thirty-one umbilical cord specimens were collected from births ranging from 24 to 40 weeks gestation. Specimens were grouped according to gestational age (less than 25, 26-30, 31-35, and 36-40 weeks). Tension tests were performed on axial and circumferential strips obtained from umbilical arteries. Stiffness, corresponding stretch values, and cross sectional tissue areas were compared using ANOVA.

Results: Stress-stretch curves displayed no apparent differences across the gestational age range. Statistical analysis of stiffness and stretch values suggested no differences between groups ($p>0.05$). Significance was shown between cross sectional areas of some groups.

Conclusions: Mechanical characterization of umbilical arteries suggests that no significant changes in material properties occur in the 24-40 week gestational age range.

Keywords: umbilical artery mechanical properties; intraventricular hemorrhage; vessel mechanics

Introduction

Premature and very low birthweight infants are susceptible to numerous injuries during and after parturition. If these injuries are neurological in nature, they can result in long-term cognitive and motor skill deficiencies. Intraventricular hemorrhage (IVH), bleeding in the ventricle region of the brain, is a common complication of prematurity and occurs in 32% of infants with birth weights less than 1500 grams and born prior to 32 weeks gestation;[1] 75% of these will experience long-term neurological disabilities.[2] Structural deficiencies in the developing cerebral vasculature are suspected to underlie the high incidence of IVH,[3-5] though this has not been confirmed.

While tissue specimens are generally difficult to obtain from neonates, a portion of the infant circulation becomes available at birth through the umbilical cord. The composition and morphology of the umbilical cord has been studied and linked with maternal and fetal diseases [6] and with anthropomorphic parameters.[7] For example, umbilical cord material properties have been shown to change in the intrauterine growth restriction lamb model.[8] These findings suggest that the umbilical cord is influenced by fetal health and therefore could potentially be used to predict susceptibility to disease or injury. Because of the limited information on correlations between umbilical cord material properties and infant health, a full understanding of its prognostic value has not been achieved.[9]

In order to better understand vascular instability during development, especially as it may apply to IVH, the objective of this study was to define the mechanical response of umbilical arteries as a function of gestational age. Although umbilical and cerebral vessels are expected to have different properties, any systemic changes evident in umbilical arteries may be relevant to cerebral vessels. Preliminary work in our lab shows that defining the properties of whole umbilical cord artery is complicated by the natural helical structure of the vessels. As a result, we here report results from tension tests on axial and circumferential strips of human umbilical cord artery from infants of various gestational ages.

Methods

Tissue Acquisition and Preparation

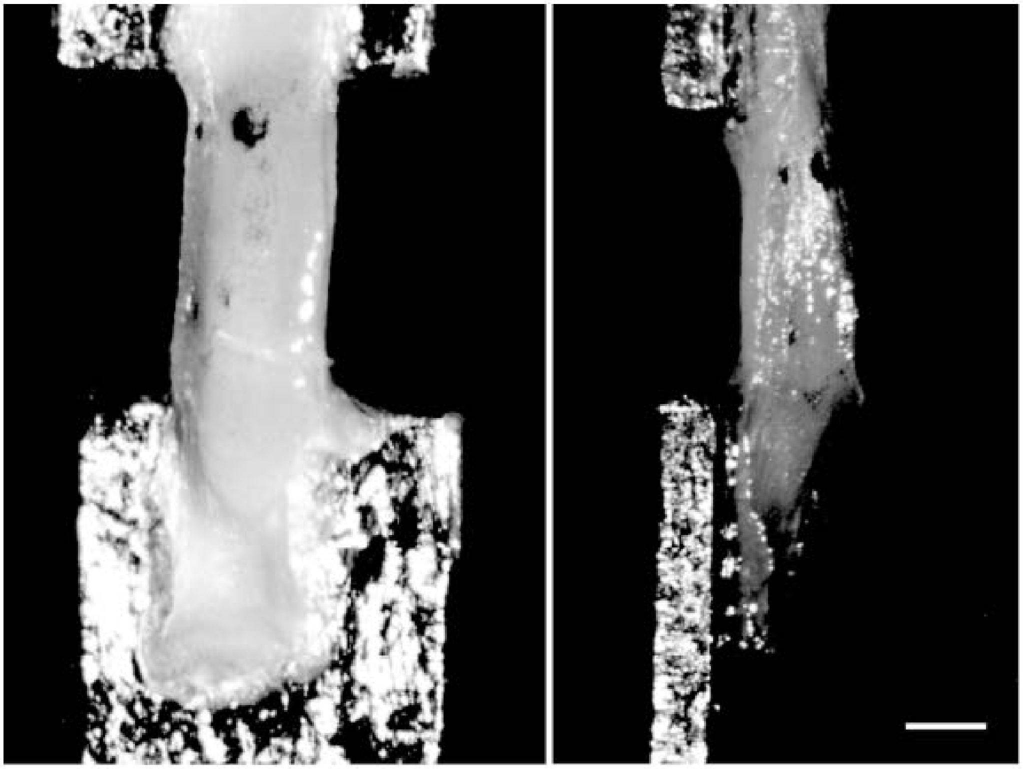
Specimens were collected from normal umbilical cords following births with 24-40 weeks of gestation at Intermountain Medical Center in Murray, Utah (IRB# 1014329). Samples were 5 cm long and were taken approximately 3 cm from the placental insertion site. The cord segments were placed in 0.9% saline solution and kept in a cold refrigerator until they were retrieved and transported to the laboratory for testing. All specimens were transported to the University of Utah on ice and tested within 24 hours of parturition.[10] Upon arrival to the lab, cord samples were placed in a dish containing cold, calcium free, phosphate buffered saline (PBS); lack of calcium precluded smooth muscle contraction during tests.

Vessels were dissected by inserting the tip of a pair of micro scissors between the artery and the surrounding Wharton's jelly and making an incision through the jelly along the path of the artery. Tissue forceps were used to hold the end of the artery, while the connective tissue surrounding the artery was cut away with micro scissors.

Once the surrounding tissue was removed, cross-sections and axial and circumferential strips were cut from the dissected vessels; both axial and circumferential strips were cut at the same width ($w=1.736\ mm$), using a prefabricated cutter, at lengths between 3 and 5 mm for circumferential strips and 8 and 10 mm for axial strips.

Experimental Setup and Procedure

To prepare each specimen for testing, it was removed from the PBS bath and excess fluid was wicked away with a low-lint wipe. The luminal side of each end was then placed onto a 5 mm X 5 mm X 1.59 mm stainless steel plate with a small amount of cyanoacrylate glue covering its surface (Figure 1). The stainless steel plates were fixed to 18 gauge needle lures, which were tightened into pre-fabricated acrylic blocks built to interface with our vessel testing system.[11] An adjustable support arm was used to fix the distance between the two acrylic blocks during specimen attachment; it was removed once the blocks were attached to the testing fixture. A 1000 g capacity load cell (Model 31 Low, Honeywell, Golden Valley, MN) was mounted in-line with the specimen. Stretching of the tissue specimen was carried out at a rate of 0.1 mm/sec with either a voice coil actuator (MGV52-25-1.0, Akribis) or a custom linear stage (Parker Automation, Cleveland, OH) driven by a stepper motor (H-2222-3, Intelligent Motion Systems, Malborough, CT). A digital camera (PL-A641, Pixelink, Ottawa, Canada) with a high magnification lens (VZM 450i, Edmund Optics, Barrington, NJ) captured side view images of specimens during testing. Test control as well as data and image acquisition were accomplished using a LabVIEW (National Instruments, Austin, TX) program written by our group. Transducer and video data were acquired at 100 and 3 Hz, respectively.



Front and side views of an axial umbilical artery strip mounted in the uniaxial tension tester. The luminal sides of the artery strips were adhered to stainless steel plates with cyanoacrylate glue. In some cases (including that shown), small black microspheres were applied to the abluminal side to enable tracking of tissue motion. Scale bar is 1 mm.

Figure 1

Once the specimen was attached to the testing machine and the support arm was removed, the estimated specimen reference length was obtained by stretching the vessel strip from an unloaded (slightly buckled) state to a stretched state in 0.1 mm increments. Stretch increments were applied to the specimen until a slight increase in force was observed, at which point the specimen was unstretched 0.1 mm and the distance between the steel plate ends was taken as the estimated reference length. During testing, PBS was periodically dripped onto the specimen to prevent dehydration. The strips were then preconditioned between 1 and 1.2 times the estimated specimen reference length through 7 cycles at a rate of 0.1 mm/sec. Given that stretch values over 2.0 were commonly observed in the subsequent failure tests, a stretch level of 1.2 times the estimated reference length was deemed to be reasonable for basic preconditioning.

Following the preconditioning cycles, the specimen was stretched from a relaxed state to failure, or until it detached from the plates.

Data Processing

Failure test data were processed to calculate the overall stress–stretch response. Specimen reference length (l_0) was defined as the distance between two natural fiducial markers in the specimen midsection. Its value was determined from an image at the beginning of the test corresponding with a tare load value of 0.0035 N. The distance between these two fiducial markers was measured in six additional, equally spaced images, up to 60 kPa in axial specimens and 30 kPa in circumferential specimens. The ratio of the distance between the markers in each image (l_i) and the reference length (l_0) was defined as the stretch ratio (λ). The calculated stretch values were observed to increase linearly with time, so a line was fit to the seven evaluated points. Intermediate stretch values were defined through interpolation to provide a one-to-one relationship with the stress data. First Piola-Kirchhoff stress (P) was calculated as the measured force (F_i) divided by the reference cross-sectional area (Eq. 1),

$$P = \frac{F_i}{t_0 w_0} \qquad \text{Eq. 1}$$

where w_0 is unstretched specimen width and t_0 is unstretched specimen thickness; t_0 was measured from the image corresponding to l_0.

The resulting stress-stretch curves were characterized by quantifying both stiffness and stretch at two levels of stress. Specimen stiffness was defined as the slope of the stress-stretch curve at stress values of 40 and 20 kPa for axial specimens and of 20 and 10 kPa for circumferential specimens. These particular stress values were chosen because all stress-stretch curves passed through them prior to the occurrence of any failure or slippage of specimens. The selected stress values also correspond well with estimated in vivo stresses. The stretch ratio associated with each stress level was also recorded.

Images of umbilical artery cross sections were taken using a microscope (Stemi 200-C, Zeiss, Thornwood, NY) with an attached digital camera (PL-A662, Pixelinx, Ottawa, Canada). Binary images of the inner and outer areas of the vessel cross-section were created in MATLAB (MathWorks, Natick, MA) using the function ‘roipoly’. Outer and inner binary images were added to obtain a single binary image of the vessel cross section. Cross-sectional area was then calculated by summing the total number of pixels in the binary image and multiplying by the determined conversion factor.

Statistical Analysis

Umbilical samples were grouped into four gestational age ranges (in weeks): 36-40, 31-35, 26-30, and 25 or less (LTE 25). One-way ANOVA was performed to evaluate the significance of gestational age on measured stiffness and stretch values and on cross sectional area. The significance of material direction was also explored. Post-hoc t-tests, using a Bonferroni correction factor, were performed where ANOVA indicated significance. Linear regression was also used to evaluate the influence of age on the measured values. In all cases, $p \leq 0.05$ was considered significant. The statistical analysis of the data was performed using StatPlus (Analystsoft Inc. Vancouver, Canada).

Results

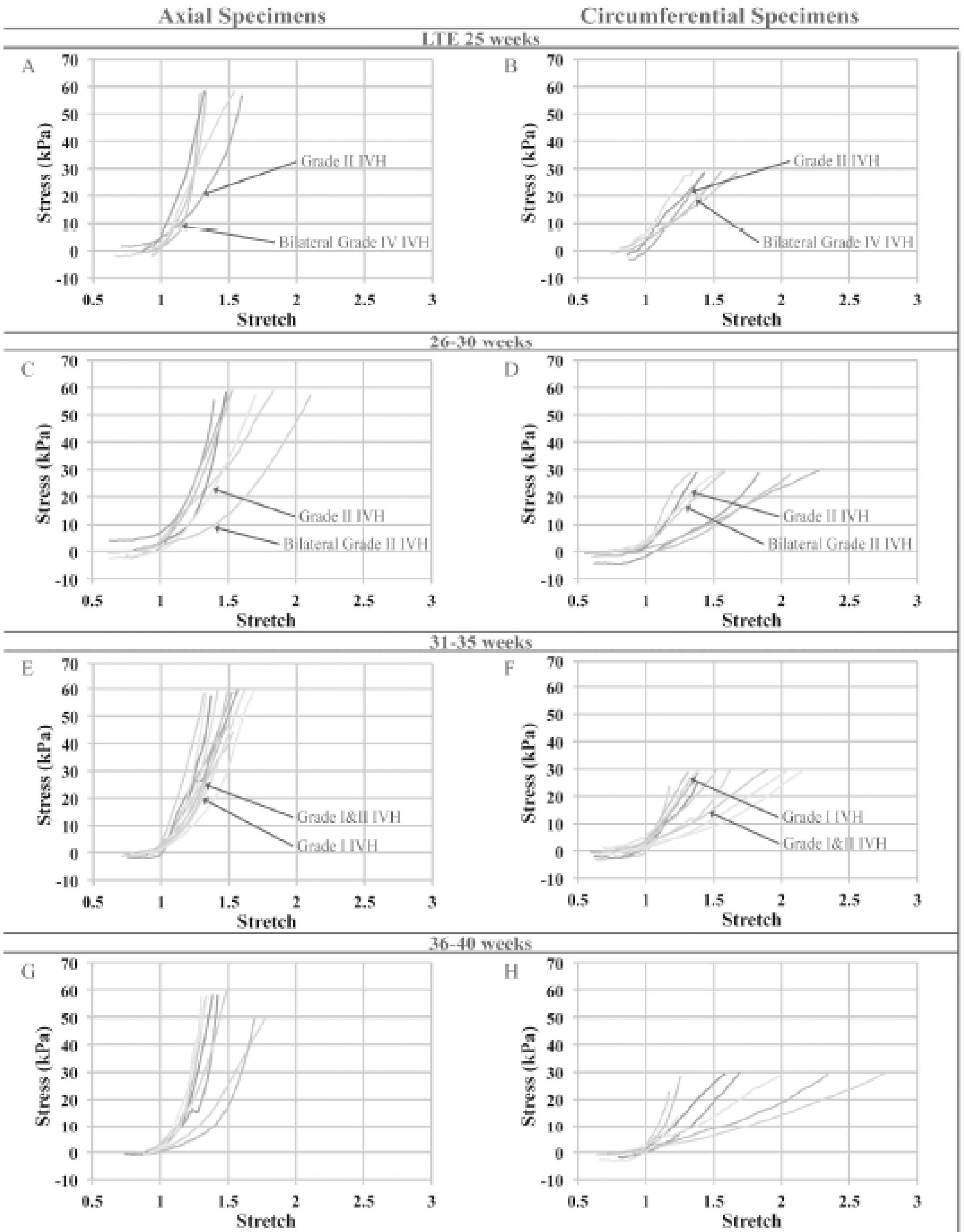
A total of 31 umbilical cord specimens was obtained (Table 1); six (all born at less than 32 weeks gestation) of the donors went on to experience some form of IVH. Handling of the cord samples suggested that those at later gestational ages were less compliant and denser than earlier samples. Arteries from later gestational ages were also generally easier to dissect compared to earlier vessels because of weaker adherence to the surrounding Wharton's jelly. Although not quantified, vessels (and cords) from older infants tended to be more coiled than those from earlier births.

Table 1) Axial and circumferential stiffness and corresponding stretch values at stress levels of 20 and 40 kPa and 10 and 20 kPa, respectively, along with p-values calculated for ANOVA and linear regression (linreg).

	Gestational Age (weeks)					
	LTE 25 (n=5)	**26-30** (n=7)	**31-35** (n=11)	**36-40** (n=8; *n=7)	**p (ANOVA)**	**p (linreg)**
			Stiffness (kPa)			
Axial (40 kPa)	285.10 ±197.16	148.68 ±66.58	181.96 ±64.10	217.45 ±68.83	0.1198	0.5291
Axial (20 kPa)	153.36 ±71.45	94.39 ±32.90	119.39 ±31.15	147.38 ±61.63	0.1214	0.7367
Circ (20 kPa)	57.89 ±11.59	55.51 ±23.68	76.53 ±47.80	80.83 ±73.33*	0.6776	0.1490
Circ (10 kPa)	58.63 ±22.76	51.26 ±26.83	57.81 ±38.46	58.29 ±38.69	0.9736	0.5370
			Stretch			
Axial	1.33±0.11	1.52±0.20	1.39±0.09	1.41±0.17	0.1728	0.8984

(40 kPa)						
Axial (20 kPa)	1.21±0.07	1.32±0.15	1.24±0.07	1.29±0.18	0.2382	0.6258
Circ (20 kPa)	1.34±0.12	1.50±0.28	1.44±0.24	1.62±0.44	0.4290	0.3341
Circ (10 kPa)	1.15±0.08	1.27±0.18	1.23±0.16	1.33±0.23	0.3416	0.2430

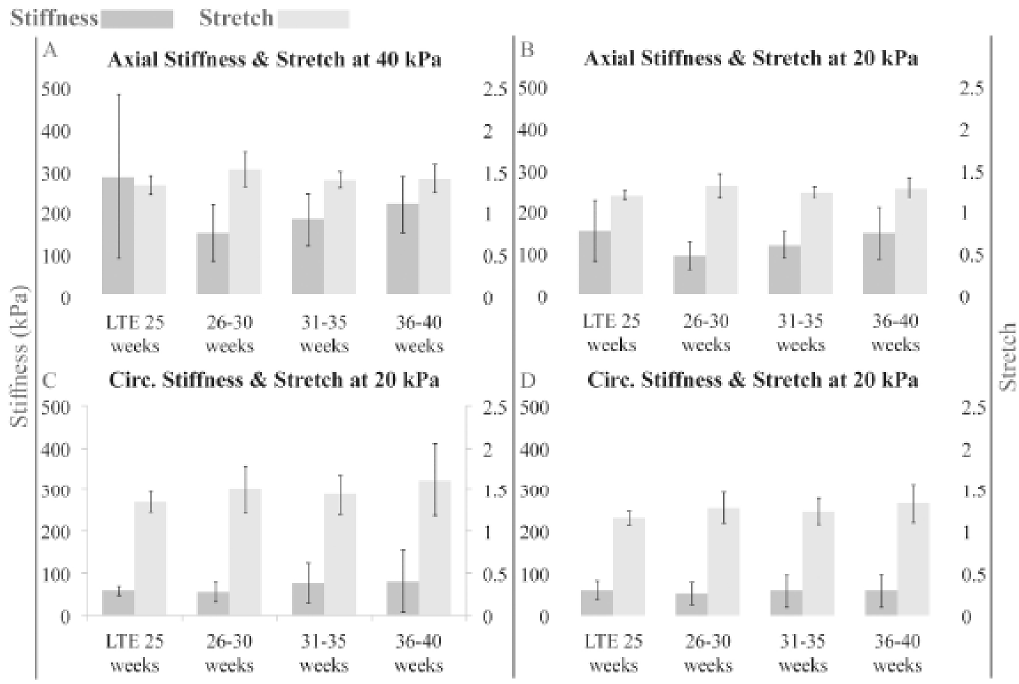
The stress-stretch curves of both the axial and the circumferential specimens under uniaxial tension were generally non-linear (Fig. 2), similar to other soft tissues. Data from each test are presented up to the maximum stress value to which fiducial markers were tracked. Although failure of the specimens was not achieved in many cases due to premature detachment from the loading plates, each specimen was stretched well beyond typical in vivo loading values (often to multiple times their unloaded length before failure; data not shown). As is common for biological tissue, there was significant scatter within each group. There were no obvious differences between curves as a function of gestational age, but circumferential specimens tended to be more extensible than axial strips. Stress-stretch curves from the small number of infants that did experience IVH demonstrated no apparent differences from those that did not, though numbers were small.



Stress-stretch curves of umbilical artery specimens. Axial (A, C, E, G) and circumferential (B, D, F, H) specimens from LTE 25 (A, B), 26-30 (C, D), 31-35 (E, F) and 36-40 (G, H) week groups. Curves from infants that experienced IVH (grades I-IV; some bilateral) are annotated.

Figure 2

Mean stiffness values of both axial and circumferential specimens are summarized by gestational age in Figure 3. Consistent with observations of the stress-stretch plots, one-way ANOVA demonstrated no statistical significance of gestational age in stiffness for either the axial (p=0.12 at 40 kPa and p=0.12 at 20 kPa) or circumferential (p=0.68 at 20 kPa and p=0.97 at 10 kPa) direction. Linear regression of stiffness versus gestational age produced similarly high p-values (Table 1).



Mean axial stiffness and stretch values evaluated at (A) 40 kPa and (C) 20 kPa and mean circumferential stiffness and stretch values evaluated at (B) 20 kPa and (D) 10 kPa.

Figure 3

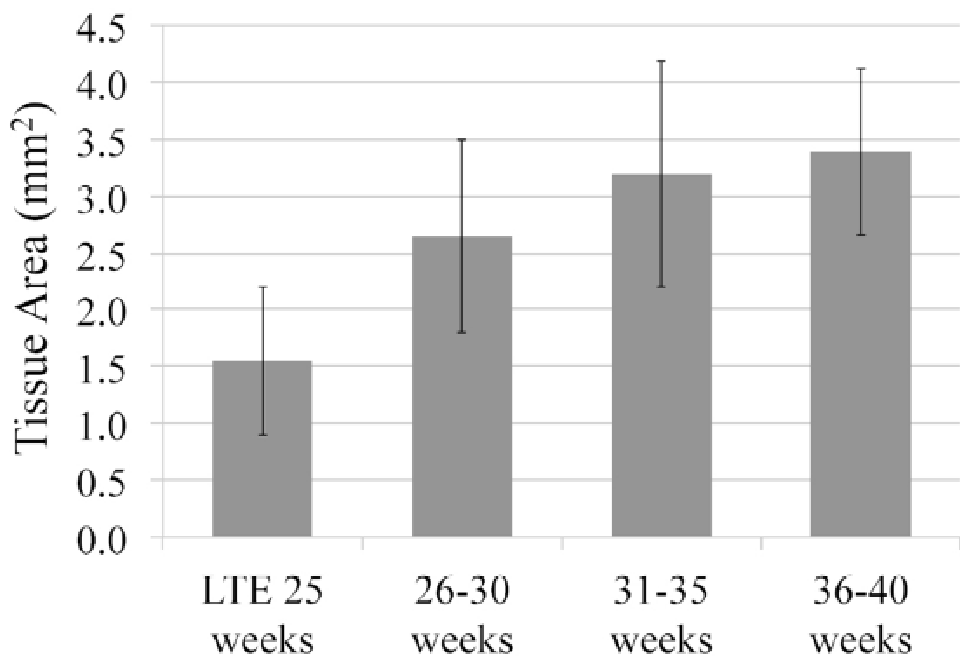
Mean stretch ratios at the evaluated stress levels are also summarized in Figure 3. As with specimen stiffness, age did not have a statistically significant effect on either axial (p=0.16 at 40 kPa and p=0.24 at 20 kPa) or circumferential (p=0.43 at 20 kPa and p=0.34 at 10 kPa) stretch. Of note, linear regression of stretch versus gestational age demonstrated a positive slope, but the trend was not statistically significant (Table 1).

In measurements corresponding to the same umbilical artery, it was possible to evaluate differences between material directions at 20 kPa (Table 2). Results show that specimens were, on average, 2.45 times stiffer and 0.88 times less extensible axially than circumferentially, with no apparent trend in these relationships with age.

Table 2) Ratios of axial to circumferential stiffness (stif ratio) and stretch (str ratio) determined at 20 kPa, along with p-values from ANOVA and linear regression evaluating directional differences between ratios within the same age group.

	Gestational age (weeks)					
	LTE 25	**26-30**	**31-35**	**36-40**	**p (ANOVA)**	**p (linreg)**
Stif Ratio	2.88 ± 1.74	2.07 ± 1.28	2.13 ± 1.34	3.04 ± 2.03	0.5365	0.9669
Str Ratio	0.90 ± 0.08	0.92 ± 0.22	0.88 ± 0.13	0.83 ± 0.17	0.7883	0.5535

As expected, the mean cross-sectional area of umbilical arteries increased with gestational age (Fig. 4). ANVOA performed between groups indicated significance (p=0.004), and post-hoc testing showed significance between LTE25 and the 31-35 and the 36-40 week groups (p=0.002 and p=0.001, respectively). Typical unloaded outer diameter and wall thickness measurements increased from approximately 1.0 to 2.3 mm and from 0.3 to 0.9 mm, respectively, from LTE25 to term.



Cross sectional tissue areas of umbilical arteries. 36-40 weeks 3.39 ± 0.73 mm^2 (n=8), 31-35 weeks 3.19 ± 1.00 mm^2 (n=12), 26-30 weeks 2.64 ± 0.85 mm^2 (n=7) and LTE 25 weeks 1.54 ± 0.65 mm^2 (n=5).

Figure 4

Discussion

The objective of this research was to investigate the effect of gestational age on the mechanical properties of umbilical cord arteries. While earlier cord samples were qualitatively different from later samples in size and coiling, results from experiments show that artery mechanical properties did not change significantly with gestational age.

The finding that properties did not change with age was unexpected. This was especially true given the observation that cross-sectional area, as well as gross cord features like apparent compliance and coiling, did change with age. Others have also reported changing blood flow rates over the same period.[12] Regardless, our findings suggest that the remodeling and growth processes in these vessels occur in such a way as to maintain a consistent mechanical response.

The observed greater stiffness in the axial, compared to the circumferential, direction of loading is notable. It is important to state that the natural curvature of circumferential specimens made identification of the reference length more challenging and possibly led to overprediction of stretch values in this direction. However, this influence was not so dramatic that it would have significantly changed conclusions regarding the relative stiffness. Functionally, the cord arteries experience consistent loading from blood pressure and flow; these loads are likely influential in vessel development, with the primary need being to accommodate circumferential loading. However, other tensile and compressive loads also occur as the fetus moves relatively freely within the uterus. The coiled nature of the cord likely helps minimize any severe longitudinal stretching of the cord material under these conditions, allowing for a greater stiffness of the material in the commonly unloaded axial direction.

A primary motivation for this study was to investigate any changes in umbilical vessel integrity with gestational age, as a possible reflection of intracranial vessel development and the incidence of IVH. It is important to note that our findings are limited to the umbilical artery and may or may not apply to intracranial vessels. This question needs to be addressed more directly. On a related note, during the course of this study, a small number of the cord samples collected were from infants that went on to experience some grade of IVH. While these cases were too limited (6 cases) to draw any firm conclusions, there was no apparent difference in their artery properties, suggesting that cord artery mechanical properties are likely not an effective predictor of IVH susceptibility. However, further exploration of cord mechanical properties and other pathological conditions that affect fetus and infant health, including cord abnormalities, may lend to more positive correlations.

While the presented results are considered reliable, some methodological limitations should be noted. First, we assumed that uniaxial tests would provide sufficient information to draw conclusions on any change in artery properties with gestational age. We have previously conducted biaxial tests of intact vessels to explore their response,[11] but the natural coiled structure of the umbilical arteries prevents direct study of pure material properties in the intact configuration. While biaxial tests provide significantly greater ability to explore mechanical behavior, there is little reason to expect that gestational age would be found to have a more significant response in biaxial tests than in uniaxial experiments. Another limitation to our study design was the bonding of samples on only the luminal side, potentially leading to non-uniform stretch through the specimen thickness. However, application of the cyanoacrylate tended to secure both the luminal and abluminal sides such that the whole of the tissue adjacent to the plate was hard to the touch. This appeared to help reduce any non-uniformity; analysis of microsphere displacement on the abluminal side of one specimen resulted in a difference of less than 10 percent between luminal and abluminal stretch ratios. Another methodological consideration is the use of a calcium-free solution in these tests. Without this, contributions from smooth muscle would likely have influenced our findings, especially in the circumferential direction. It has been shown that contractility of umbilical vessels is altered by factors such as diabetes, smoking[13] and arterial flow velocity,[14] so smooth muscle behavior appears to have prognostic value in some conditions. However, the use of calcium-free solution in the present study was necessary to allow isolated study of passive vessel response.

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