Published in final edited form as:

Eur J Obstet Gynecol Reprod Biol. 2013 July ; 169(2): 207–212. doi:10.1016/j.ejogrb.2013.03.010.

Determination of hyperelastic properties for umbilical artery in preeclampsia from uniaxial extension tests

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Abstract

Objective—Preeclampsia often results in altered hemodynamics and structurally remodeled umbilical arteries in the fetus – alterations that may be associated with arterial stiffening. We therefore hypothesized that the mechanical function of preeclamptic (PE) umbilical arteries had increased stiffness compared to control.

Study design—Umbilical arteries were collected from control ($n = 9$) and PE ($n = 6$) pregnancies without any other complications. Samples were tested uniaxially in axial and circumferential directions for the passive mechanics. The umbilical artery was modeled as a fiber reinforced hyperelastic material in both control and PE conditions.

Results—The PE arteries were stiffer than control arteries at stresses of 20–160 mmHg in the axial direction and 65–200 mmHg in the circumferential direction ($P < 0.05$). The PE umbilical arteries exhibited a 58% and 48% increase in circumferential moduli at the systolic and diastolic blood pressure respectively compared to the controls ($P < 0.05$). A hyperelastic model showed a substantial increase in both isotropic and anisotropic contribution in the mechanical behavior. Collectively, the changes observed correlated to a higher collagen fiber density in the PE group with increased hyperelastic material parameters ($P < 0.05$).

Conclusion—PE umbilical arteries demonstrated stiffer biomechanics compared to the controls due to the change in collagen fiber content. These altered biomechanical and structural changes provide a potential snapshot into systemic vasculature remodeling occurring in the newborn.

Keywords

Biomechanics; Vascular remodeling; Extracellular matrix; Collagen

1. Introduction

Maternal diseases of pregnancy have been found to detrimentally affect the fetal circulatory system, with consequences lasting well into adulthood. In 1995, Barker introduced the idea

that major disorders of adult life (including coronary heart disease, hypertension, stroke and diabetes) arise not only through an interaction between factors in our lifestyle (such as a high-fat diet, obesity, and smoking) and a genetically determined susceptibility, but also through development *in utero* [1]. Epidemiological evidence continues to support the notion that adult cardiovascular disease (CVD) has fetal origins [1–6], with the environment *in utero* playing an important role in phenotypic expression of vascular disease.

Adult CVD is characterized by increased stiffening of arteries. The extracellular matrix (ECM) which comprises the structural strength of the artery rapidly forms during late gestation and in the newborn [7–10]. Alterations in vascular formation *in utero* could directly impact downstream adult cardiovascular health. Additionally, Burkhardt has shown that the maternal disease of intrauterine growth restriction (IUGR) plays a major role in human umbilical artery (UA) stiffness [7]. To the best of our knowledge, however, the human UA has not yet been studied in preeclampsia (PE), a maternal disease that affects 8% of pregnancies.

Preeclampsia is a disease of pregnancy in which hypertension arises in association with significant amounts of protein in the urine and is characterized by placental dysfunction. It is well established that PE fetuses develop altered umbilical cord hemodynamics, morphology, and composition. *Ex vivo* measurements have shown that preeclamptic umbilical arteries have reduced internal circumference, reduced transverse cross-sectional area, and reduced wall mass [11]. Biochemical characterization has shown that the PE UA contains decreased levels of collagen-degrading enzymes including matrix metalloproteinases: MMP-1 (collagenase 1), MMP-2 (gelatinase A), MMP-3 (stromlysin 1), and MMP-9 (gelatinase B) [12]. The reduced availability of MMPs to degrade collagen may contribute to the altered collagen-elastin ratio observed in preeclampsia along with increased amounts of sulfated proteoglycans concomitant with decreased hyaluronic acid [12–18]. The collective pathologies of the umbilical cord structures in PE may result from and/or contribute to altered hemodynamics.

The biomechanical changes to the UA associated with the altered structure in preeclampsia have thus far received little attention. As an extension of the fetal cardiovascular system, studying the human umbilical arteries may provide a unique opportunity to understand the fetal vasculature through vessels that branch directly from the iliac arteries [19]. Study of the biomechanics of the UA provides an important characterization of the physiologic arterial function in health and disease. The objective of this work was to assess the mechanical response of human umbilical arteries isolated from both normal and preeclamptic pregnancies and to interpret our findings in the context of numerous published studies that document alterations in hemodynamics and arterial extracellular matrix composition [12–18,20]. We hypothesize that PE infants have increased UA stiffness compared to control infants and that the biomechanical function can be related to the ECM structure through use of a phenomenologic model.

2. Materials and methods

2.1. Tissue collection and preparation

Umbilical cords were collected from consented subjects at the University of Colorado Hospital (Aurora, CO; COMIRB 06-1159) and the Boulder Community Hospital (Boulder, CO; IRB 1007.16) during March 2010 to February 2012 from control ($N = 9$) and preeclamptic patients ($N = 6$: 5 severely preeclamptic, 1 mild preeclamptic) without any other complications. Umbilical cords were collected from babies with weight appropriate for gestational age. Pregnancies with intrauterine growth restriction (IUGR) were excluded. The research goals of this work are intended to examine only those subjects with PE. Therefore,

only subjects with classical presentations as assessed by our clinical partners were accepted into the study. Subjects with other complications of pregnancy were excluded from this study. The UA were isolated from the region of umbilical cord halfway between the placental and fetal insertion, avoiding any areas showing physical abnormality, and Wharton's Jelly was carefully removed by sharp dissection. The UA was cut into successive circumferential and axial test strips ($N = 3$ axial and 3 circumferential test specimens per artery). The specimen initial gage length (l_0), width (w_0) and thickness (t_0) were measured prior to testing using digital calipers.

2.2. Mechanical testing

Uniaxial tests were performed within 72 h of delivery using an MTS Insight II (MTS Systems, Eden Prairie, MN) equipped with a 5-N load cell and environmental chamber filled with calcium- and magnesium-free phosphate buffered saline (PBS, pH 7.4, 37 °C). Uniaxial extension was applied at a constant crosshead speed of 0.5 mm/s, executing five successive preconditioning cycles to a prescribed elastin and collagen activation strain. The tensile force and specimen length were continuously recorded (10 Hz, Test-works 4 software).

2.3. Histology

Test specimens were fixed in 10% neutral-buffered formalin. Representative samples (control $N = 3$ and PE $N = 3$) were paraffin embedded, sectioned and stained for collagen and elastin using Elastic-Van Gieson (EVG) stain. Bright-field images were captured using Zeiss Axioskop 40FL (Carl Zeiss Inc.; Germany) at 40 $\times$ equipped with a Nikon SPOT RT-900 camera (Nikon Instruments; Melville, NY).

Three representative test samples of control and PE arteries were processed for second harmonic generation (SHG) imaging. Test samples were fixed using 10% neutral buffered formalin, ethanol dehydrated, and rehydrated using PBS. The SHG imaging was completed as previously described [21]. Images were quantified for in ImageJ (NIH, Bethesda, MD) for fiber area density by image threshold and collagen fiber orientation using a custom analysis macro OrientationJ [22].

2.4. Data analysis

The final loading cycle of the uniaxial preconditioned sample was analyzed. From the recorded data, the Cauchy stress (σ) was calculated by dividing the measured force (F) by the unstressed area (A_0 = initial width, w_0 , by initial thickness, t_0) and the stretch ratio (λ):

$$\sigma = \frac{F}{A_0 \lambda} = \frac{F}{w_0 t_0 \lambda} \quad (1)$$

where the stretch ratio (λ) is the current length (l) divided by the initial length (l_0). Stress-stretch data were fitted using a 9th order polynomial. Significance of each coefficient was determined using the Linfield and Penny method [23] examining the Student t -ratio and variance-influencing factor (VIF) of the least squares fit. Data were interpolated at constant stresses to compare control and preeclamptic groups. The elastic modulus (E) was calculated as the first derivative of the stress (σ) over stretch (λ):

$$E = \frac{d\sigma}{d\lambda} \quad (2)$$

and determined at stresses of 52 mmHg and 42 mmHg, which correspond to the systolic and diastolic pressures in the human UA [24]. Data were compared using a Mann-Whitney non-

parametric test to examine individual data points and a two-way ANOVA to compare repeated measures significance at $P < 0.05$.

The artery was modeled as a single layer, hyperelastic material using a phenomenologic hyperelastic model developed by Holzapfel and Gasser [25]. The hyperelastic model characterizes the material anisotropy present within the arterial structure by describing the contribution of collagen fibers to the mechanical response. The orientations of two symmetric families of fibers are defined in the reference, or initial, configuration through two unit vectors $\mathbf{a}$ and $\mathbf{a}'$, transposed with an angle $\pm\gamma$ from the vessel axis assuming for simplicity there was no fiber distribution (Fig. 1). The strain energy function proposed is as follows [25]:

$$\psi = \frac{\mu}{2}(I_1 - 3) + \frac{k_1}{2k_2} \sum_{i=4,6} [\exp(k_2(I_i - 1)^2) - 1] \quad (3)$$

where I_1 is the first invariant of $\mathbf{C}$ ($I_1 = \text{tr}(\mathbf{C})$), the following two invariants are defined as $I_4 = \mathbf{a} \cdot \mathbf{C} \cdot \mathbf{a}$ and $I_6 = \mathbf{a}' \cdot \mathbf{C} \cdot \mathbf{a}'$, and μ , k_1 , k_2 , γ are material parameters (all positive values). The equation describes the macroscopic response of the material to mechanical loading. In Eq. (3), the first term represents a neo-Hookean isotropic response and describes the elastin and ground substance response to loading. The remaining terms describe the anisotropic behavior of the material with directions of the collagen fibers defined.

The previous hyperelastic model has no closed form solution for the Cauchy stress σ_1 (stress in the direction 1 with loading) and the related stretch λ_1 . Therefore a straightforward derivation based upon the work by Garcia-Herrera [26] was used to determine the material parameters. The material response as determined by using uniaxial testing is governed by

$$\sigma_1 = \mu \left(\lambda_1^2 - \frac{1}{\lambda_1^2 \lambda_2^2} \right) + 2 \frac{d\psi}{dI_4} \lambda_1^2 \cos^2(\gamma - \alpha) + 2 \frac{d\psi}{dI_6} \lambda_1^2 \cos^2(\gamma + \alpha), \quad (4)$$

$$\sigma_2 = \mu \left(\lambda_2^2 - \frac{1}{\lambda_1^2 \lambda_2^2} \right) + 2 \frac{d\psi}{dI_4} \lambda_1^2 \sin^2(\gamma - \alpha) + 2 \frac{d\psi}{dI_6} \lambda_1^2 \sin^2(\gamma + \alpha) = 0, \quad (5)$$

where α denotes the sample orientation ($\alpha = 0^\circ$ and 90° is axial and circumferential samples respectively). The system of equations was fitted using the methods previously described [26] to find the material properties μ , k_1 , k_2 , and γ .

3. Results

Maternal patients who contributed placental tissues for this study exhibited no other complications of pregnancy other than preeclampsia (Table 1). In this limited population, PE patients tended to be older and carried pregnancies to a shorter gestational age, with no significant difference observed between controls and PE patients.

Preliminary EVG sections confirmed what previous research had shown: reduced collagen to elastin content noted by a less pronounced staining for elastin in preeclamptic artery compared to the normal arteries (Fig. 2). This further motivated our study of the biomechanics of the PE arteries. The UA exhibited an increased stiffness in PE infants compared to the control infants as shown in the mean representative stress-strain plots for tests in the axial and circumferential tests (Fig. 3). PE arteries were significantly stiffer at equivalent stresses 2.7–21.3 kPa (20–160 mmHg) for the circumferential direction and 8.7–

26.7 kPa (65–200 mmHg). The moduli (mean $\pm$ standard error of the mean) at systolic/diastolic pressures in the axial direction were 178.9 ± 8.6 kPa/ 139.9 ± 7.3 kPa for control infants and 187.3 ± 15.2 kPa/ 151.6 ± 14.4 kPa for PE infants, with no significant change ($P = 0.23/0.17$), while in the circumferential direction the moduli were 171.0 ± 6.7 kPa/ 139.5 ± 6.4 kPa for control infants and 252.7 ± 41.2 kPa/ 220.0 ± 41.0 kPa for PE infants, which constitute a significant change ($P < 0.05/0.05$) (Fig. 4).

The constitutive parameters μ , k_1 , k_2 , and γ based upon the macroscopic mechanical response are summarized in Table 2 for both control and PE arteries. The experimental mean data *versus* the predicted response are shown in Fig. 5. The predicted constitutive parameters demonstrate consistent fiber orientation (γ) across groups, and an alteration in the isotropic (μ) and anisotropic (k_1) nature of the arteries. The SHG images confirm consistency in fiber angle (γ) of $45.1 \pm 1.5^\circ$ for control samples and $44.1 \pm 0.7^\circ$ for PE samples (Fig. 6). The fiber density of PE is higher than that of the control arteries, however, indicating a higher concentration of collagen (CON = $69.9 \pm 1.7\%$ *versus* PE $77.4 \pm 0.9\%$, $P < 0.05$) (Fig. 6).

4. Discussion

Our results describe an increased stiffness of the UA with a structural increase of collagen fiber density in the maternal disease of PE. The hyperelastic model describes the constitutive biomechanical, macroscopic response of the PE umbilical artery and demonstrates a remodeled ECM. The hyperelastic model shows an increase in both the isotropic and anisotropic contribution to the mechanics. Histology indicates this change in modulus occurs *via* a higher density of collagen found in the artery, as confirmed by SHG imaging. PE disrupts the collagen to elastin ratio in the UA, increasing the overall stiffness. Our observed change in biomechanics in conjunction with arterial structure remodeling may result from and contribute to altered hemodynamics.

Arterial stiffening in adolescents and adults has been shown to contribute to CVD, leading to high pulsatility indices, increased vascular resistance, and increased cardiac workload [27–29]. Limited studies, however, have examined the role of neonatal arterial stiffening as a potential contributor to the development of CVD. In the absence of a large animal corollary of PE, the UA provides a natural glimpse into the systemic vasculature of the newborn. Our previous work has shown that the UA is a predictor of systemic vascular remodeling, with umbilical stiffening occurring concomitant with systemic carotid artery stiffening in a sheep model of intrauterine growth restriction (IUGR) [19]. Therefore, a reasonable hypothesis may be derived from these collective findings, that infants whose mothers experience PE may be predisposed to later development of CVD.

Our research fits within a large body of literature that UA remodeling quantified through biochemical assays in PE leads to stiffening. Research by Galewska et al. has shown the PE UA has decreased levels of collagen degrading enzymes including matrix metalloproteinases: MMP-1, MMP-2, MMP-3 and MMP-9 [12]. We show a higher density of collagen fibers within the axial-circumferential plane contributing to the anisotropic stiffening, where collagen accumulation may result from diminished enzymatic activity. In addition, others have shown that increased amounts of sulfated proteoglycans occur concomitant with decreased hyaluronic acid [12–18]. The addition of proteoglycans and reduction in hyaluronic acid would likely increase the isotropic stiffness seen in our constitutive hyperelastic model. Finally, the significant increase in modulus in the circumferential direction agrees with altered hemodynamics, specifically increased pulsatility index, which are observed during routine clinical Doppler ultrasound assessment of PE [20].

Systemic development is signaled through important biomechanical stimuli with the arterial system growing in response to alterations in biomechanical stimuli, which come from blood pressure and flow. Compensatory changes in ECM composition in response to altered biomechanical stimuli are fundamental in cardiovascular physiology and pathophysiology [30,31]. Evidence continually suggests that blood pressure should now be considered an important indicator of vascular remodeling [32] and may play a role in the vascular remodeling seen in PE fetuses. Altered hemodynamics, increased shear stress and possible increased pressures due to placental vascular resistance could play a critical role in altering the vascular development in the PE UA.

Several limitations of our study must be acknowledged. For this study of PE, human subjects were used since they are the most clinically relevant study population. Therefore, no fetal blood pressure or hemodynamics measurements were made *in utero*. Many studies, however, have characterized the alteration of hemodynamics with PE through Doppler ultrasound. Doppler ultrasound has shown that developed PE exhibited increased resistance with reduced velocity and volume flow [33–36]. Additionally, PE UA flows demonstrate increased pulsatility indices [34]. These routinely seen clinical findings are consistent with the arterial stiffening we observed. Likewise, no quantitative biochemical assays were used to characterize the umbilical arteries. These measures are also well characterized in literature, however, and are consistent across multiple studies showing decreased MMP activity, increased sulfated proteoglycans and reduced hyaluronic acid [12–18]. The main purpose of the current study was to develop an understanding of the functional behavior of a critical arterial tissue in PE and we interpreted our results in the context of a large body of previously published data.

In conclusion, we have demonstrated stiffer umbilical arteries in PE infants compared with control infants. It is likely that this difference in arterial tissue function is due to a structure with higher concentration of collagen fibers in the PE arteries and significantly influences *in vivo* function and hemodynamics. This change with altered hemodynamics in PE may alter ECM content and stiffness in the connecting systemic vessels. Therefore, increased arterial vessel stiffness may provide a link between PE and cardiovascular disease later in life.

Acknowledgments

The authors would like to thank Dr. Michael Plotnick of Boulder Medical Center (Boulder, CO) for providing expertise and assistance in collecting umbilical cords. Additionally, we would like to thank Dr. Virginia Winn and Anita Kramer of the University of Colorado Denver Anschutz Medical Campus for their support in collecting samples. SHG images were taken with the expertise at the Advanced Light Microscopy Core, School of Medicine, University of Colorado Denver.

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Fig. 1. Uniaxial testing. Uniaxial strip test showing Holzapfel fiber families (**a** and **a'**) based upon the axis of the test.
Recreated from [26].

Fig. 2.

Elastic-Van Gieson stain of umbilical arteries. EVG staining of preliminary tissue showed reduced number and articulation in elastin fiber rings in PE (b) compared to the control (a) samples.

Fig. 3.

Experimental data curves. Mean experimental data curves with standard error of the means for control (●) and PE (○) umbilical arteries. In the axial direction (a), the PE arteries are significantly stiffer than controls at stresses from 2.7 to 21.3 kPa (20–160 mmHg) ($P < 0.05$). The circumferential tests (b) show the PE arteries significantly more stiff than control arteries for stresses 8.7–26.7 kPa (65–200 mmHg) ($P < 0.05$).

Fig. 4.

Elastic moduli. The axial and circumferential moduli for control (●) and PE (○) umbilical arteries at the mean diastolic (42 mmHg/5.6 kPa) and systolic (52 mmHg/6.9 kPa) blood pressures based upon previous measurements by Margolis [24]. The umbilical moduli are much higher in the circumferential direction of the PE arteries compared to the control, which implies altered biomechanical tissue behavior.

Fig. 5. Data fit. Mean experimental data for control (●) and PE (○) umbilical arteries with the hyperelastic model applied.

Fig. 6.

SHG collagen fibers. Second harmonic generation imaging confirmed that fiber angles did not change between groups with fiber angle (γ) of $45.1 \pm 1.5^\circ$ for control samples and $44.1 \pm 0.7^\circ$ for PE samples and it in good agreement with the hyperelastic model. However, collagen quantity was increased in PE compared to the control with an area density of fibers $77.4 \pm 0.9\%$ in PE and $69.9 \pm 1.7\%$ in control arteries.

Table 1

Patient group data.

Variable	Control (N = 9)		PE (N = 6; 5 severe PE, 1 mild PE)		P value
	Mean ± SEM	(Median [range])	Mean ± SEM	(Median [range])	
Maternal age (years)	29.5 ± 1.8	(31 [22–37])	33.7 ± 4.2	(36 [20–43])	0.52
Gestational age (weeks)	39.1 ± 0.5	(39 [36–42])	37.7 ± 1.2	(37 [34–40])	0.42
5 min APGAR	8.7 ± 0.2	(9.0 [8.0–9.0])	8.7 ± 0.2	(9.0 [8.0–9.0])	0.89
	Number	Percent	Number	Percent	
Infant sex					
Female	5	56	2	33	
Male	4	44	4	67	
Delivery type					
Vaginal	5	56	2	33	
C-Section	4	44	4	67	

Table 2

Hyperelastic model coefficients. Data fit coefficients using the anisotropic hyperelastic model of the umbilical artery in control (CON) and preeclamptic (PE) fetuses showing increased isotropic (μ) and anisotropic (k_1) contribution to stiffening.

Group	μ (kPa)	k_1 (kPa)	k_2	γ (Deg)
CON	5.87	16.6	137.8	44.1
PE	12.1	30.2	214.7	43.0