Biomechanical Characterization of Umbilical Cord Arteries in Normotensive and Preeclamptic Pregnancies

by

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Thesis directed by Professor Virginia L. Ferguson, PhD

Abstract

The umbilical cord (UC) delivers oxygenated blood and nutrients to the fetus *in utero*. As an extension of the fetal cardiovascular system, the UC blood vessels provide information about fetal cardiovascular development. However, little is known about the relationship between the structure and the biomechanical function of tissues within the UC. This thesis establishes normative data on the uniaxial tensile response of healthy UC arteries. The primary objective of this thesis is to establish data for healthy UC arteries in order to provide a benchmark for studying disease states in pregnancy.

It is well established that the maternal vasculature system during pregnancy plays an important role in the development of a healthy fetal cardiovascular system. This thesis also examines the biomechanical and morphological changes exhibited with preeclampsia (PE). PE is the most common complication during pregnancy and is characterized by the onset of hypertension and proteinuria after 32 weeks gestation. PE accounts for 76,000 maternal and 500,000 neonatal deaths annually [1, 2]. Understanding how PE affects the development of the fetus has a high potential to impact many lives by providing targets for the development of therapeutics and other

bioengineering interventions. The secondary objective of this thesis is to understand how PE affects the mechanical response and function of UC arteries.

Uniaxial tensile testing was performed on UC arteries from healthy (n=11) and preeclamptic (n=4) pregnancies. Tissues exhibited a non-linear stress-strain response, which was characteristic of elastin and collagen behaving as the primary load-bearing constituents in UC arteries. Arteries were strained until the collagen-dependent stress-strain response was exhibited. Elastic moduli corresponding to the initial and late linear regions were determined. Results showed that the initial and late elastic moduli for healthy UC arteries was 5.03 ± 1.82 kPa and 1358 ± 388 kPa, respectively. The mode of delivery (vaginal versus Cesarean section) did not affect the mechanical response of UC arteries. Mean length of the UC did not change with PE, whereas mean UC width decreased significantly (P = 0.04) by 22.5%. Initial elastic modulus exhibited a significant increase (P = 0.003) of 86% with PE. The late elastic modulus, however, did not change significantly. Preliminary histological and scanning electron microscopy results showed that elastin content decreased and vessel walls thickened in UC arteries with PE. Overall, UC arteries exhibited a stiffer response at physiological strains.

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Chapter 1

Introduction

1.1 Fetal Circulatory System

1.1.1 Circulatory System *in Utero*

The human fetal circulatory system begins to develop approximately 20 days after fertilization [3]. The most critical development stage of the fetal heart occurs 20 to 50 days after fertilization. During the fifth week, the fetus is connected to the placenta via the umbilical cord (UC). The fetal circulatory system functions very differently than that of born humans. The most important difference is that the fetal lungs are not functional. Oxygen and nutrients are supplied to the fetus via the UC and the placenta. About half the blood traveling to the fetus enters the liver while the remaining blood travels through the ductus venosis. The ductus venosis merges with the inferior vena cava, which leads to the right atrium of the heart (Figure 1.1).

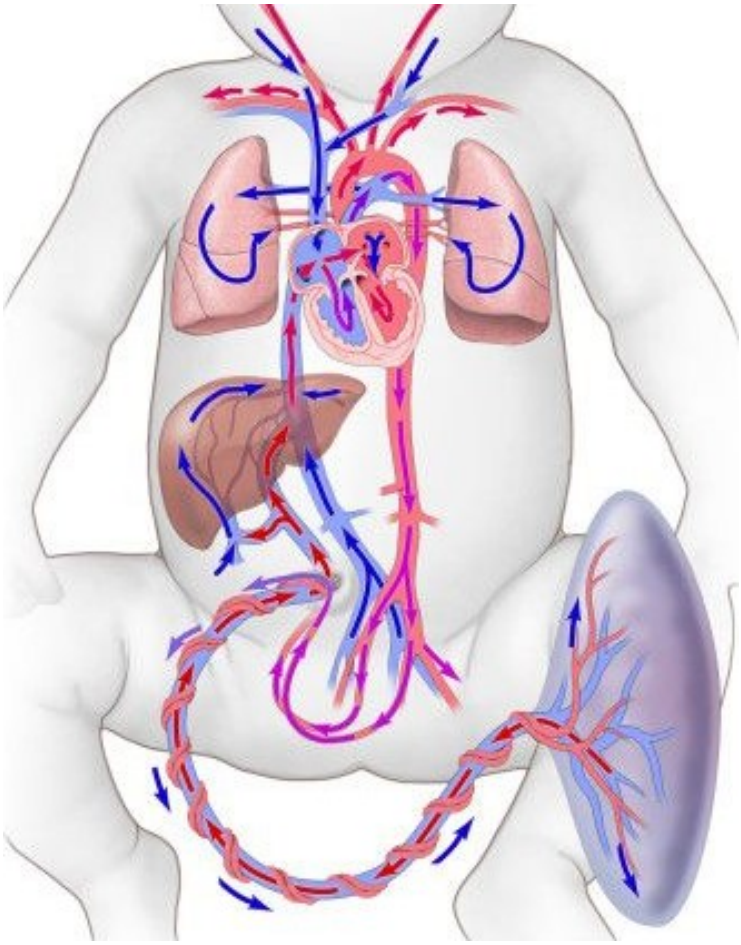

Figure 1.1: Fetal circulatory system *in utero. Taken from: www.gettyimages.com.*

The fetal heart contains three valves which are not present in born humans. These are the foramen ovale, the ductus arteriosis and the ductus venosis. The foramen ovale allows blood to bypass the lungs by travelling directly from the right atrium to the left atrium. The ductus arteriosis connects the fetal pulmonary artery to the aortic arch, also allowing blood to bypass the lungs. The ductus venosis causes oxygenated blood from the placenta to bypass the liver and instead directs it to the fetal brain, where blood is more critical [3].

1.1.2 Neonatal Circulatory System

Within minutes following birth, specific circulatory system changes occur which allow the newborn's lungs to function properly outside of the womb. The umbilical arteries and vein close with cord clamping and cessation of blood flow from the placenta. An increase of pressure in the left atrium and decrease of pressure in the right atrium causes tissue flaps to close over the foramen ovale, thereby fusing the valve and causing blood to circulate through the lungs. The ductus arteriosis also fuses shut as the lungs become functional. In healthy newborns, the ductus venosis closes within one week after birth, allowing blood to travel through the now functional liver. The arterial blood pressure of newborns increases almost 20% shortly after birth [4].

1.2 UC Structure and Function

The UC begins to form approximately 13 days after fertilization [3] and connects the fetus to the placenta during development *in utero*. A normal UC consists of one vein which supplies oxygenated blood to the fetus and two arteries which carry deoxygenated blood back to the placenta. In normal cases, the vessels

coil around each other throughout the length of the cord (Figure 1.2). These three vessels are encased in a gelatinous material called the Wharton's jelly (WJ) which functions to protect and cushion the blood vessels (Figure 1.3).

Because blood is pumped through the umbilical arteries to the placenta by means of the fetal heart, examining these vessels can provide important information about cardiovascular development *in utero*.

Figure 1.2: Umbilical cord blood vessels coiled around each other. *Photo by Megan Schroeder, University of Colorado, 2007.*

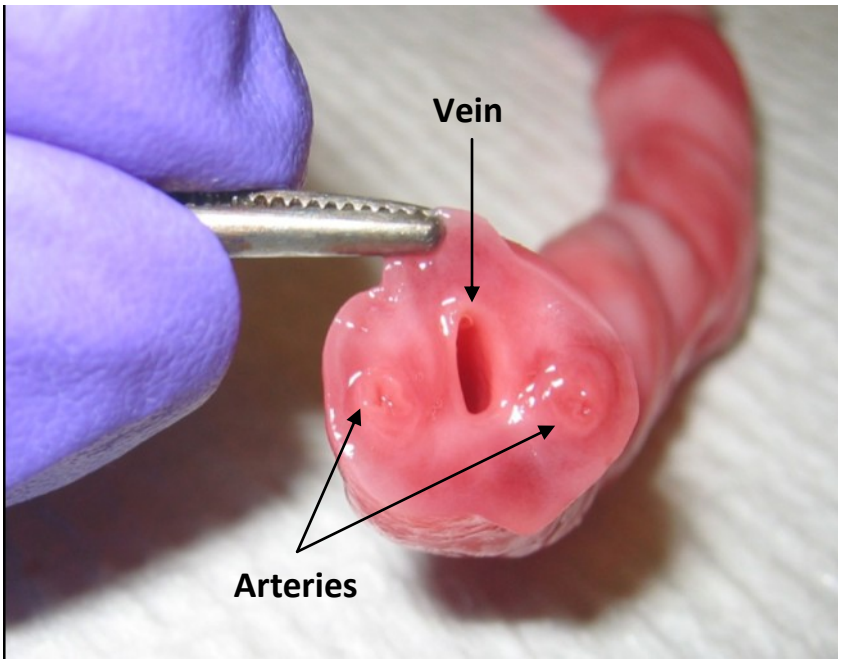

Figure 1.3: Cross section of umbilical cord showing one vein and two arteries. *Photo by Megan Schroeder, University of Colorado, 2007.*

1.3 Structure and Function of Arteries

The primary role of arteries is to distribute oxygenated blood from the heart to the body. Two types of arteries, elastic and muscular, are present in the body. Elastic arteries are responsible for carrying blood to different areas of the body where it will then be distributed by muscular arteries. As the heart pumps between ventricular systole and diastole, the total volume of arterial blood changes. In order to ensure a constant blood flow rate during this change in blood pressure, elastic arteries contain many structural proteins with allow the vessel to radially expand and contract. This distensibility allows arteries to maintain a constant flow of blood [5]. Muscular arteries are further downstream from the heart and do not experience significant

variations in pressure as in elastic arteries. Therefore, muscular arteries contain fewer structural proteins which allow for vessel distension.

The structure of UC arteries is different than that of blood vessels found elsewhere in the body [6]. Most arterial walls consist of three layers: the *tunica intima*, the *tunica media*, and the *tunica adventitia* (Figure 1.4). UC arteries, however, contain no *tunica adventitia*. Instead, the *tunica media* joins directly to the WJ [7].

The innermost layer, the *tunica intima*, surrounds the vessel lumen and consists primarily of endothelial cells and elastin. The function of the endothelial cells is to provide a smooth surface, thereby reducing turbulence in blood flow. This layer also acts as a selective barrier between the vessel lumen and the surrounding tissue by controlling the passage of water, electrolytes, sugars and other molecules into and out of the blood stream [8].

The middle layer of muscular arteries, the *tunica media*, is the largest portion of the arterial wall. This layer differs from elastic arteries because it is made up primarily of smooth muscle cells (SMC). Few elastic fibers are present in muscular arteries and are not arranged into sheets as observed in elastic arteries [9]. Smooth muscle in the arterial wall contracts and relaxes as controlled by the autonomic, or involuntary, nervous system. This functions to regulate blood flow through the artery.

The ability for arteries to function properly depends largely on the mechanical response of the vessels under load. Mechanical properties of muscular arteries are determined by both active (i.e. – smooth muscle contraction) and passive

components. Elastin and collagen are the most significant biomolecular components affecting the passive mechanical response of arteries [10]. These structural proteins account for 50% of arterial dry weight [11].

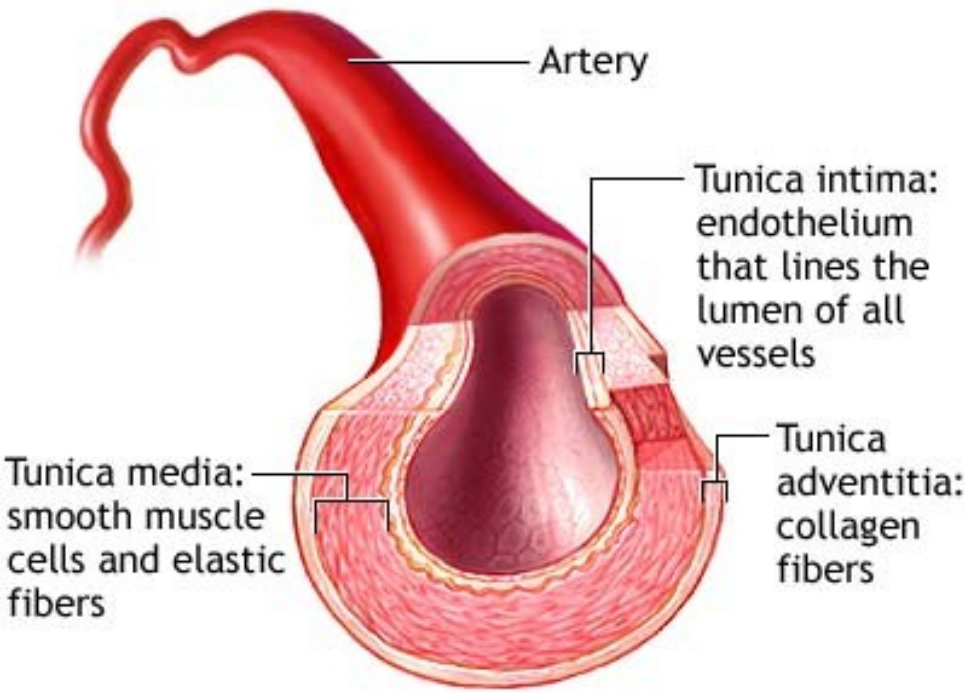

Figure 1.4: Muscular Artery Cut Section. *Taken From: U.S. National Libraries of Medicine, National Institutes of Health*.

1.3.1 Structure and Function of Elastin

Elastic fibres are found throughout the arterial wall and form concentric lamellae. Elastin makes up 90% of elastic fibres found in connective tissue [12]. The

lamellae are slack at low pressured and become taut at physiological pressures [10] (Figure 1.5). This allows the molecules to stretch and recoil after deformation. Elastin is therefore important in tissues which must maintain their shape after stretching such as the lungs, bladder, skin and blood vessels. Although large elastic arteries such as the aorta are rich in elastin, muscular arteries such as those in the UC contain relatively few elastin fibers [6].

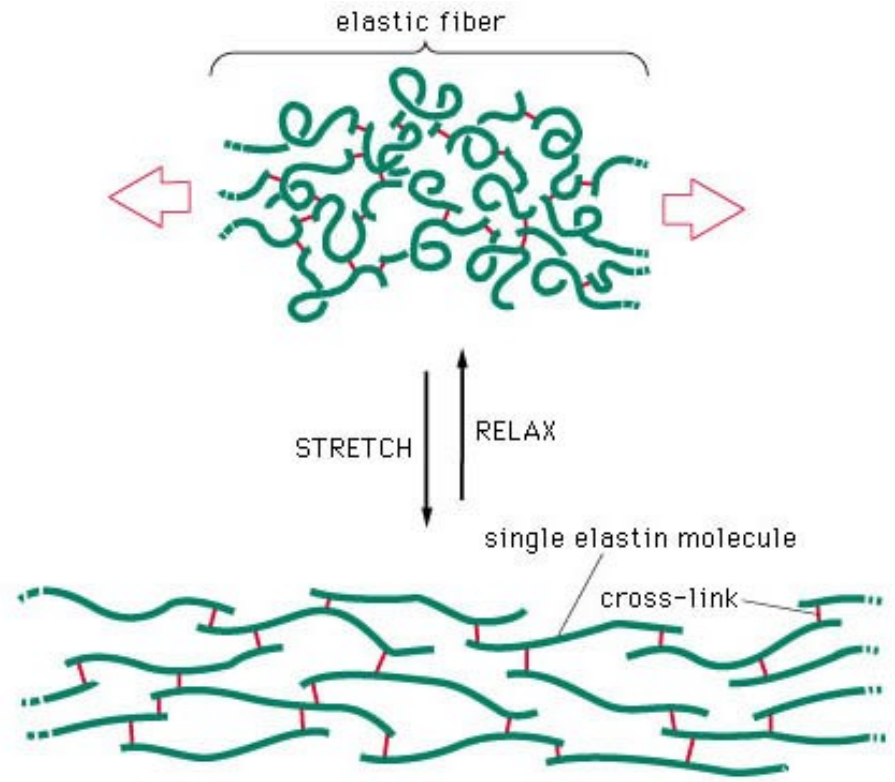

Figure 1.5: Elastic fiber made up of cross-linked elastin molecules. *Essential Cell Biology – 2nd Edition, Garland Science, London (2004)* [13].

1.3.2 Structure and Function of Collagen

Collagen is a protein found in connective tissues throughout the body. It is strong in tension and is therefore an important component of tissues experiencing tensile forces such as tendons, ligaments and blood vessels. Collagen exhibits a

triple-helix polypeptide structure [10, 14] which combine to form a collagen fiber (Figure 1.6). Within the arterial wall, collagen is arranged circumferentially in the *tunica media* and *tunica adventitia* [10]. UC arteries, however, lack the collagen-rich *adventitial* layer. Collagen is slack at both low and physiological pressures. It is only taut above physiological pressures. It is more than 1000 times stiffer than elastin and is important in limiting vessel distention [9, 10].

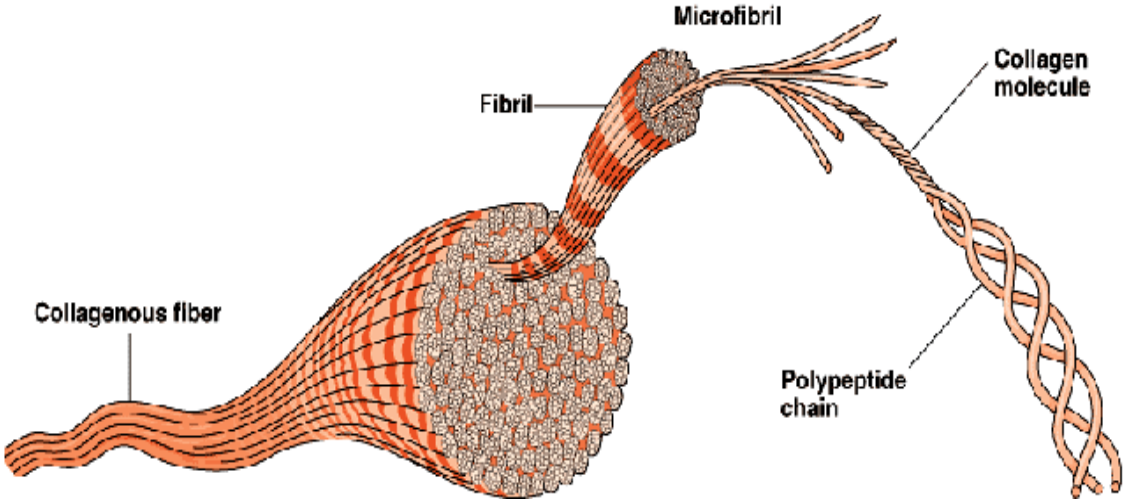

Figure 1.6: Collagen molecules exhibit a triple-helix structure. *Pearson Education, Inc., Benjamin Cummings Publishing, 2006.*

1.3.3 Vascular Remodeling with Hypertension

Previous human vasculature studies have described the affects of hypertension on blood vessels. Multiple studies have shown that increased blood pressure causes changes in the vascular structure as it compensates for increased blood pressure [12]. In particular, vascular remodeling due to hypertension has been shown to cause both

narrowing and stiffening of arterial walls. This occurs as levels of elastin decrease and levels of collagen increase, leading to higher arterial wall stiffness in hypertensive individuals. Some studies hypothesize that hypertension may be due to a factors occurring during development *in utero* which predispose an individual to arterial stiffening [12].

1.4 Tensile Testing of Biological Soft Tissues

Mechanical characteristics of arteries directly influence hemodynamics of the tissue [10]. It is known that biological soft tissues exhibit non-linear elasticity when placed in uniaxial tension [9, 15-17] and that two distinct stiffness moduli are exhibited. The initial stiffness modulus occurs at low strain values and is attributed to the extension of elastin fibers in the tissue. The second modulus occurs at higher strain values and is attributed to the engagement of collagen fibers (Figure 1.7).

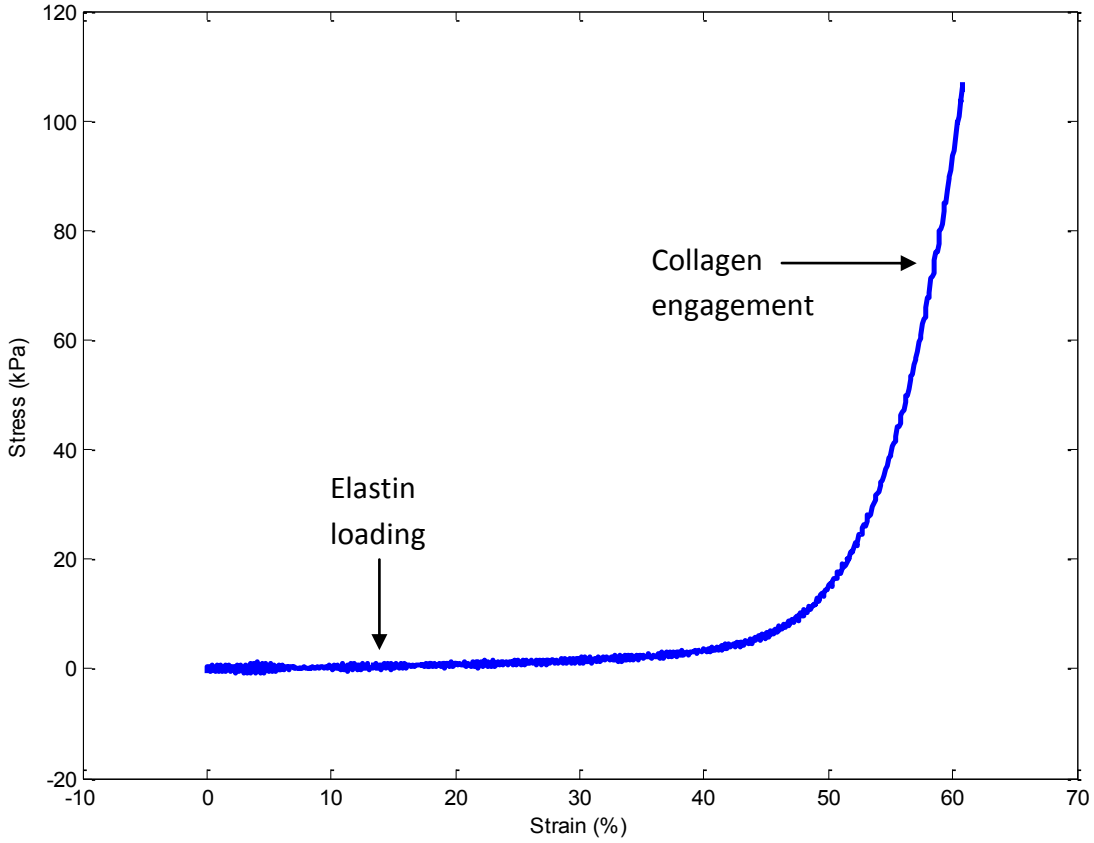

Figure 1.7: Representative stress-strain curve of UC artery showing non-linear response corresponding to elastin loading and collagen engagement regions.

1.5 Factors Affecting Biomechanics of Umbilical Cord

1.5.1 Factors in Pregnancy

Several factors during pregnancy can potentially affect on the biomechanical properties of UC vessels. These include smoking, previous health history, twins or other multiple birth, age of mother and gestational age of the baby [18].

1.5.2 Physiological Changes at Birth

Other factors which may affect UC biomechanical properties are the physiological changes which occur at birth. Within minutes after delivery, the UC arteries constrict to slow blood flow and prevent loss of fetal blood. Arterial narrowing is activated by the release of bradykinin, a hormone causing contraction of the smooth muscle cells in the arterial wall [19]. This physiological modification occurs after birth and may change the overall properties of the UC arteries from that experienced *in utero*. It is uncertain, however, if this would affect the passive mechanical properties of the tissue.

1.5.3 Mode of Delivery

Factors during delivery (i.e. – vaginal versus Cesarean section delivery) may affect the biomechanical properties of UC vessels. In Cesarean section deliveries, the doctor manually removes the placenta from the accessible uterus. In vaginal deliveries, the UC is commonly pulled to assist removal of the placenta. Consequently, greater mechanical strain may be placed on the umbilical cord during vaginal deliveries. Although it has been shown that mode of delivery has no affect on neonatal cerebral blood flow [20], this applied mechanical strain during delivery may affect the mechanical properties of the UC. Scanning electron microscopy (SEM)

results have shown that the coverage of endothelial cells in the umbilical vein is unchanged between Cesarean section and vaginal deliveries [21]. Histological results have also shown that the endothelial layer and smooth muscle structure of the umbilical vein are not affected by the mode of delivery [21]. Although the endothelial cell layer is not influenced, the passive mechanical properties of the arteries may be affected by the mode of delivery.

Changes in the biomechanical properties of the UC may also be introduced in labored versus non-labored Cesarean section deliveries. It is expected that labored Cesarean section deliveries may cause compression of the cord because the fetus may press against the cord during contractions of the uterus [22]. Doctors in the delivery ward may also affect the biomechanical properties of the UC vessels because different methods may be used between doctors for delivery of the placenta.

1.6 Preeclampsia

1.6.1 Description of Disease State

Preeclampsia (PE) is the most common complication during pregnancy, affecting 5-8% of pregnant women, including about 300,000 women in the United states annually [23]. This condition is characterized by an onset of hypertension during pregnancy in previously normotensive (NT) women and proteinuria, or elevated levels of protein in the urine. Symptoms can also include severe headache, sudden weight gain, nausea, confusion, and swelling in the hands and feet. Risk factors for PE include overweight women, age, smoking, diabetes, multiple births, and health history [18, 24]. In severe cases, PE can lead to congestive heart failure,

making it the leading cause of maternal death. PE is also thought to directly impact on the developing cardiovascular system of the fetus [25].

1.6.2 Diagnosis Criteria for Preeclampsia

PE is diagnosed by elevated blood pressure (systolic > 140 mmHg, diastolic > 90 mmHg) and proteinuria (greater than 300 mg of protein in 24 hour urine sample) after 32 weeks gestation [1]. Symptoms occurring before 32 weeks gestation are considered early onset PE and can indicate severe complications during pregnancy.

1.6.3 Incidence of Preeclamptic Pregnancies

During the past 10 years, the incidence of PE has risen by 40% [18]. This increase is likely caused by older mothers giving birth, a higher frequency of multiple births, and a higher incidence of diabetic and overweight mothers. According to the Preeclampsia Foundation, over 30% of women of reproductive age are obese, indicating that obesity is the leading cause of preeclamptic pregnancies [18].

1.6.4 Outcomes of Preeclamptic Pregnancies

The only known treatment for PE is to deliver the baby, making this disease is the most common cause of premature births. Worldwide, PE accounts for 12% of deaths during pregnancy and childbirth. This includes about 76,000 maternal and 500,000 neonatal deaths each year [1, 2]. Hypertensive disorders of pregnancy are more prevalent in low-income countries due to limited maternity and neonatal care [26].

PE also indicates serious maternal health problems later in life [27]. Women with a history of PE are four times more likely to develop hypertension and two times more likely to experience ischaemic heart disease and stroke [1]. Additionally,

women with early onset PE (less than 32 weeks gestation) are more likely to experience recurrent PE in later pregnancies [1, 28].

Environmental factors experienced by the fetus *in utero* can negatively affect post-natal health and may predispose offspring to disease later in life [25, 29, 30]. Children born to preeclamptic mothers are at a higher risk for cardiovascular disease during adolescence. At 13 to 19 years of age, offspring of PE-complicated pregnancies have higher systolic blood pressure and a higher incidence of obesity [31].

Chapter 2

Motivation and Objectives

2.1 Normative Motivation and Objectives

UC arteries are directly downstream from the fetal heart and this study considers these vessels as an extension of the fetal cardiovascular system. However, very little is known about the mechanical properties or the structure-function relationship of UC arteries. The primary motivation of this study is to establish normative data for UC arteries in order to provide a benchmark for studying disease states during pregnancy. Although this motivation is not hypothesis-drive, the primary goals of this study are to establish an understanding of healthy biomechanical function of UC arteries and to compare this data to published results for porcine, sheep, canine and calf arteries.

2.2 Preeclamptic Motivation and Objectives

The secondary motivation of this study is to understand how PE affects the mechanical properties of UC arteries. Understanding how hypertensive disorders such as PE affects fetal development is an important field of research with a high potential to impact many lives. Fewer than half of expectant mothers in the United States are informed about the symptoms and risks of this potentially life-threatening

disease [32]. This lack of information likely leads to increased incidence of PE as well as negative maternal and neonatal outcomes.

Changes in the mechanical response and biomolecular components of the umbilical cord in pregnancies complicated with PE may indicate alterations in arterial flow. These changes may reveal irregularities throughout the developing fetal cardiovascular system. The secondary goal of this study is to determine how PE affects the biomechanical properties of UC arteries. This study hypothesizes that PE causes stiffening in the mechanical properties of umbilical cord arteries when compared to that of healthy cords.

> *"Hypertensive disorders of pregnancy collectively represent a significant public health problem in the United States and throughout the world".*
>
> The National Institute of Health [33]

Chapter 3

Previous Studies

3.1 Study of UCs from Normotensive Pregnancies

Little is known about the relationship between function and structure of the UC. Pennati et al. [16] performed uniaxial tensile tests and stress-relaxation tests on longitudinal and circumferential sections of UC veins and on longitudinal sections of WJ. Mechanical testing results showed a typical non-linear response for biological tissues which contain elastin and collagen as the primary load-bearing proteins. For longitudinal sections of UC vein, the stress remained below 100 kPa for strains below 10%. This low-stiffness region corresponds to the recruitment and stretching of elastin fibers, whereas the collagen fibers are still in a relaxed state. As strain was increased above 10%, however, the applied stress values increased from 100 kPa to 700 kPa. This high-stiffness region corresponds to extension and recruitment of the strong collagen fibers. It was also shown that the UC vein acted anisotropically by exhibiting a higher stiffness longitudinally than circumferentially. It was concluded that the UC contains mechanisms meant to prevent excessive elongation and prevent interference with UC circulation. The UC vein and WJ also acted n a viscoelastic manner during stress-relaxation tests. Results showed that tissue viscosity is an important contribution to tissue stiffness. This study is important in understanding

how collagen and elastin affect the mechanical properties of the UC vein. Mechanical testing of the UC arteries, however, was omitted due to the small size of the vessels.

The correlation between anatomical location in the UC and biomechanical response of the UC vein has also been studied. Li et al. [34] defined the pressure-strain modulus as $E_p=\Delta P/\Delta R \cdot R$, where P is internal vessel pressure and R is vessel radius. This group showed that pressure-strain modulus varied along the length of the UC and was stiffest as the fetal end. UC arteries also exhibit a shorter stress-relaxation time and a stiffer elastic modulus when collected from the placental end of the UC [35]. Results of these studies show that the biomechanics of UC vessels vary along the length of the cord.

Imaging of the normotensive UC can also provide information about its structure-function relationship. Scanning electron microscopy on the gross anatomy of the UC showed distinct interstitial cavities in the WJ and in the UC vessel walls [36]. This spacing in the tissue likely allows for vessel distension and cord compression and twisting with minimal disruption to fetal blood flow.

Although the results of previous studies are significant, they do not compare UCs from healthy and hypertensive pregnancies. Also, most studies do not test the mechanical properties of UC arteries which are directly downstream from the fetal heart and may provide valuable information about the fetal cardiovascular system. Therefore, further testing is necessary to understand how PE affects the mechanical properties of UC arteries.

3.2 Previous Preeclamptic UC Studies

Numerous studies have suggested that maternal hypertension leads to altered UC arterial blood flow and may lead to abnormal fetal cardiovascular development or fetal hypoxia [25, 30, 37-43]. The UC arterial wave-form changes in pathologic conditions of pregnancy such as intrauterine-growth-restriction and PE [41, 43]. This change indicates an increased vascular resistance in the placenta as compared to healthy pregnancies.

Napolitano et al. [44] found significantly higher levels of homocysteine in UC blood in pregnancies with PE than in normotensive pregnancies. High levels of this biomolecule in the blood has been associated with an increased risk of stroke and heart attack and has been shown to damage the endothelial cell lining the lumen of blood vessels. This study suggested that the increased levels of homocysteine in UC blood with PE may damage the endothelial cell lining of UC blood vessels.

It has been shown that the biomolecular components of the UC are altered in cases of PE [37, 38, 40]. Gogiel et al. [40] found that WJ had fewer proteoglycans in pregnancies with PE. This may reduce the elasticity of the WJ and inhibit its ability to cushion and protect the UC blood vessels. Such a change in the mechanical properties of WJ may decrease the ability of cells in the blood vessels to regulate their diameter and cause disturbance in fetal blood flow.

Histological studies showed that the smooth muscle cells in umbilical arteries with PE contain an increased amount of endoplasmic reticulum [37, 38]. This indicated an increase of metabolically activated smooth muscle cells which were able to produce large amounts of collagen protein fibers. Studies also showed that the

layer of endothelial cells lining the arterial lumen tended to protrude into the vessel lumen and even detach from the adjacent smooth muscle cells in PE [37]. This led to areas of luminal surface which were lined with smooth muscle cells rather than endothelial cells. These alterations in the endothelial cell lining may increase turbulence and wall shear stress in the UC arterial blood, thereby affecting the fetal cardiovascular system.

It was also shown that UC artery walls were 15% thicker in PE-complicated pregnancies than in normotensive pregnancies [38]. This thickening was seen in both the tunica intima and the tunica media. Additionally, the relative elastin content between NT and PE was evaluated through graduated intensities of orcein staining in UC arteries. Elastin content decreased with PE [38] and the few exiting elastic fibers did not form lamellae as seen in healthy UC arteries [9]. An elastin assay analysis showed that elastin content in the UC arterial wall decreased by 10% in hypertensive cases [45]. Reduced elastin protein in the arterial wall was hypothesized to affect the mechanical properties of UC arteries by reducing their compliance [45]. Similarly, UC arteries from pregnancies with PE exhibited twice as much collagen and significantly less elastin than in healthy pregnancies [42]. This remodeling likely stiffens the arteries and may cause a decrease in arterial blood flow in the fetus and lead to altered fetal blood circulation.

Chapter 4

Materials and Methods

4.1 Clinical Research Consent

Umbilical cords (n=15) were obtained from deliveries at the Boulder Community Hospital (BCH) in Boulder, Colorado. Obstetricians at the Boulder Medical Center collected UC tissues from subjects seeking routine obstetric care. Expectant mothers were informed of this research study and agreed to participate at will. Each participant signed her informed consent (HRC# 1007.16) before the UC was collected. For each study participant, a BCH obstetrician completed a clinical data form indicating the health history of the mother and any complications during delivery. This study did not affect normal prenatal or neonatal care.

4.2 Umbilical Cord Samples

Of the 15 cords obtained, 11 were from normotensive pregnancies while 4 were from preeclamptic pregnancies with no additional complications of pregnancy. 8 newborns were female and 7 newborns were male. All cords were from full-term births with an average gestational age of 38.7 weeks (range 37-40 weeks) and 39.5 weeks (range 38-40 weeks) for normotensive and preeclamptic pregnancies, respectively. Mothers had no history of premature deliveries. The average maternal

age was 34.2 years (range 29-40 years). All cords were obtained from singleton births. Of the normotensive pregnancies, 5 cords were collected from vaginal deliveries and 6 were collected from non-labored Cesarean section deliveries. Of the preeclamptic pregnancies, 1 cord was collected from vaginal delivery and 3 cords were collected from labored Cesarean section deliveries.

4.3 Tissue Collection and Transport

Each cord was stored in a plastic biohazard bag in a 2 ˚C refrigerator at BCH immediately after birth. Bags were labeled with a patient number corresponding to the clinical data sheet for each mother. Cords were transported to the University of Colorado in an insulated cooler filled with ice and placed in a 2 ˚C refrigerator before dissection. In order to prevent additional changes in mechanical properties, UC tissues were never frozen [46].

4.4 Tensile Testing

4.4.1 Dissection and Preservation

Mechanical properties of fresh tissue are unchanged up to 3 days after harvesting [47]. Cords were dissected within 72 after birth to be well within this range. Each cord was rinsed briefly in deionized water to remove excess blood both externally and within the vessels. UC length and average width was measured and recorded for each sample. Dissection was performed on a frozen dissecting plate in order to keep the tissue at a cool temperature. Cords were continually irrigated with calcium-free and magnesium-free phosphate buffered saline (PBS, pH 7.4) during

dissection to prevent drying. Both arteries were carefully dissected from the middle of each cord in order to minimize affects of cord location on mechanical response of the tissue (Figure 4.1). Care was taken to remove all WJ from arteries and to avoid any areas where blood clotting was observed or other irregularities were present. Arteries were then cut axially into rectangular sections 20-35 mm in length (Figure 4.2) and immersed in PBS on a cold dissecting plate. Width and thickness for each artery was measured using digital calipers.

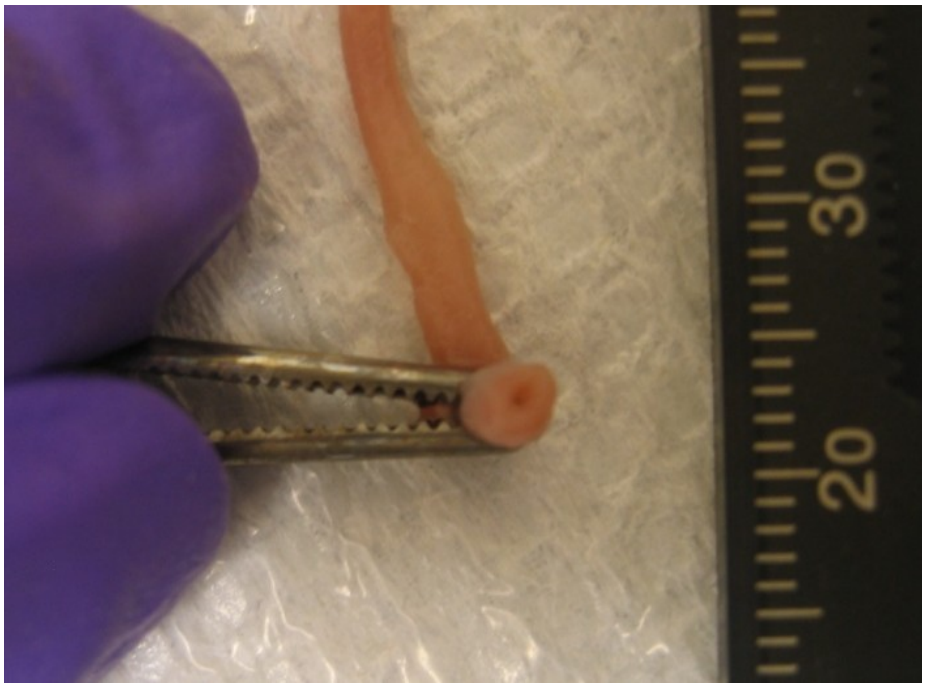

Figure 4.1: Dissected UC artery before axial cutting. *Photo by Megan Schroeder, University of Colorado, 2007*.

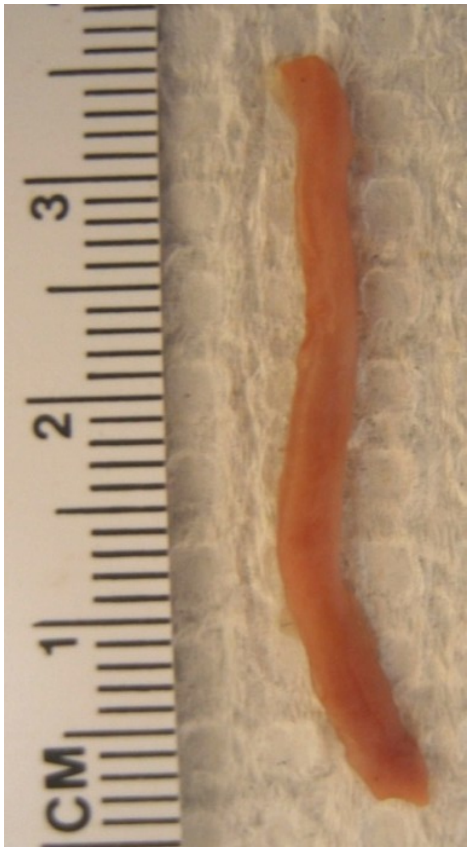

Figure 4.2: Dissected UC artery after axial cutting. *Photo by Megan Schroeder, University of Colorado, 2007*.

4.4.2 Test Setup

Mechanical testing was performed using an MTS Insight II (MTS Systems, Eden Prairie, MN) tensile testing device equipped with a 5-N load cell and environmental chamber (Figure 4.3). UC arteries were secured between the grips of the MTS device (Figure 4.4). Initial gage length between grips was measured using digital calipers at a 5-mN preload. The environmental chamber was filled with PBS at 37 ˚C to simulate *in vivo* conditions of human body pH and temperature. PBS and the time elapsed after delivery ensured that the tissue did not actively contract during testing.

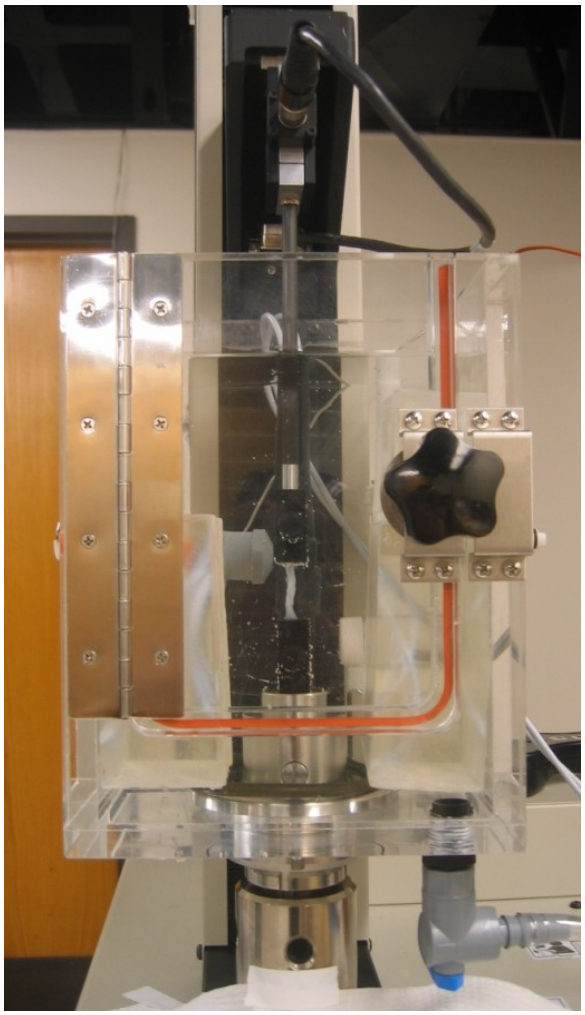

Figure 4.3: MTS tensile testing machine setup with environmental chamber. *Photo by Megan Schroeder, University of Colorado, 2009.*

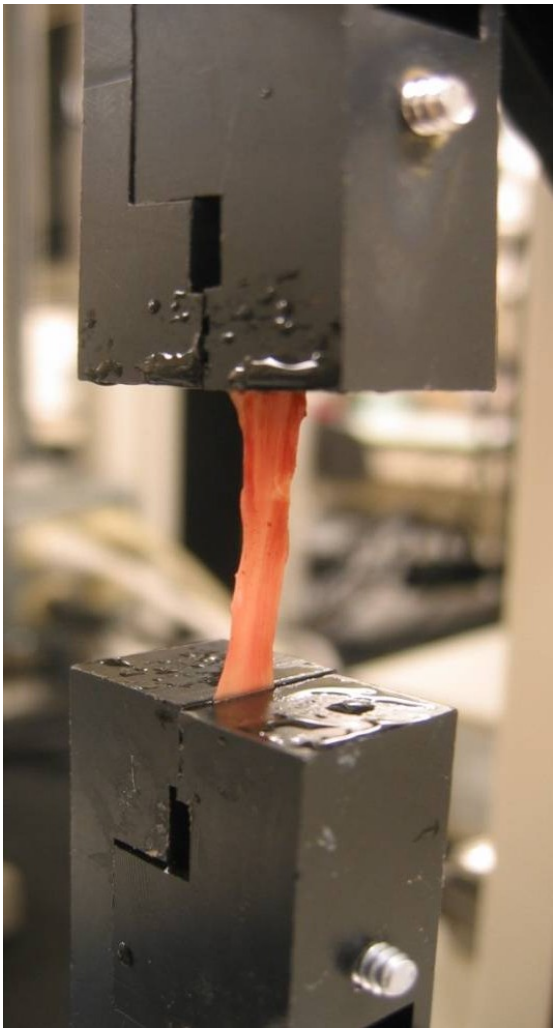

Figure 4.4: Dissected UC artery in grips before tensile testing. *Photo by Megan Schroeder, University of Colorado, 2007.*

4.4.3 Mechanical Testing Procedure

Grips were separated at a constant rate of 0.5 mm per second. In order to prevent damage to the tissue, each sample was first strained to 35%. The strain was incrementally increased by 5% until the collagen dependent stress-strain response was exhibited. The tissue was then preconditioned to this prescribed strain for 10 extension-relaxation cycles. Preconditioning has no affect on the orientation and special distribution of elastin and collagen [48]. Load and elongation data were collected at 10 Hz using Testworks 4 software (Software Research, Inc., San Francisco, CA). Data was omitted if tissue slipped from or failed at either grip.

4.4.4 Data Analysis

Engineering stress (σ), engineering strain (ε), and elastic modulus (E) were calculated using the following equations. All calculations were based upon the initial tissue width (W) and thickness (T).

$$\sigma = \frac{F}{WT}$$

$$\epsilon = \frac{L - L^{\circ}}{L^{\circ}}$$

$$E = \frac{\Delta\sigma}{\Delta\epsilon}$$

F is the force applied to the tissue, L° is the initial gage length and L is the instantaneous gage length. Both the initial and the late elastic moduli were determined from the preconditioned state (cycle 10) for each sample. These moduli correspond to the two linear regions of the stress versus strain curves. Data analysis was performed using custom-written software (Matlab R2008b, The Math Works, Natick, MA; written by R. Blair Dodson, University of Colorado).

Data was disregarded for the whole cord (n=2) if the initial or late elastic modulus values for either artery was outside of ± 3 standard deviations (SD) from the mean. Error bars indicate ± 1 SD from the mean. Statistical analysis was performed using one-way ANOVA. Results were considered statistically significant for *P*-values < 0.05.

4.5 Histology

Fresh UC whole cross-sections were cut in sections approximately 1 cm in length. Each section was rinsed quickly in PBS and placed in a 50-mL vial with 10% neutral-buffered formalin solution and stored at 2 ˚C.

Slides were paraffin-embedded and sectioned at Premier Laboratory LLC (Longmont, CO) using standard techniques. Tissue was stained with elastin Verhoeff-van Gieson (EVG), providing a visual distinction between elastin and collagen proteins. Slides were examined using a Zeiss Axioskop 40 FL brightfield microscope equipped with a digital SPOT camera.

4.6 Scanning Electron Microscopy

Tissue preparation for scanning electron microscopy (SEM) was modified slightly from previously-established methods [36]. Whole UC sections approximately 10-cm in length were fixed by injecting gluteraldehyde into the vessels using a surgical syringe. Sections were immersed in gluteraldehyde for 48 hours followed by rinsing in tap water for 3 hours. Samples were then immersed in 2*N*-NaOH for 12 days and washed in tap water for 1 day.

Cords were then cut using a scalpel into sections of approximately 4 mm thickness and dehydrated through a graded ethanol series (75% EtOH for 24 hrs., 85% EtOH for 24 hrs., 90% EtOH for 24 hrs., 95% EtOH for 12 hrs., 100% EtOH for 12 hrs.). In order to ensure removal of all liquid but maintain tissue structure, UC samples were processed in a critical point dryer (Samdri pvt 3; Tousimis Research Corporation, Rockville, MD). Finally, samples were mounted to conducting stubs and gold plated. Samples were examined with a JEOL low-voltage scanning electron microscope (model JSM-6480 LV).

Chapter 5

Results

5.1 Normative Results

The mean elastic modulus for the elastin-loading region of UC arteries was 5.03 kPa (SD = 1.82 kPa), as shown in Figure 4.1, while that for the collagen-engagement region of UC arteries was 1358 kPa (SD = 388 kPa), as shown in Figure 4.2. The mean elastic modulus for the elastin-loading region from vaginal and Cesarean section deliveries was 5.4 kPa (SD = 2.2 kPa) and 4.8 kPa (SD = 1.6 kPa), respectively (Figure 4.3). The mean elastic modulus for the collagen-engagement region from vaginal and Cesarean section deliveries was 1415 kPa (SD = 300 kPa) and 1325 kPa (SD = 532 kPa), respectively (Figure 4.4). There was no statistical difference in initial modulus (P = 0.5) or late modulus (P = 0.7) for vaginal versus Cesarean section deliveries.

The moduli from both arteries from each UC were analyzed for equality. Results demonstrated that in all cases, one artery exhibited a stiffer modulus over the other from the same UC. Values were compared by calculating the percent difference between the weaker modulus and the stiffer modulus for each UC. The average difference between arterial moduli was 18.5% for the elastin-loading region and 26% for the collagen-engagement region (Figure 4.5).

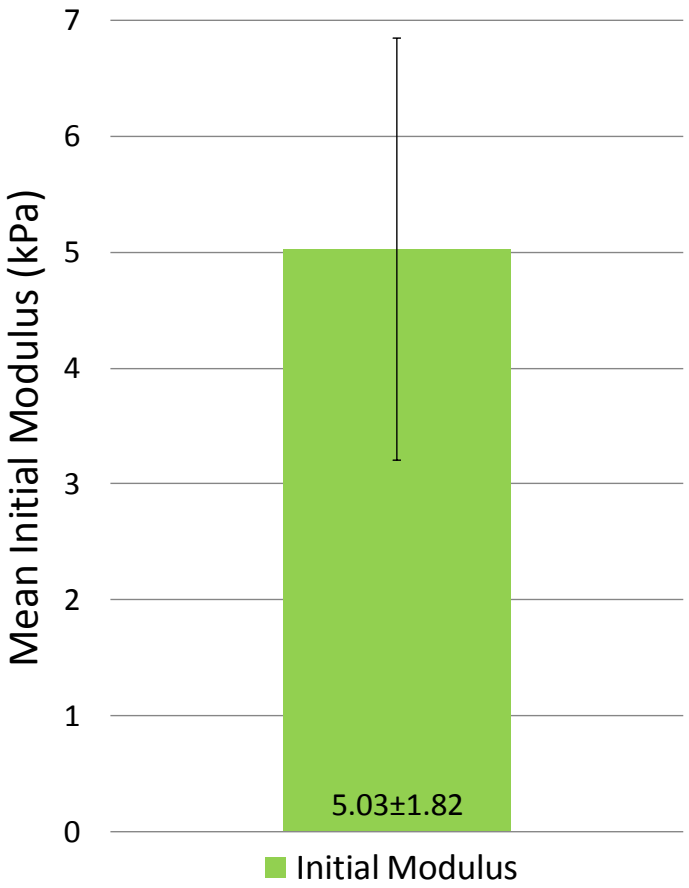

Figure 5.1: Mean elastic modulus from elastin-loading region of UC arteries from normotensive pregnancies. Data are presented as mean ± one standard deviation.

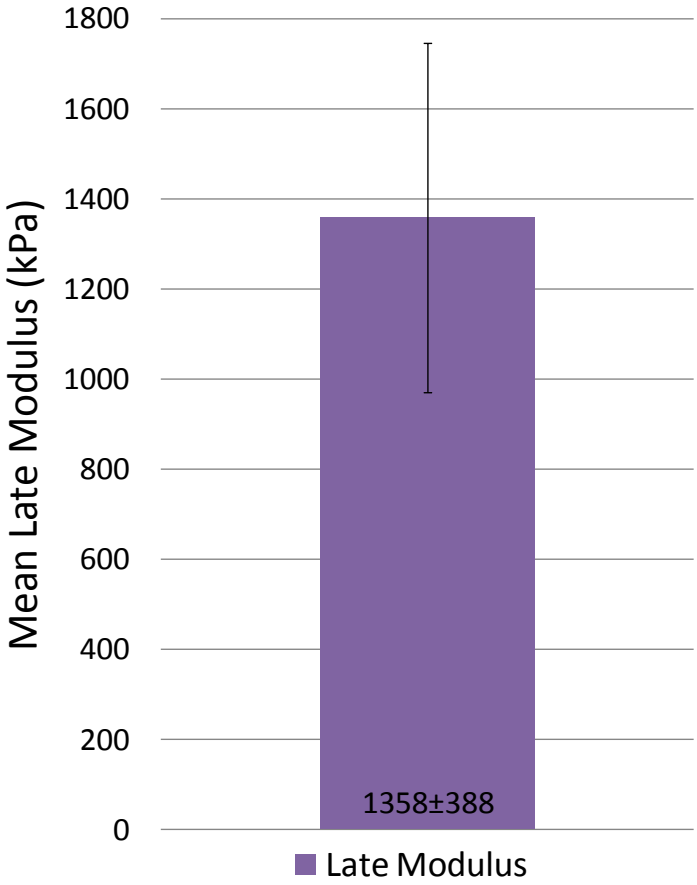

Figure 5.2: Mean elastic modulus from collagen-loading region of normotensive UC arteries. Data are presented as mean ± one standard deviation.

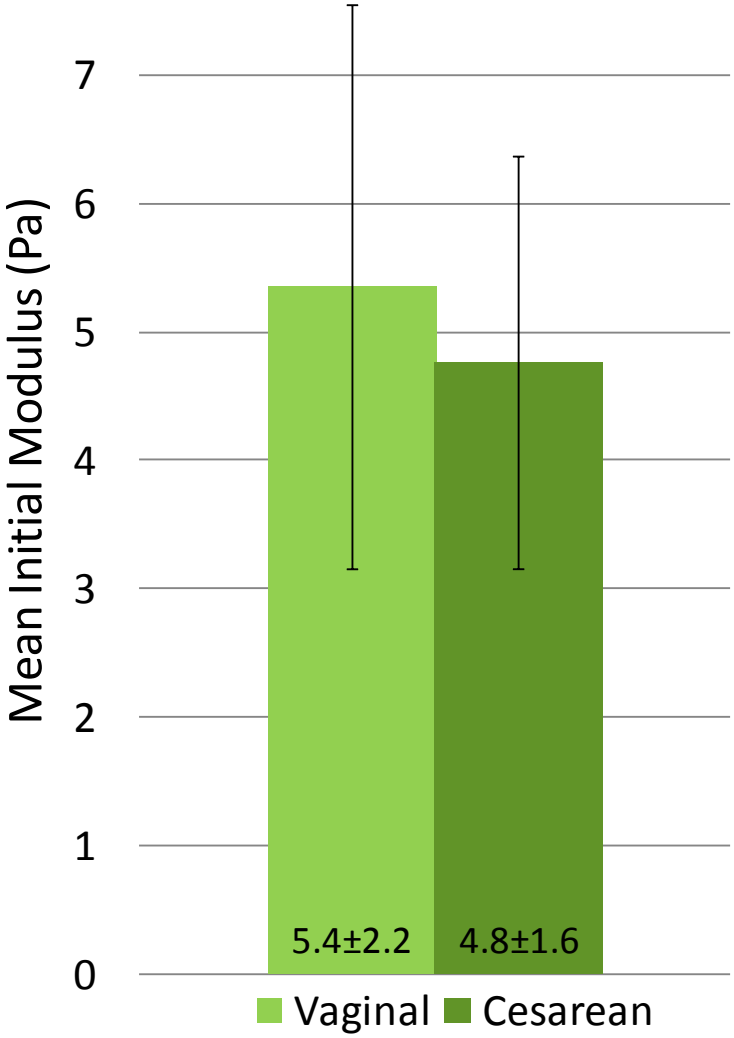

Figure 5.3: Mean elastic modulus from elastin-loading region of normotensive UC arteries for vaginal and Cesarean section deliveries. Groups are not statistically different ($P = 0.5$). Data are presented as mean ± one standard deviation.

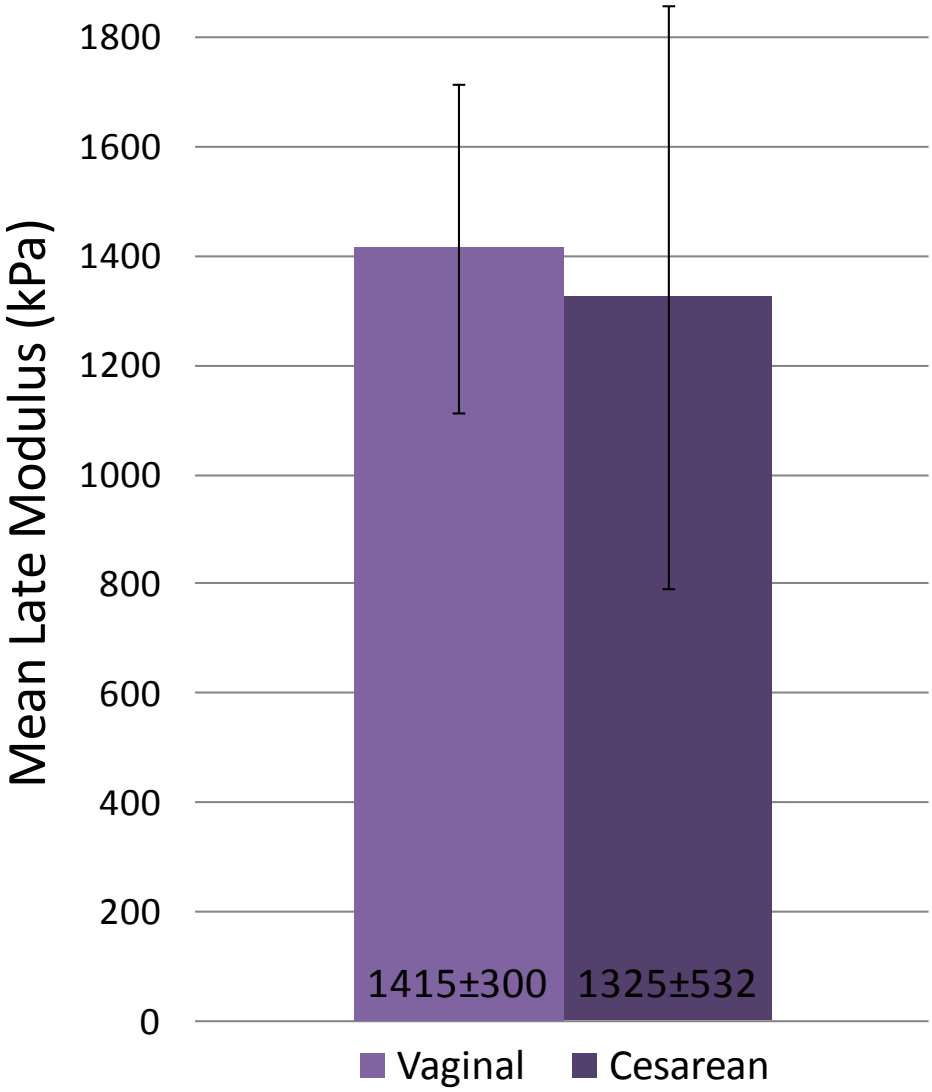

Figure 5.4: Mean elastic modulus from collagen-loading region of normotensive UC arteries from vaginal and Cesarean section deliveries. Groups are not statistically different ($P = 0.7$). Data are presented as mean ± one standard deviation.

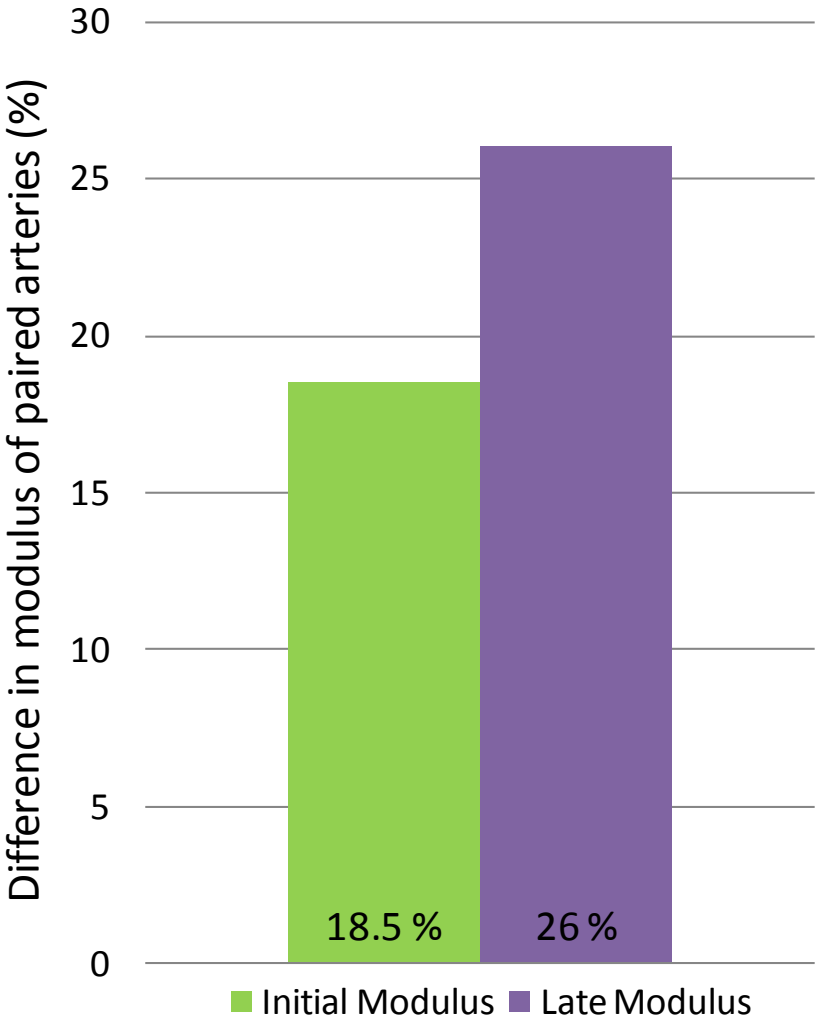

Figure 5.5: Mean percent difference in elastic modulus between paired arteries for elastin-loading and collagen-loading regions.

5.2 Preeclamptic Results

The average normotensive UC length was 41.8 cm (SD = 10.4 cm) and the average preeclamptic UC length was 40.3 cm (SD = 13.4) as shown in Figure 5.6. There was no statistical difference (P = 0.82) between the lengths of normotensive and preeclamptic cords.

The average normotensive UC width was 1.2 cm (range 0.8-1.5 cm) and the average preeclamptic UC length was 0.93 cm (range 0.70-1.0 cm) as shown in Figure 5.7. Results showed a statistically significant (P = 0.04) decrease in cord width with PE.

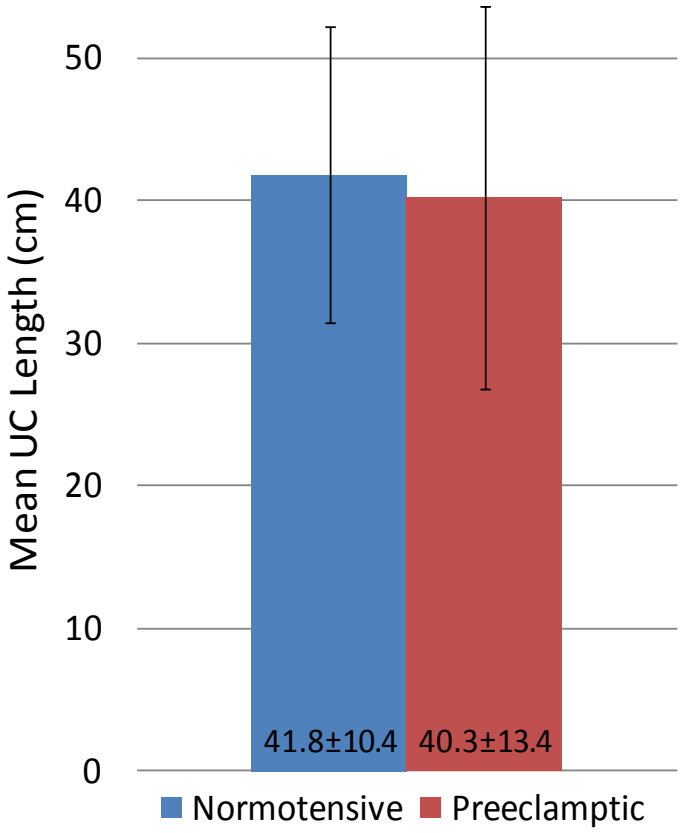

Figure 5.6: Mean UC length from normotensive and preeclamptic pregnancies. Groups are not statistically different (P = 0.82). Data are presented as mean ± one standard deviation.

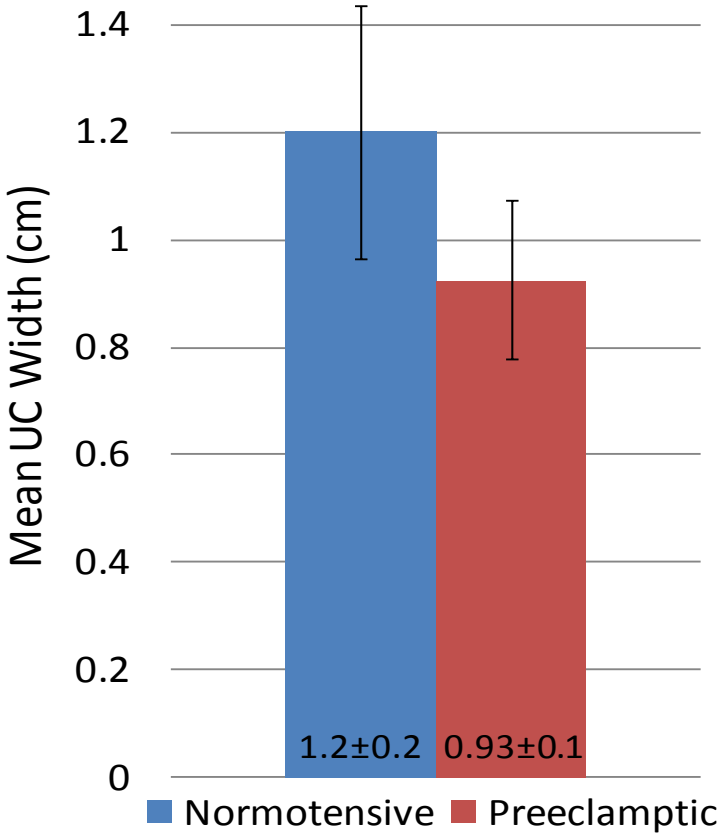

Figure 5.7: Mean UC width for normotensive and preeclamptic pregnancies. Groups are statistically different (P = 0.04). Data are presented as mean ± one standard deviation.

The average initial elastic modulus was 5.03 kPa (SD = 1.82 kPa) for normotensive UC arteries and 9.33 kPa (2.50 kPa) for preeclamptic UC arteries (Figure 5.8). Results showed a statistically significant (P = 0.003) increase in initial elastic modulus with PE.

The average elastic modulus in the collagen-engagement region was 1358 kPa (SD = 388 kPa) for normotensive UC arteries and 1544 kPa (SD = 286) for preeclamptic UC arteries (Figure 4.9). There was no statistical difference (P = 0.4) in late modulus between normotensive and preeclamptic cases.

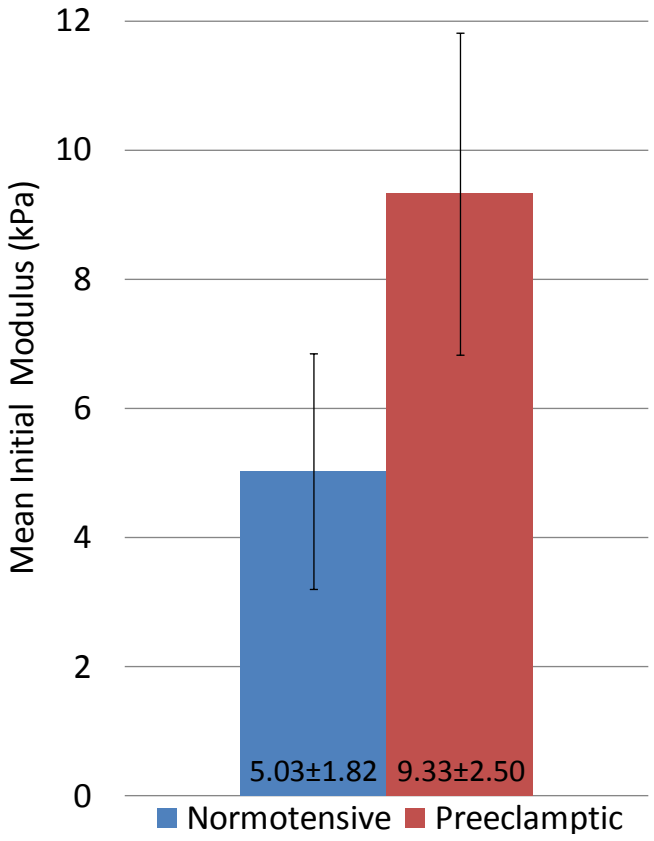

Figure 5.8: Mean elastic modulus for elastin-loading region of normotensive and preeclamptic UC arteries. Groups are statistically different (P = 0.003). Data are presented as mean ± one standard deviation.

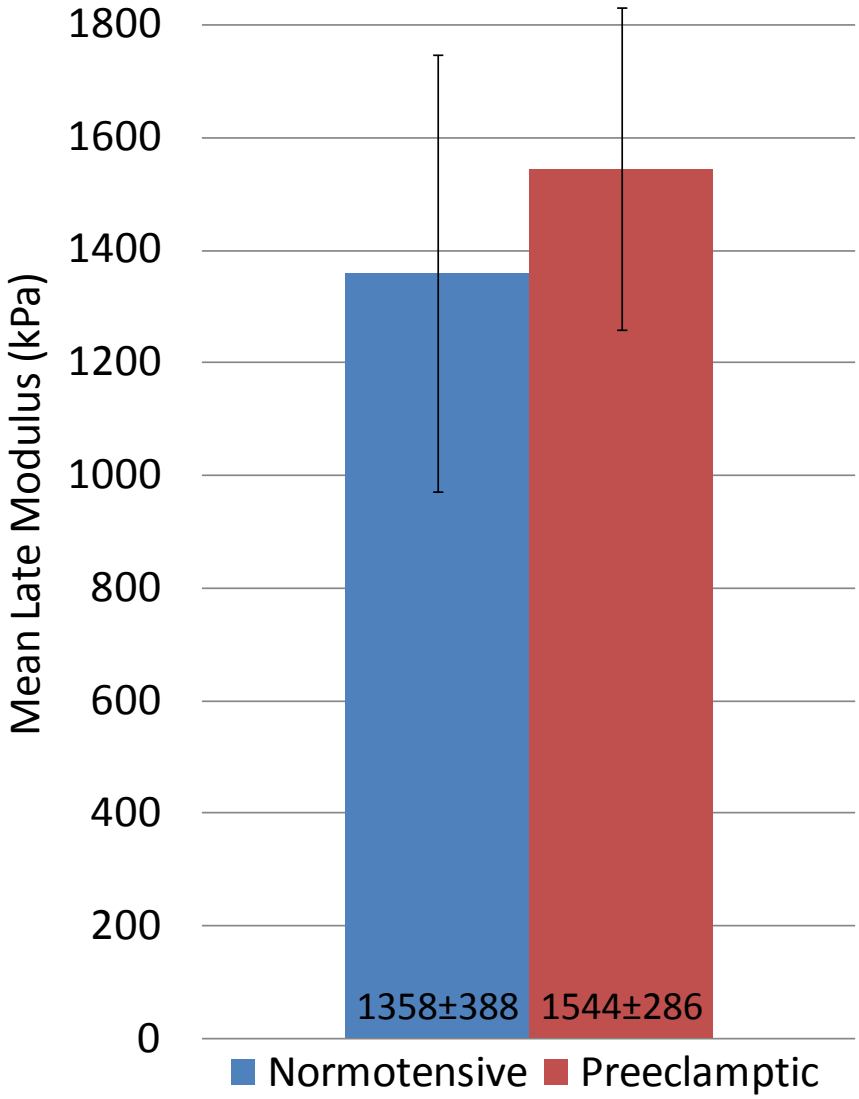

Figure 5.9: Mean elastic modulus from collagen-loading region of normotensive and preeclamptic UC arteries. Groups are not statistically different (P = 0.4). Data are presented as mean ± one standard deviation.

Representative microphotoraphs from normotensive UC arteries are shown in Figures 5.10 and 5.11. Preeclamptic UC arteries are shown in Figures 5.12 and 5.13.

Black staining indicates elastin bands, as shown. Preliminary qualitative results indicated that elastin was more abundant in normotensive than in preeclamptic UC arteries.

Representative SEM images from normotensive and preeclamptic UC arteries are shown in Figures 5.14 and 5.15, respectively. Interstitial spacing was seen in the arterial wall for both cases. In a comparison of two samples (normotensive n=1 and PE n=1), the artery wall thickness increased from approximately 200 μm in the normotensive case to approximately 400 μm with PE.

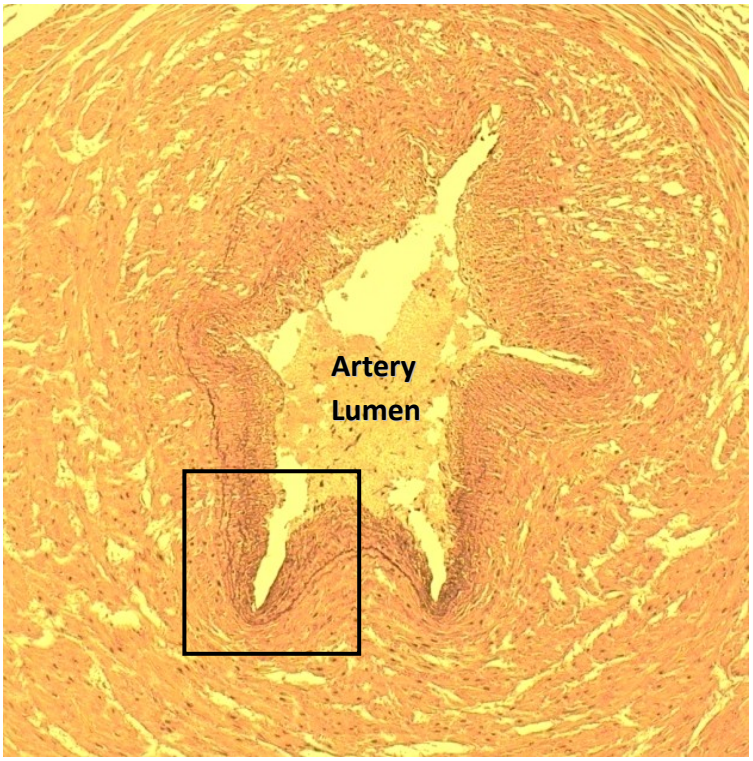

Figure 5.10: Normotensive UC artery stained with EVG (20X). Close up of boxed region shown in figure 5.11.

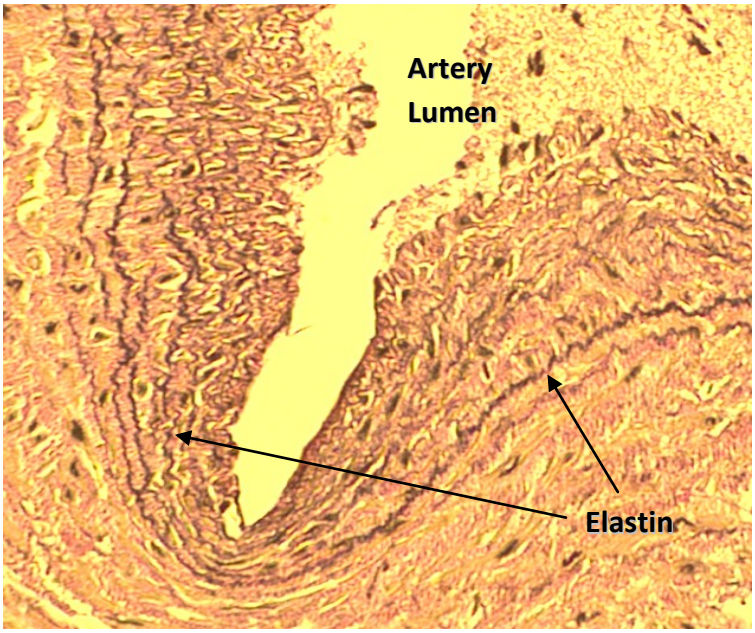

Figure 5.11: Normotensive UC artery stained with EVG (40X). Black staining indicate elastin bands.

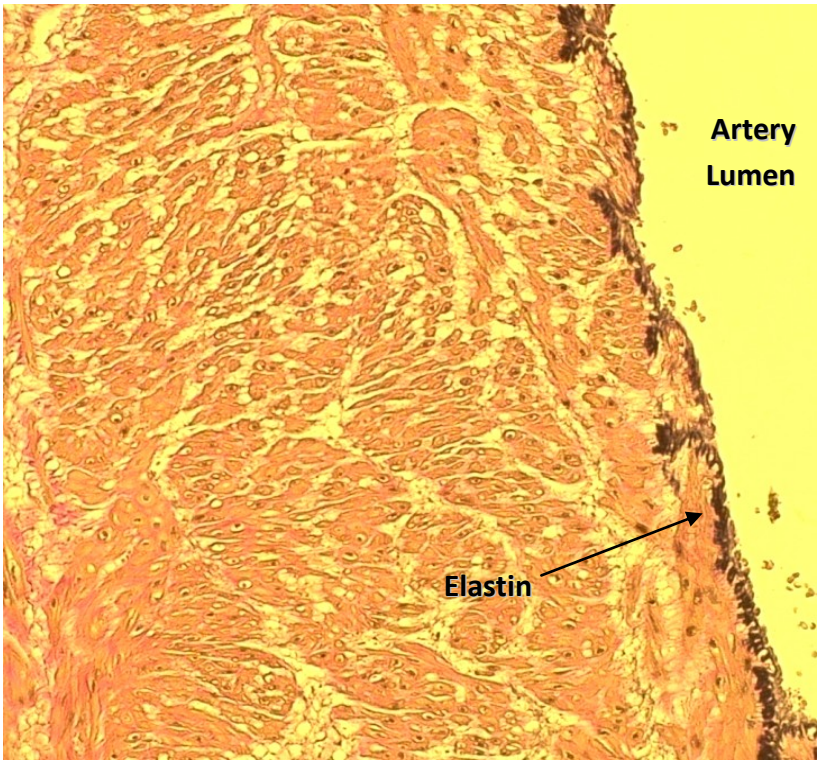

Figure 5.12: Preeclamptic UC artery stained with EVG (40X). Black staining indicates elastin bands.

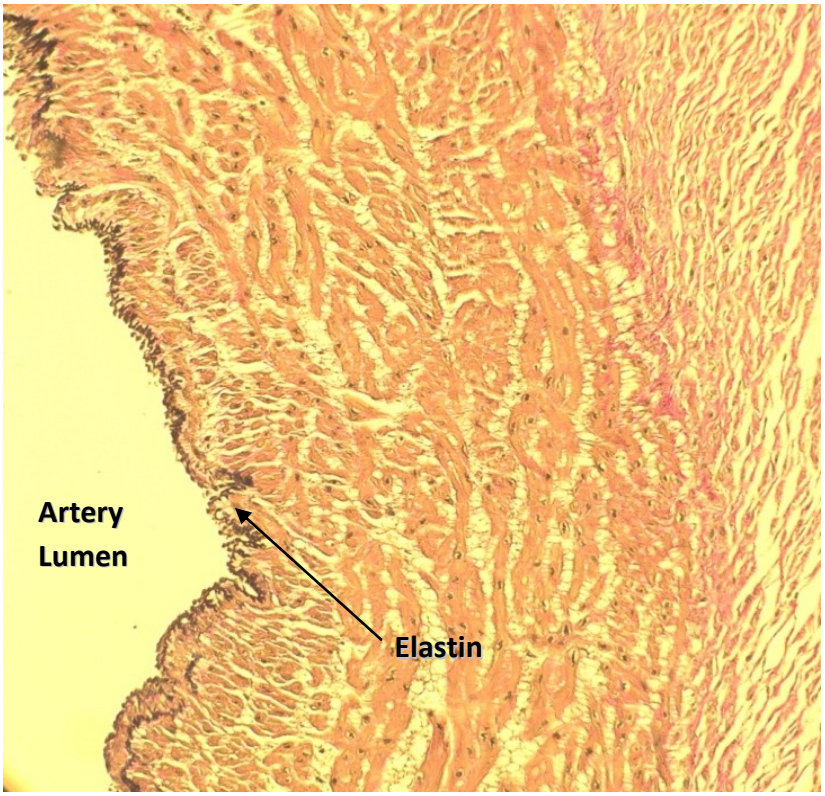

Figure 5.13: Preeclamptic UC artery stained with EVG (40X). Black staining indicates elastin bands.

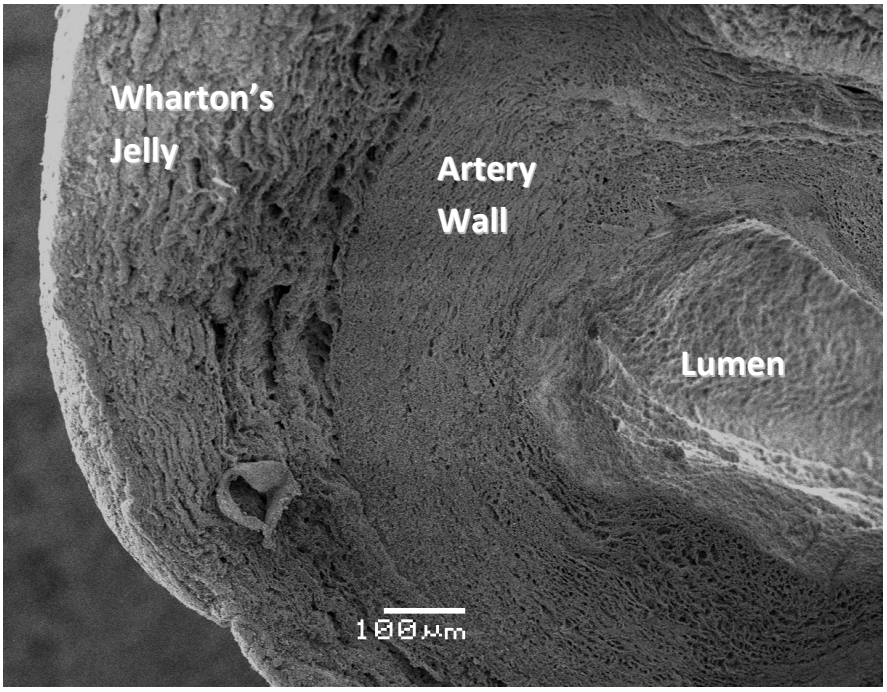

Figure 5.14: Scanning electron microscopy image of normotensive UC artery.

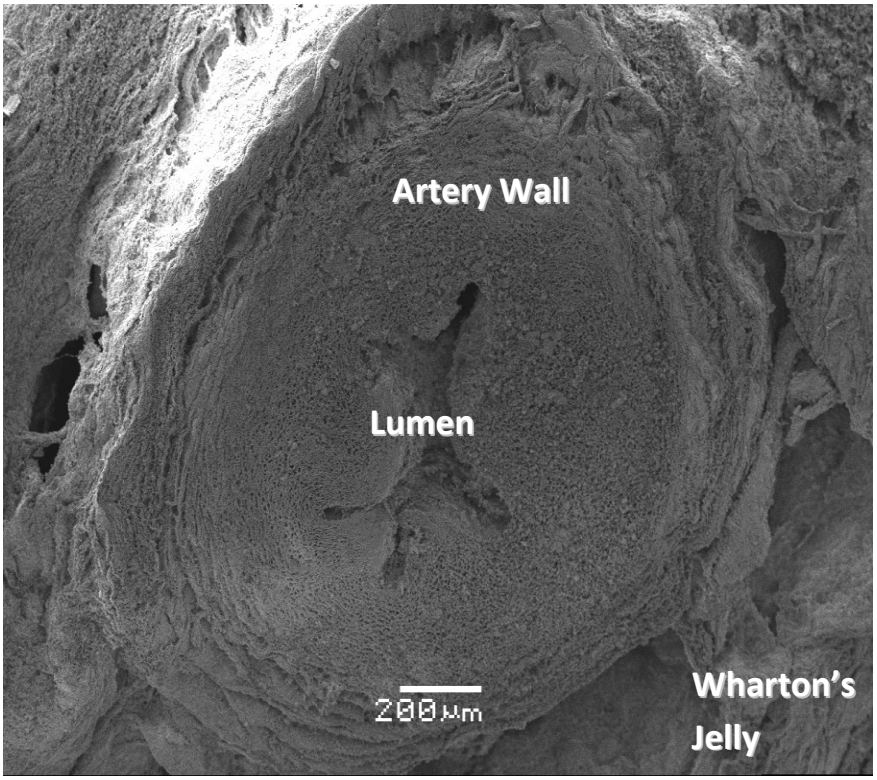

Figure 5.15: Scanning electron microscopy image of preeclamptic UC artery.

Chapter 6

Discussion

6.1 Discussion of Normative Results

Mean UC length were lower than published values [49, 50] (Figure 6.1). This was likely due to the fact that published studies reported the UC length immediately after delivery and before the cord was cut. Also, it has been shown that the UC decreases up to 7 cm in length within one hour after birth [51]. Because cords used for the current study were not measured within this one hour time frame, the UC lengths presented herein are expected to be shorter than published values. Within the current analysis, UC length did not change with PE.

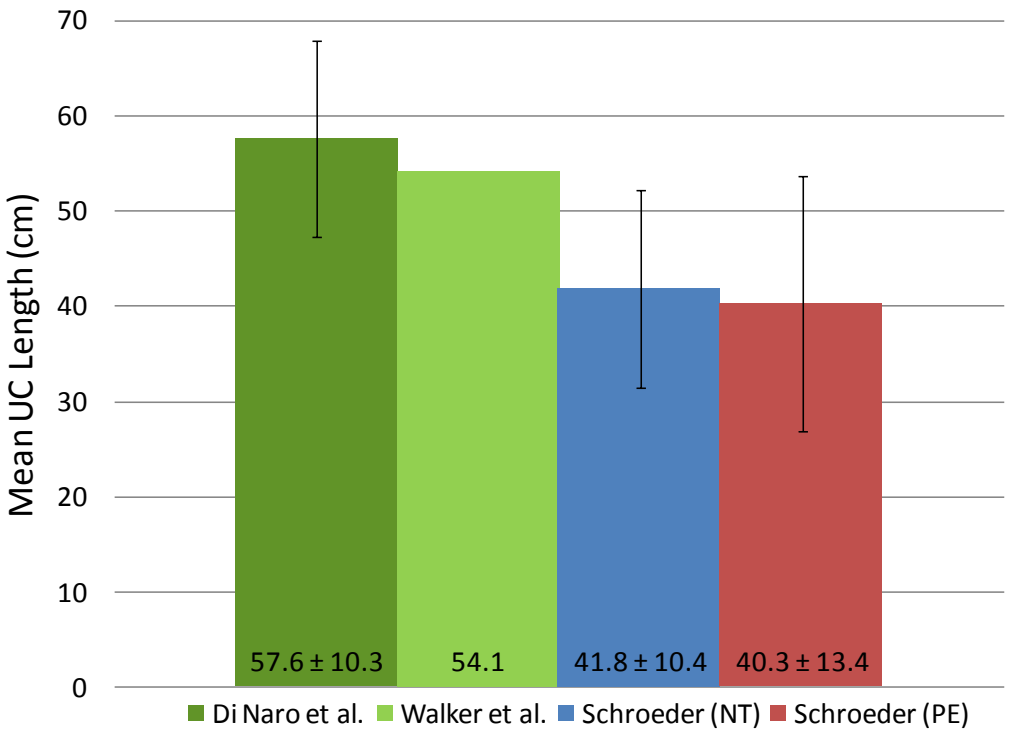

Figure 6.1: Mean UC length compared to published data [49, 50]. Data are presented as mean ± one standard deviation.

The mode of delivery did not affect the measured mechanical properties of UC arteries. This result agreed with previous published studies showing that the endothelial layer and smooth muscle cells of the UC vein are unchanged with mode of delivery [20, 21]. Therefore, UC vessels from either vaginal or Cesarean section deliveries may be used for future studies, although the influence of delivery mode should be considered with a larger sample size.

When comparing the behavior of two arteries from a single UC, one artery exhibited a higher elastic modulus over the other in all UCs for both the initial and the late moduli. Previous clinical studies showed a 10.4% difference in the systolic to diastolic ratio of paired arteries in the same UC [52]. This dissimilarity in moduli may indicate that one artery is more resistant to blood flow than the other. For future studies, it will be essential to test both arteries from each UC. Also, stiffer arteries and less stiff arteries should be treated as distinct groups and should be compared separately.

The mean initial modulus for UC arteries ($E = 5.03 \pm 1.82$ kPa) was lower than that of compared data. Sokolis et al. showed an initial elastic modulus of 100 kPa for porcine upper descending thoracic aorta [15] and Bergel et al. showed an initial elastic modulus of 120 kPa for the canine femoral artery [53]. The lower initial modulus for UC arteries, as compared to these published values, is attributed to three factors. First, UC tissue is only 32-35 weeks from its origin, whereas the published values are from adult vessels. Because it has been shown that arteries stiffen with age [14, 54], it is reasonable to believe that the very young age of UC arteries would contribute to a lower modulus when compared to other arterial tissue. Second, it has

been shown that the initial elastic modulus in fetal sheep aorta nearly doubles in adults [55]. It is expected that the initial elastic modulus of UC arteries would be similarly low compared to that of adult animal arteries. Third, although the UC is not under longitudinal tension *in vivo* as in other arteries, the UC does undergo longitudinal stretching *in utero* due to fetal movement [6]. It can be concluded that UC arteries are extensible at physiological strains to ensure constant blood flow to the fetus during longitudinal stretching. This agrees with previous studies which concluded that the UC contains mechanisms to prevent excessive longitudinal elongation [16].

A wide variability exists for late elastic modulus data. Mean late elastic modulus ($E = 1358 \pm 388$ kPa) for UC arteries was higher than that of compared calf main pulmonary arteries ($E \approx 50$ kPa) and right pulmonary arteries ($E \approx 100$ kPa) [47].

The different in late modulus for UC arteries and calf pulmonary arteries are likely due to two factors. First, the wall-to-thickness ratio for UC arteries is lower than that of the calf arteries. Second, it has been shown that the mean arterial pressure (MAP; defined as $MAP=P_{diastolic}+1/3(P_{systolic}-P_{diastolic})$) of a human fetus is 84 mmHg whereas the MAP for a calf is 27 mmHg [47]. Smaller dimensions, coupled with increased MAP, has been shown to increase the elastic modulus of arterial vessels [55] and may explain the higher late elastic modulus of UC arteries.

The late modulus of UC arteries were in agreement with that of fetal sheep thoracic aorta and previous UC artery data. Wells et al. found that the late elastic modulus for fetal sheep thoracic aorta was 1300 kPa (SD ≈ 200 kPa) [55]. A

previous UC study showed a mean late elastic modulus of 1128 ± 694 kPa for UC arteries [35]. It is important to note that the blood pressure in normotensive UC arteries is 9.3-10.6 kPa [56]. Therefore, stresses experienced by the tissue in the late modulus region are not experience *in vivo*.

6.2 Discussion of Preeclamptic Results

Mean UC width agreed with published data [50, 57] (Figure 6.2). It was shown that mean UC width decreased significantly (P = 0.04) by 22.5% with PE. Because UC artery and vein walls thicken with PE [38, 39], this decrease in overall cord width was likely due to a decrease in WJ. Because WJ is responsible for protecting and cushioning the UC vessels, one reason for adverse fetal outcomes in PE pregnancies may be due to a reduced WJ content. Additionally, decreased WJ may reduce the stiffness of the overall cord structure. This may cause UC vessels to stiffen in order to compensate for the decreased stiffness of the surrounding material.

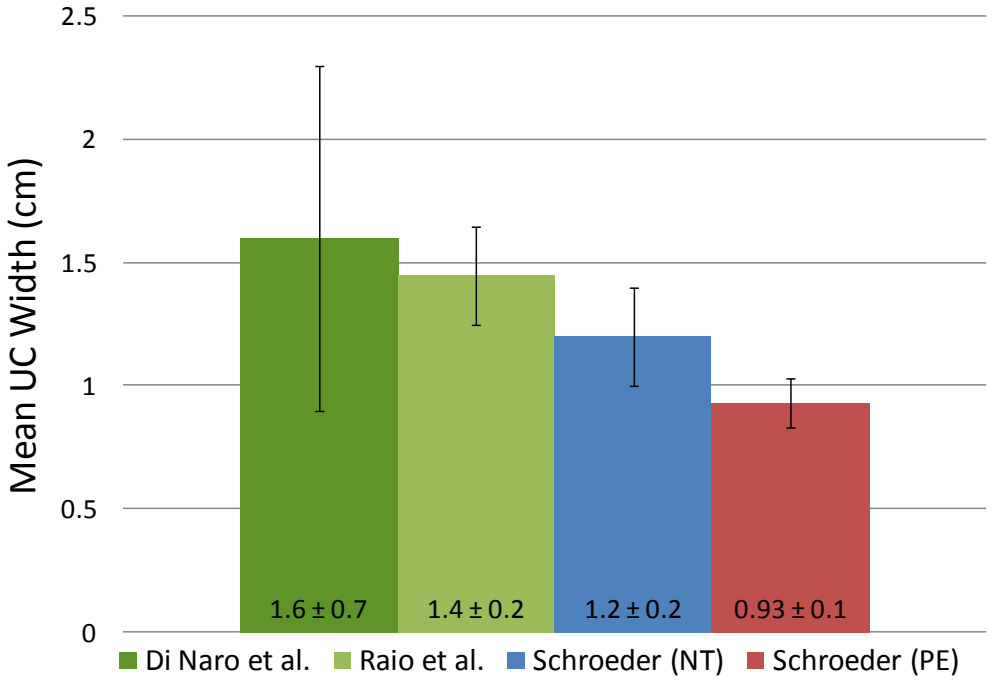

Figure 6.2: Mean UC width compared to published data [50, 57]. Normotensive and preeclamptic groups are statistically different ($P = 0.04$). Data are presented as mean ± one standard deviation.

The mean initial elastic modulus increased significantly (P = 0.003) by 86% with PE. This change was attributed to the increased physiological pressure in UC arteries with PE. It is known that the systolic to diastolic ratio in UC arteries increases by more than 50% with PE [58]. As seen in other arteries [12], UC arteries may remodel to compensate for the higher physiological pressure experienced in PE. This stiffening at physiological strains indicated a decrease in elastin content in the artery wall with PE. Results agreed with previous studies which showed that elastin content decreased with PE and may cause a stiffening in the tissue [45, 59].

The mean late elastic modulus increased by 14% with PE. Although this result was not statistically significant, the increased modulus was attributed to increased collagen content of UC arteries with PE [37, 38].

Histology and SEM images agreed with published results showing that elastin content decreases with PE and that the artery wall thickens with PE [38]. However, these were preliminary results. Further histology and SEM studies are necessary to be certain of these changes in UC arteries with PE.

Chapter 7

Conclusions

Little is known about the relationship between the structure and function of UC arteries. Studying the biomechanical response of healthy UC arteries is an essential first step in understanding the function of these vessels. This thesis established normative mechanical data as a benchmark for studying disease states during pregnancy. Mode of delivery (vaginal versus Cesarean section) had no discernible effect on the mechanical response of UC arteries. Also, mechanical response differed between the arteries in the same UC. This indicates that blood may flow preferentially through one artery over the other.

To the best of the author's knowledge, this was the first study to compare the mechanical properties of UC arteries in normotensive and preeclamptic pregnancies.

Because PE is the most common complication during pregnancy, it is important to establish an understanding about how this disease affects fetal development. This thesis provided a foundation for studying the mechanical response of UC arteries in normotensive and preeclamptic pregnancies. Mechanical response of UC arteries exhibited a stiffer response with PE. Also, UC width decreased with PE. These changes may be due to decreased elastin content or decreased hydration in

UC arteries. These factors likely alter UC hemodynamics and may lead to changes in fetal cardiovascular development.

Chapter 8

Future Work

Further work is needed to fully understand the biomechanics of UC arteries in normotension and preeclampsia. Recommendations for future work include increasing sample size of UC tissues for both normotensive and PE cases. In addition, more clinical data should be collected concerning the health of the mother and the newborn. For example, length and weight of the newborn, maternal blood pressure throughout pregnancy and a record of fetal heart rate to observe deceleration during delivery [22]. These clinical factors are usually monitored during pregnancy and childbirth and should be easily obtainable.

Although care was taken to accurately measure the tissue dimensions using digital calipers, an improved method for measuring artery width and thickness is recommended. Use of a laser beam micrometer as in previous studies [15] or similar method may improve the accuracy of artery width and thickness measurements. In addition, a method for measuring the UC artery lumen diameter is recommended, as this is an important indicator of vessel hemodynamics.

A method to visualize collagen alignment in the UC arteries during mechanical testing, such as that described by Tower et al. [60], is also recommended. This technique would provide a correlation between the mechanical response of the

tissue and fiber orientation. In addition, a method to quantify specific biomolecular components such as elastin, collagen, proteoglycans and glycosaminoglycans would provide an understanding of how these biomolecules affect the mechanical response of UC arteries.

The final recommendation for future work is to mechanically test UC arteries in the circumferential direction. *In vivo*, Arteries primarily exhibit circumferential strains rather than longitudinal strains [10]. For this reason, it will be beneficial to establish data for UC arteries in the circumferential direction. This will provide a better comparison to other circumferential arterial data and will establish a better understanding of UC artery behavior.

Future work will improve upon the current understanding of the structure-function relationship in UC arteries. Correlations between the mechanical response, anatomical structure, biomolecular structure and hemodynamics should be established. A detailed understanding of UC structure and function has the potential to impact many lives and may provide improved solutions in cases of adverse fetal health.

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