Thoracic and abdominal aortas stiffen through unique extracellular matrix changes in intrauterine growth restricted fetal sheep

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INTRAUTERINE GROWTH RESTRICTION (IUGR) is a clinical complication that places the fetus and newborn infant at significant risk for morbidity and mortality (8, 19, 29). Epidemiological studies have reported long-term chronic complications in the fetus such as adult hypertension, heart disease, stroke, and diabetes (2, 3, 18, 28). Currently, researchers have hypothesized the mechanism of cardiovascular disease (CVD) is arterial dysfunction characterized by “premature aging” (27). This theory is based upon extracellular matrix (ECM) formation during the fetal and neonatal period with protein depositions of collagen and elastin, the largest contributors to vessel compliance, highest during this developmental window (9, 15, 42, 43) and glycosaminoglycans (GAGs), important proteins in vessels ECM development and viscoelastic strength, also deposited during this period (13, 35). Disruption of the synthesis and deposition of vascular collagen, elastin, and GAGs in utero is thought to be a major mechanism in adult CVD in previously IUGR individuals.

Hemodynamic stress in adult arteries has been shown as an important modulator in vascular ECM composition and stiffness (12, 20). The fetal cardiovascular system in utero receives growth and development signaling from hemodynamic forces during the fetal and neonatal period (6, 7) and is highly susceptible to abnormal vascular constitutive remodeling due to abnormal hemodynamic stress (1). Hypoxic IUGR fetuses redistribute cardiac output altering the hemodynamics forces along the arterial tree (5). Changes in the thoracic aorta and abdominal aorta in the healthy neonatal lamb between 131 days gestation and 2–3 wk postpartum show that hemodynamics play a critical role in determining arterial structure (6). In IUGR newborns, a sustained change in hemodynamics has been shown through increased aortic pulse wave velocity indicating vascular stiffening in low birth weight babies as young children (31). Proximal aortic vascular stiffening is thought to be an important contributor to the progression of CVD (22, 36, 37).

Our group has used a fetal sheep model of placental insufficiency IUGR (PI-IUGR) that replicates many of the key features of human IUGR to study the pathogenesis of placental insufficiency complications (5). The sheep model of PI-IUGR has proven a useful model for studying underlying mechanisms in human PI-IUGR and has played an integral part in our current understanding of both normal and complicated pregnancies (4). Previously, we showed carotid and umbilical stiffening through different remodeling mechanisms in smaller more muscular arteries (10). However, the extent of proximal remodeling in the large elastic arteries has yet to be determined. In adolescents and adults, increased stiffness in the proximal large elastic arteries is a significant contributor to the progression of CVD increasing vascular resistance and heart afterloading (22, 36, 37).

Therefore, our goal was to test the biomechanics of the great elastic vessels, the thoracic and abdominal aortas, and measure the wall composition and structural organization to assess the degree and site-specific changes in proximal remodeling. We hypothesized PI-IUGR thoracic and abdominal aortas would

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exhibit increased stiffness due to increased collagen content and reorganized structure compared with controls. To test this hypothesis, we used aortas harvested from near-term control and PI-IUGR animals and assessed passive compliance, biochemical composition, and histomorphological structure to model the stiffening mechanism.

MATERIALS AND METHODS

Animal model. This study was approved by the University of Colorado Denver Institutional Animal Care and Use Committee and performed at the Perinatal Research Center, an accredited facility by the National Institutes of Health, the United States Department of Agriculture, and the American Association for Accreditation of Laboratory Animal Care. A total of 22 mixed-breed Columbia-Rambouillet ewes with time-dated singleton pregnancies were used for this study. Ten ewes were housed in an environmental chamber for maternal hyperthermic exposure (40°C for 12 h; 35°C for 12 h) for 80 days beginning at 35 days of gestational age (term = 147 days) as previously described (4, 5, 10, 32, 33). Following the hyperthermic exposure, ewes were housed in a normothermic environment (20°C) for the remainder of the study. Twelve control ewes were housed in a normothermic environment for the duration of the study.

Mechanical testing. Fetal and placental weights and lengths were obtained and recorded as previously described (10, 11). The thoracic aortas (control n = 11; PI-IUGR n = 10) were dissected from the base of the aortic arch to the diaphragm. The abdominal aorta (control n = 11; PI-IUGR n = 9) was dissected from the top of the diaphragm to the branches of the iliac and umbilical arteries. The aortas were stored in calcium-free phosphate-buffered saline (PBS) at 4°C until testing.

Vessel morphology was assessed before testing by measuring a stress-free 2-mm-long ring portion of the dissected arteries measured for thickness and inner diameter optically. The dissected aortas were tested for stiffness using transmural pressure and diameter as previously described (10, 11). In brief, the aorta was cannulated and sutured into a custom arteriography chamber and tested for passive compliance measuring transmural pressure, axial stretch, and vessel diameter. Aortas were tested for compliance using the ISO 7198.8.6 standard for the determination of a pressurized artery. Aortas were inflated from 5 to 10–200 mmHg in 10-mmHg increments and from 200 to 300 mmHg in 20-mmHg increments at prescribed axial stretches from 0% to 40% in 10% increments. Following mechanical testing, segments were collected and frozen for biochemical assay or fixed for histological examination as described below.

In vitro biochemical assay. An ~20-mg portion/test sample was lyophilized and weighed for dry mass. The dried tissue was hydrolyzed in 200 μl of 6 M HCl and dried using a speedvac. The amounts of elastin and collagen in the ECM were quantified using standard measurements of tissue desmosine and hydroxyproline content, respectively (38, 39). An additional ~20-mg portion/test sample was dried and weighed for dry mass. Sulfated GAG content was measured using a standard dimethylmethylenew acid assay with chondroitin-6-sulfate (Sigma-Aldrich, St. Louis, MO) as the standard (14).

Histology. Mechanical test samples of control and PI-IUGR aortas were processed for histology. The histological samples were formalin fixed, paraffin embedded, and sectioned at 5 μm with each artery stained with Verhoeff-van Gieson (VVG) for collagen and elastin content and hematoxylin and eosin (H&E) for general structure and cell number. Three representative fixed test samples of control and PI-IUGR thoracic and abdominal aortas were processed for imaging. Samples were rehydrated using PBS and then dissected and oriented for imaging in the circumferential-axial plane and imaged using laser confocal second harmonic generation (SHG) to visualize collagen and elastin content as previously described (11). Images were quantified for collagen fiber orientation and dispersion using a previously validated custom-written ImageJ analysis macro OrientationJ (30). An effective fiber angle was determined through a weighted mean and dispersion through the standard deviation from the mean.

Data analysis. The aortic walls were analyzed using the Gasser et al. constitutive model as previously described (11, 17). Experimental arterial walls were modeled as single layer fiber reinforced isochoric hyperelastic material. A strain energy function constitutively relates the stress required to deform a fiber composite hyperelastic artery. It is composed of an isotropic function (Ψ^isotropic) that describes the ground substance and elastin dominant portion of the vessel low load response and an anisotropic function (Ψ^anisotropic) that accounts for collagen fiber orientation and engagement at higher loads with an exponential increase in material stiffness at high strains as collagen recruitment takes over the load response.

The isotropic function (Ψ^isotropic) employs a neo-Hookian model that describes the behavior of isotropic rubber-like materials. In the circumferential and axial planes this term becomes:

\[ Ψ^\text{Isotropic} = \frac{c}{2}(I_1 - 3), \]

where \( I_1 \) is the first invariant, \( c \) is a fitting constant, and \( \lambda_n \) and \( \lambda_\theta \) are the stretches in the circumferential and axial directions, respectively.

The constant \( c \) is related to the shear modulus of the tissue. The anisotropic function (Ψ^anisotropic) describes the exponential increase in load as collagen engagement begins, and the strain energy function is appropriately in exponential form:

\[ Ψ^\text{Anisotropic} = \frac{k_1}{k_2} \left( \exp\left\{ k_2 \left[ (1 - 3k_1)I_1 - 1 \right] \right\} - 1 \right), \]

in which the constant \( k_1 \geq 0 \) is a stress-like parameter, \( k_2 \) is dimensionless, \( \kappa \) describes the fiber dispersion \((\kappa \neq 0 \ \text{fibers ideally anisotropically aligned}, \ \kappa = 1/3 \ \text{fibers isotropically distributed})\), and the collagen fiber angle, \( \gamma \). The invariant, \( \hat{I}_n \), characterizes the constitutive response of the fibers to mechanical loading.

To model the experimental response, the pressurization of a thin-walled tube was considered with an initial wall thickness \( \hat{h} \) and mean vessel radius \( R \) axially stretched across the experimentally prescribed conditions with no applied axial load and an internal pressure, \( p_i \), resulting in boundary conditions described in the axial and circumferential directions (17):

\[ f_x = \lambda_x \frac{\partial \hat{I}}{\partial x} - \frac{\lambda_x \lambda_\theta (\hat{h}/2\lambda_\theta)}{2HR} p_i = 0, \]

\[ f_\theta = \lambda_\theta \frac{\partial \hat{I}}{\partial \theta} - \frac{\lambda_\theta \lambda_x (\hat{h}/2)}{H} p_i = 0, \]

where \( f_x \) and \( f_\theta \) denote the axial and circumferential functions.

Equations 3 define a system of nonlinear equations that can be solved numerically for a prescribed internal pressure, \( p_i \).

The hyperelastic constitutive model was fit to experimental data by optimizing the correspondence between model predicted behavior and experimental behavior provided from the experimental pressure-diameter tests, by minimizing the stress-based nonlinear error function

\[ f_{\text{error}} = \sum_{i=1}^{n} \left( w_1 f_1^2 + w_2 f_2^2 \right) \]

where \( n \) is the number of experimental data points and the weighting factors are \( w_1 \) and \( w_2 \). Error function minimization was performed using Matlab (The Mathworks, Natick, MA) across a continuous spectrum of circumferential stretches and at five discrete axial stretches, corresponding to the available experimental data. Constitu-
tive parameters were found for both the thoracic and abdominal aorta across all sets of data minimizing the error function.

The degree of agreement between the experimental data and the constitutive model was assessed by a goodness of fit test. The F distribution (F) was measured based upon the value of the residual sum of the squares (Res SS) of the considered constitutive model for the individual experimental data points ($y_i$) and the residual points ($\hat{y}_i$) and defined

$$ F = \frac{\text{Res SS}/(n-q)}{\text{Reg SS}/q} = \sqrt{\frac{\sum_{i=1}^{n} (y_i - \hat{y}_i)^2/(n-q)}{\text{Reg SS}^2}} \quad (5) $$

where $n$ is the number of considered data points, $q$ is the number of parameters of $\Psi$, and $n-q$ is the number of degrees of freedom. The regression sum of the squares (Reg SS) is the sum of all the wall stresses for the experimental data point divided by the number of all data points.

**RESULTS**

**Anatomical findings.** PI-IUGR fetuses exhibited a 40% reduction in fetal weight ($P < 0.05$), a 33% reduction in placental weight ($P < 0.05$), and a 14% reduction in crown to rump length ($P < 0.05$) compared with the control fetuses (Table 1). The PI-IUGR thoracic aorta morphology exhibited no change in the inner diameter and a reduction of the wall thickness by 30% ($P < 0.05$) compared with the control, increasing the ratio of inner diameter to wall thickness. Abdominal aorta morphology in PI-IUGR had a reduced inner diameter by 18% ($P < 0.05$) and wall thickness by 19% ($P < 0.05$), maintaining the ratio of inner diameter to wall thickness. Wall composition measured by biochemical assays demonstrated vessel-specific changes. In the thoracic aorta, the collagen (hydroxyproline) and elastin (desmosine) content increased by 24% ($P < 0.05$) and 30% ($P < 0.05$), respectively, whereas sulfated GAG content was decreased by 33% ($P < 0.05$) in PI-IUGR (Table 1). The abdominal aorta contained increased collagen by 30% ($P < 0.05$) and decreased sulfated GAG by 38% ($P < 0.05$) in PI-IUGR.

**Biomechanical findings.** The thoracic aorta exhibited reduced outer diameter, inner diameter, and wall thickness across all transmural pressures (5–300 mmHg) in PI-IUGR ($P < 0.05$) (Fig. 1). Similarly, the abdominal aorta had decreased outer and inner diameters ($P < 0.05$) across all transmural pressures (5–300 mmHg) in PI-IUGR but retained a mean wall thickness similar to the controls ($P = 0.50$). Both thoracic and abdominal aortas showed increased stiffness demonstrated by the reduced circumferential stretch in response to transmural pressure in PI-IUGR (Fig. 1). In PI-IUGR, the thoracic aorta showed an increased stiffness at transmural pressures between 5–20 and 190–300 mmHg ($P < 0.05$), whereas the abdominal aorta demonstrated an increased stiffness at transmural pressures between 100 and 300 mmHg in PI-IUGR ($P < 0.05$).

Figure 2 shows the stiffening trend with reduced outer diameter and circumferential stretch as axial stretch were applied from 1.0 to 1.2 and finally to 1.4. Across an axial stress step, the abdominal aorta PI-IUGR artery increases in stiffness much more than the control artery. As axial stretch increased, a reduction of the mean thoracic aorta outer diameter and circumferential stretch was observed of 5% (range = 2–7%) in control arteries and 5% (range = 2–10%) in PI-IUGR arteries. In the abdominal aorta, the mean outer diameter and circumferential stretch were reduced, varying from 6% (range =

Table 1. Necropsy data

<table>
<thead>
<tr>
<th></th>
<th>Control ($n = 12$)</th>
<th>PI-IUGR ($n = 10$)</th>
<th>Change, %</th>
<th>$P$ Value</th>
</tr>
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<tbody>
<tr>
<td>Necropsy data</td>
<td></td>
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</tr>
<tr>
<td>Fetal wt, g</td>
<td>3.391 ± 228.0</td>
<td>2.044 ± 163.8</td>
<td>↓ 40%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Placental wt, g</td>
<td>324.8 ± 34.6</td>
<td>218.9 ± 178.8</td>
<td>↓ 33%</td>
<td>0.03*</td>
</tr>
<tr>
<td>Age at Necropsy, days</td>
<td>134 ± 1</td>
<td>133 ± 1</td>
<td></td>
<td>0.7</td>
</tr>
<tr>
<td>Crown to rump length, mm</td>
<td>468 ± 13</td>
<td>403 ± 9</td>
<td>↓ 14%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Female n (%)</td>
<td>4 (33%)</td>
<td>4 (45%)</td>
<td></td>
<td></td>
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<tr>
<td>Male n (%)</td>
<td>8 (67%)</td>
<td>6 (55%)</td>
<td></td>
<td></td>
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<tr>
<td>Morphology</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Thoracic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ID, mm</td>
<td>3.98 ± 0.23</td>
<td>3.52 ± 0.16</td>
<td></td>
<td>0.21</td>
</tr>
<tr>
<td>Thickness, mm</td>
<td>1.99 ± 0.07</td>
<td>1.38 ± 0.08</td>
<td>↓ 30%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ID-to-thickness ratio</td>
<td>1.93 ± 0.14</td>
<td>2.61 ± 0.19</td>
<td>↑ 35%</td>
<td>0.008</td>
</tr>
<tr>
<td>Cross-sectional area, mm²</td>
<td>37.6 ± 2.2</td>
<td>21.6 ± 2.1</td>
<td>↓ 43%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Abdominal</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>ID, mm</td>
<td>4.58 ± 0.81</td>
<td>3.75 ± 0.16</td>
<td>↓ 18%</td>
<td>0.01</td>
</tr>
<tr>
<td>Thickness, mm</td>
<td>0.67 ± 0.13</td>
<td>0.54 ± 0.09</td>
<td>↓ 19%</td>
<td>0.02</td>
</tr>
<tr>
<td>ID-to-thickness ratio</td>
<td>7.08 ± 0.63</td>
<td>7.12 ± 0.64</td>
<td></td>
<td>0.96</td>
</tr>
<tr>
<td>Cross-sectional area, mm²</td>
<td>11.1 ± 2.8</td>
<td>7.3 ± 1.3</td>
<td>↓ 34%</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Biochemical composition</td>
<td></td>
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<tr>
<td>Thoracic</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Hydroxyproline, μg/mg dry tissue</td>
<td>54.0 ± 3.3</td>
<td>66.8 ± 2.9</td>
<td>↑ 24%</td>
<td>0.01</td>
</tr>
<tr>
<td>Desmosine, μg/mg dry tissue</td>
<td>3.00 ± 0.39</td>
<td>3.90 ± 0.15</td>
<td>↑ 30%</td>
<td>0.05</td>
</tr>
<tr>
<td>sGAG, μg/mg dry tissue</td>
<td>32.9 ± 1.7</td>
<td>21.9 ± 1.3</td>
<td>↓ 33%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Abdominal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxyproline, μg/mg dry tissue</td>
<td>78.0 ± 6.0</td>
<td>101.4 ± 6.0</td>
<td>↑ 30%</td>
<td>0.010</td>
</tr>
<tr>
<td>Desmosine, μg/mg dry tissue</td>
<td>2.80 ± 0.31</td>
<td>2.94 ± 0.39</td>
<td></td>
<td>0.78</td>
</tr>
<tr>
<td>sGAG, μg/mg dry tissue</td>
<td>28.4 ± 2.1</td>
<td>17.5 ± 1.1</td>
<td>↓ 38%</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of ewes. ID, inner diameter; sGAG, sulfated glycosaminoglycans; ↓, decrease; ↑, increase. Animals show reduced fetal size in the placental insufficiency intrauterine growth restriction (PI-IUGR) model. PI-IUGR vessels have reduced geometry and remodeled wall composition. *Mann-Whitney Nonparametric test.

H431
2–7%) in control arteries and 8% (range = 4–10%) in PI-IUGR as axial stretch was increased from 1.0 to 1.2. As axial stretch was increased from 1.2 to 1.4 in the abdominal aorta, the mean outer diameter and circumferential stretch were reduced, varying from 8% (range = 5–10%) in the control arteries and 11% (range = 8–12%) in the PI-IUGR arteries. The PI-IUGR abdominal aortas had an increased stiffening as shown in reduced circumferential stretch ($P < 0.05$) due to applied axial stretching compared with the control arteries.

The constitutive model response (curves) was obtained from a fit to the experimental data (points) of circumferential stretch vs. circumferential stress shown across axial stretches of 1.0, 1.2, and 1.4 for the thoracic and abdominal aortas (Fig. 3). The goodness of fit values for the thoracic aorta obtained from the error for the $F$ distribution were $3.6 \times 10^{-6}$ and $4.3 \times 10^{-6}$ for the control and PI-IUGR arteries, respectively, whereas for the abdominal aorta these values were $9.4 \times 10^{-7}$ and $4.4 \times 10^{-6}$ for the control and PI-IUGR arteries, respectively. These minimal $F$ distribution errors indicate the constitutive model fit parameters are statistically significant ($P < 0.05$). The constitutive parameters $c$, $k_1$, $k_2$, $\gamma$, and $\kappa$ based upon macroscopic response are summarized in Table 2. The thoracic aorta showed an increase in the isotropic and anisotropic contribution concomitant to collagen fibers aligned circumferentially ($\gamma$) creating stiffer vessels in PI-IUGR. The abdominal aorta exhibited increased anisotropic contribution with more circumferentially oriented collagen fibers ($\kappa$) stiffening in PI-IUGR vessels.

**Histomorphology.** Histology samples were examined for both morphological quantitative and qualitative differences. H&E image analysis from thoracic and abdominal aortas showed an increase in cellular density in the PI-IUGR vessels ($P < 0.05$) (Fig. 4). Changes in cellular shape and distribution were also noted between the two groups. PI-IUGR vessels exhibited densely clustered cells near the external elastic lamina and adventitia. Histomorphometric analysis of VVG-stained sections in the thoracic aorta showed significant increased elastin content in PI-IUGR ($P < 0.05$) (Fig. 5). However, histomorphometric analysis of the abdominal aorta showed no significant changes in elastin content. Observed qualitative differences were noted: elastic lamellae (black) structure in the PI-IUGR appeared more globular and less organized than the control samples that exhibited concentric elastic bands. The predicted fiber angle values ($\gamma$) and fiber dispersion ($\kappa$) were validated using SHG (Fig. 6). Thoracic and abdominal aorta images con-

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**Fig. 1.** A and B: pressure vs. diameter for control (closed symbols) and placental insufficiency IUGR (PI-IUGR, open symbols) at a stretch of 1.2. Thoracic aorta (A) has a significantly reduced outer diameter (triangles), inner diameter (diamonds), and wall thickness from 5 to 300 mmHg ($P < 0.05$). Abdominal aorta (B) has a significantly reduced outer diameter (triangles) and inner diameter (diamonds) from 5 to 300 mmHg ($P < 0.05$). C and D: pressure vs. circumferential stretch for control (closed triangles) and PI-IUGR (open triangles) at a stretch of 1.2. Thoracic aorta (C) is significantly different at pressures between 5–20 and 190–300 mmHg ($P < 0.05$). Abdominal aorta (D) is significantly different at pressures between 100 and 300 mmHg ($P < 0.05$).
Our major findings showed that the proximal, elastic arteries of the thoracic and abdominal aortas stiffen in response to PI-IUGR, while displaying different structural etiologies. The PI-IUGR thoracic aorta had a reduction in sulfated GAG content, increases in both collagen and elastin content, and increased collagen fiber alignment ($\gamma$) in the circumferential direction. The increased elastin and collagen contributed to isotropic and anisotropic stiffening, respectively. The PI-IUGR abdominal aorta had a reduction in sulfated GAG content, an increase in collagen, and decreased collagen fiber dispersion ($\kappa$) creating more aligned anisotropic fibers, increasing circumferential stiffness. At the end of gestation, the extracellular content and organization is remodeled in the PI-IUGR fetal sheep systemic vasculature increasing aortic stiffness.

These findings are important in understanding changes in the functional response of stiffened proximal vessels under diseased conditions, namely a reduction in the functional elastic reservoir, due to ECM remodeling. This important change is an often neglected contributor compared with the well-known increases in resistance and altered hemodynamics in the PI-IUGR model. Vascular remodeling indicates vascular dysfunction in PI-IUGR altering systemic cardiovascular growth and development. The increase in systemic stiffening represents a novel finding and possible mechanism of increased CVD of IUGR infants as adults. This work demonstrates that the intrauterine stress environment shapes vascular growth and development and can be altered in response to maternal disease. Building upon our previous work, we show that increases in stiffness occur along the arterial tree, but the pathophysiological remodeling mechanism differs with location, which may be due to the complex changes in hemodynamic blood flows to organ beds in PI-IUGR. Through the PI-IUGR model, our work moves toward understanding preclinical molecular events that may enhance the ability to detect patient risk and has the unique benefit of determining early life influences on later cardiovascular health.

During the neonatal transition, the thoracic and abdominal aortas develop differently in response to the changes in hemodynamic stress (23, 24). Our previous work showed that remodeling occurs differently in the carotid and umbilical arteries concomitant with increased stiffness with increases in collagen in the carotid artery and decreased sulfated GAGs in the umbilical artery in PI-IUGR (10, 11). This work further
examines the role of PI-IUGR alterations in systemic vascular development in the proximal aortas. CVD is thought to start vascular remodeling with changes in resistance at the organ level and progress to the proximal artery stiffness, which has recently received interest as an important indicator of morbidity and mortality (36). The work presented here shows that newborns with IUGR are at risk for increased proximal artery stiffness that will more directly affect left heart afterload and systemic hemodynamics than the carotid stiffness. Hypoxia has been shown to be a strong modulator of structural vascular remodeling in fetal sheep thoracic aorta resulting in increased collagen content, transforming growth factor-β (TGF-β1), matrix metalloproteinases, α-actin, and E-selectin and reduced elastin content (41). Remodeling in fetal aortic development due to IUGR stress in utero leads to structural remodeling resulting in aortic stiffening in the guinea pig as an adult (40). Therefore, both hemodynamics and oxygen tension appear to

Table 2. Data fit coefficients using the anisotropic hyperelastic model of the thoracic aorta and the abdominal aorta in control and IUGR near-term fetuses

<table>
<thead>
<tr>
<th></th>
<th>c, kPa</th>
<th>$k_1$, kPa</th>
<th>$k_2$</th>
<th>$γ$, degree</th>
<th>$κ$</th>
</tr>
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<tbody>
<tr>
<td>Thoracic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.7</td>
<td>26.5</td>
<td>2.2</td>
<td>23.4</td>
<td>0.118</td>
</tr>
<tr>
<td>PI-IUGR</td>
<td>25.3</td>
<td>34.1</td>
<td>4.3</td>
<td>15.7</td>
<td>0.126</td>
</tr>
<tr>
<td>Abdominal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.1</td>
<td>84.3</td>
<td>2.9</td>
<td>29.5</td>
<td>0.121</td>
</tr>
<tr>
<td>PI-IUGR</td>
<td>1.0</td>
<td>94.5</td>
<td>15.9</td>
<td>27.4</td>
<td>0.085</td>
</tr>
</tbody>
</table>

IUGR, intrauterine growth restriction; $c$, fitting constant; $k_1$, stress-like parameter constant; $k_2$, dimensionless constant; $γ$, collagen fiber angle; $κ$, fiber dispersion.

Fig. 3. Experimental data for control (●) and PI-IUGR (○) with experimental fit for control (solid line) and PI-IUGR (broken line). Axial stretches of 1.0 (A), 1.2 (B), and 1.4 (C) for the thoracic aorta and axial stretches of 1.0 (D), 1.2 (E), and 1.4 (F) for the abdominal aorta.

Fig. 4. Hematoxylin and eosin (H&E) histology and analysis. H&E-stained cross sections for thoracic aorta (A) and abdominal aorta (B). Top in A and B: representative control (CON) images. Bottom in A and B: representative PI-IUGR images. Quantification for no. of nuclei/500 mm$^2$ for both the thoracic aorta and abdominal aorta showed increased cell nuclei per area in PI-IUGR ($^*P < 0.05$).
pulmonary arteries with disruption in the NO, VEGF, and ECM gene expression. Future studies may include coherent analysis of biomechanical and biomolecular factors to identify key pathways in systemic vascular remodeling in IUGR.

In addition, several potential limitations in our study must be acknowledged. First, in this study we examined the role of PI-IUGR in the near-term time point. This work has important consequences for understanding vascular dysfunction and disease that may be present in the IUGR newborn. Furthermore, we hypothesize that intrauterine stress has long-term consequences based upon the Barker hypothesis literature, and acknowledge that exploration of this hypothesis will require future work in understanding the sequelae of CVD following intrauterine stress. By studying the end of gestation, the disease initiation and progression of vascular disease is not clearly understood, but this work represents an initial study into an underlying mechanism that altered vascular development and provides a potentially novel therapeutic target in the newborn treating vascular remodeling before the onset of CVD. Also, we present here the static and passive stiffness. Further studies should examine the dynamic compliance and vasoactive tone; however, static passive tests for compliance are the gold standard and basis for relating anatomical structure and physiological function. Finally, this work represents an investigation of the role of PI-IUGR in vascular remodeling. The underlying cellular and extracellular mechanisms of fetal vas-

easured through decreased growth, migration, tube formation, and loss of a highly proliferative population. We also have preliminary data of disrupted myogenic tone concomitant to vascular stiffening. Furthermore, our current in vitro studies indicate that hemodynamics may alter fetal endothelial cell phenotype by altering vasoactive mediators, angiogenic, inflammatory, and ECM gene expression. Future studies may include proper analysis of biomechanical and biomolecular factors to identify initial pathways in systemic vascular remodeling in IUGR.

be important modulators of fetal vascular development and that development clearly has far-reaching implications beyond gestation.

Altered hemodynamics, increases in flow and pressure with the intrinsic stretch, have been shown to increase vascular stiffening and cause vascular remodeling in the mature vasculature (12, 16, 20). The mechanotransduction pathways for the vascular remodeling have been well established in the adult where small increases in flow have been observed to stimulate production of nitric oxide (NO), prostacyclin, nitric oxide synthase, platelet-derived growth factor receptor (PDGR)-A, platelet-derived growth factor (PDGF)-B, fibroblast growth factor (FGF)-1, FGF-2, TGF-β, interleukin-1 and -6, and intercellular adhesion molecule-1 while downregulating vascular cell adhesion molecule-1 (21). Small increases in pressure are also observed to stimulate the production of PDGR-A, PDGF-B, FGF-1, FGF-2, and thromboxane (21). However, large pathological increases in hemodynamic flow and pressure in the mature vasculature have been shown to disrupt production of biomolecules of vasoactive mediators, angiogenesis, and inflammation (25, 26). These mechanisms of hemodynamic stress are relatively well established in the mature vasculature, but have received little attention in the fetal and neonatal developing vasculature. While the possible mechanisms outlined were not examined in the current study, it is probable that these factors play a role in the different vascular mechanical stiffening observed along the vascular tree. Our group has shown impaired endothelial function in the IUGR pulmonary arteries with disruption in the NO, VEGF, and protein kinase B production in IUGR (34). With preliminary data gathered in ongoing studies, we speculate that PI-IUGR may lead to systemic artery endothelial cell dysfunction as measured through decreased growth, migration, tube formation, and loss of a highly proliferative population. We also have
ular remodeling are still not understood and will require more research to understand the vascular pathogenesis in PI-IUGR.

Placental insufficiency in fetal sheep creates increased vascular stiffening through remodeling at the end of gestation, which in turn sets the stage for possible altered vascular growth and development. Changes in fetal development in response to placental insufficiency may be a mechanism for CVD later in life, the Barker hypothesis (2). The observed vascular changes in this study help define the pathogenic link between placental insufficiency and adult hypertension. Timely clinical recognition and treatment of disadvantageous fetal programming development may provide novel approaches for diagnosing and alleviating increased incidence of hypertension in adulthood.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: R.B.D., P.J.R., K.S.H., and V.L.F. conception and design of research; R.B.D. and K.S.H. analyzed data; R.B.D., P.J.R., K.S.H., and V.L.F. interpreted results of experiments; R.B.D. performed experiments; R.B.D., P.J.R., K.S.H., and V.L.F. performed experiments; R.B.D., P.J.R., and C.C.P. interpreted results of experiments; R.B.D. and K.S.H. wrote the first draft of the manuscript; R.B.D., P.J.R., K.S.H., and V.L.F. critically revised the manuscript; R.B.D. and K.S.H. contributed important intellectual content; R.B.D., P.J.R., K.S.H. and V.L.F. approved the final version of the manuscript.

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