Chronic effects of pulmonary artery stenosis on hemodynamic and structural development of the lungs

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Razavi H, Stewart SE, Xu C, Sawada H, Zarafshar SY, Taylor CA, Rabinovitch M, Feinstein JA. Chronic effects of pulmonary artery stenosis on hemodynamic and structural development of the lungs. Am J Physiol Lung Cell Mol Physiol 304: L17-L28, 2013. First published October 5, 2012; doi:10.1152/ajplung.00412.2011.—Pulmonary artery (PA) stenosis is a difficult obstructive defect to manage since clinicians cannot know a priori which obstructions to treat and when. Prognosis of PA stenosis and its chronic effects on lung development are poorly understood. This study aimed to characterize the hemodynamic and structural effects of PA stenosis during development. Fourteen male Sprague-Dawley rats underwent left PA (LPA) banding at age 21 days, and 13 underwent sham operation. Hemodynamic and structural impacts were studied longitudinally at 20, 36, 52, 100, and 160 days. Chronic LPA banding resulted in a significant reduction in LPA flow (P < 0.0001) and size of both proximal LPA (P < 0.0001) and distal LPA (P < 0.01), as well as a significant increase in flow and size of the right PA (P < 0.05) throughout development. Flows and sizes adapted such that normal levels of wall shear were restored after banding. At 160 days, LPA banding resulted in a significant decrease in left lung volume and an increase in right lung volume but no significant differences in total lung volume. There was an elevation of proximal LPA pressure as well as right ventricular hypertrophy in the banded animals. The banded lung exhibited arterial disorganization, loss of vessels, and enlargement of its bronchial arteries, whereas the contralateral lung showed signs of vascular pathology. There are consequences on development of both lungs in the presence of an LPA stenosis at young age. These results suggest that early intervention may be necessary to optimize left lung growth and minimize right lung vascular pathology.

CONGENITAL HEART DEFECTS (CHDs) are abnormalities of the heart and the great vessels that affect ~1 in 100 live births (14). They are the most common cause of developmental anomalies and non-infectious infant mortalities (9). A large number of CHD patients exhibit diffuse or focal obstructions in their pulmonary arteries resulting from an abnormal extension of ductal tissue (42), postoperative damage after surgical repair of other CHD lesions as in the case of tetralogy of Fallot (24), or genetic disorders such as Alagille or Williams syndromes (8, 19). The long-term effects of pulmonary artery stenosis, whether native or postoperative, are poorly understood and are only based on anecdotal evidence. Pulmonary artery stenosis is difficult to manage because no recommendations exist on the optimal strategies for intervention (2), and the isolated effects of these obstructions and their treatments are still largely unknown (7). In particular, physicians have no way of knowing a priori whether a particular stenosis should be treated and what the optimal timing for an intervention would be.

Previous studies have used models of pulmonary obstruction that are not directly applicable to the case of children with CHD. In particular, most previous studies have investigated the effects of a complete pulmonary arterial obstruction or ligation in both neonates (4, 10–12) and adults (17, 21–23, 31, 37–39) in a variety of mammalian species. The case of a partial pulmonary arterial obstruction was studied by Rabinovitch et al. (27), who described the acute effects of left pulmonary artery (LPA) banding in adult rats. To elucidate the chronic changes that occur in response to a partial pulmonary arterial obstruction in neonates, as most closely related to clinical CHD cases such as repaired Tetralogy of Fallot, we banded the LPA of neonatal rats and studied the functional and structural changes that ensued as the animals grew to adulthood. In this study, we aimed to assess the impact of pulmonary artery stenosis on lung development using new imaging techniques recently developed and validated by our group (29). We describe how increased and decreased pulmonary flows result in major structural changes and provide insight for clinicians in care of children with CHDs.

MATERIALS AND METHODS

Phase-contrast magnetic resonance imaging (PCMRI) was used to make longitudinal measurements of 1) cardiac output (CO), 2) right and left pulmonary arterial flows and sizes, 3) right-left lung flow split, 4) pulmonary arterial distensibility, and 5) wall shear rate. Echo was used to measure heart rate as well as main pulmonary artery (MPA) and aortic diameters. Arterial casting and high-resolution micro-computed tomography (CT) techniques as well as histological evaluations were used to assess structural changes in the pulmonary vasculature and the alveoli. Catheterization with Radi pressure wires (St. Jude Medical, St. Paul, MN) under fluoroscopy was used to measure pulmonary arterial pressure, and the effects on the heart were evaluated by measuring right ventricle (RV) mass compared with the left ventricle and the septum (LV + S). Hemogram analyses were conducted to check for changes in hematocrit levels.

Animals

All imaging and animal procedures were approved by Stanford University’s Institutional Animal Care and Use Committee. Male Sprague-Dawley rats were obtained from Charles River Laboratories (Wilmington, MA) at 19 days of age and were housed three per cage until 36 days of age, at which point they were separated into individual cages.
Study Design

PCMRI and echo measurements were made at 20, 36, 52, 100, and 160 days of age. At 21 days, the rats randomly underwent either an LPA banding (n = 14) or a sham operation (n = 13). Hemogram analyses were conducted at 22 and 160 days. Pulmonary arterial pressure measurements were made at 160 days (Table 1). At euthanasia, the animals were randomly assigned to undergo either pulmonary arterial casting (n = 7 for banded, n = 6 for sham) or perfusion fixation for histological preparations (n = 5 per experimental group).

A separate group of banded and sham-operated animals were used to evaluate RV hypertrophy at 100 and 160 days (n = 6 per group per time point).

Pulmonary Arterial Banding

To prepare the band, tantalum wire of diameter 0.127 mm (Sigma-Aldrich, Milwaukee, WI) was wound under tension around the core of a 27-gauge needle to form a helix. Cuts were made parallel to the longitudinal axis of the needle to obtain a number of rings with an inner diameter of ~0.4 mm. The bands were smoothed with fine sandpaper and opened slightly to allow placement around the LPA. After sedation, the animal was ventilated by use of 0.3 ml of oxygen and 0.4 mm. The bands were smoothed with fine sandpaper and opened slightly to allow placement around the LPA. After sedation, the animal was ventilated by use of 0.3 ml of oxygen.

Table 1. Characteristics of each experimental group

<table>
<thead>
<tr>
<th>Experiment Group</th>
<th>20 days</th>
<th>36 days</th>
<th>52 days</th>
<th>100 days</th>
<th>160 days</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Banded</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of animals</td>
<td>n = 13*</td>
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<tr>
<td>Body weight, g</td>
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<td>152 ± 10</td>
<td>276 ± 12</td>
<td>503 ± 40</td>
<td>583 ± 46</td>
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<tr>
<td>Heart rate, beats/min</td>
<td>455 ± 27</td>
<td>430 ± 25</td>
<td>392 ± 21</td>
<td>344 ± 25</td>
<td>333 ± 24</td>
</tr>
<tr>
<td>MPA diameter, mm</td>
<td>0.18 ± 0.02</td>
<td>0.26 ± 0.02</td>
<td>0.29 ± 0.03</td>
<td>0.38 ± 0.02</td>
<td>0.39 ± 0.02</td>
</tr>
<tr>
<td>Aorta diameter, mm</td>
<td>0.15 ± 0.01</td>
<td>0.25 ± 0.01</td>
<td>0.28 ± 0.01</td>
<td>0.33 ± 0.02</td>
<td>0.34 ± 0.02</td>
</tr>
<tr>
<td><strong>Sham-operated</strong></td>
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<tr>
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<td>n = 12</td>
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<tr>
<td>Body weight, g</td>
<td>43 ± 6</td>
<td>120 ± 14</td>
<td>278 ± 17</td>
<td>504 ± 52</td>
<td>590 ± 63</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>439 ± 42</td>
<td>434 ± 25</td>
<td>415 ± 28</td>
<td>320 ± 49</td>
<td>333 ± 37</td>
</tr>
<tr>
<td>MPA diameter, mm</td>
<td>0.18 ± 0.02</td>
<td>0.26 ± 0.02</td>
<td>0.29 ± 0.02</td>
<td>0.37 ± 0.03</td>
<td>0.40 ± 0.03</td>
</tr>
<tr>
<td>Aorta diameter, mm</td>
<td>0.15 ± 0.01</td>
<td>0.24 ± 0.01</td>
<td>0.30 ± 0.03</td>
<td>0.33 ± 0.02</td>
<td>0.35 ± 0.02</td>
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</tbody>
</table>

RV hypertrophy (different groups of animals at different time points)

<table>
<thead>
<tr>
<th>Experiment Group</th>
<th>20 days</th>
<th>36 days</th>
<th>52 days</th>
<th>100 days</th>
<th>160 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Banded</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of animals</td>
<td>n = 6</td>
<td>n = 6</td>
<td>n = 6</td>
<td>n = 6</td>
<td>n = 6</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>528 ± 58</td>
<td>609 ± 39</td>
<td>609 ± 39</td>
<td>609 ± 39</td>
<td>609 ± 39</td>
</tr>
<tr>
<td>RV mass, g</td>
<td>0.23 ± 0.01</td>
<td>0.23 ± 0.02</td>
<td>0.23 ± 0.02</td>
<td>0.23 ± 0.02</td>
<td>0.23 ± 0.02</td>
</tr>
<tr>
<td>LV + S mass, g</td>
<td>0.89 ± 0.09</td>
<td>0.97 ± 0.08</td>
<td>0.97 ± 0.08</td>
<td>0.97 ± 0.08</td>
<td>0.97 ± 0.08</td>
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<tr>
<td><strong>Sham-operated</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Number of animals</td>
<td>n = 6</td>
<td>n = 6</td>
<td>n = 6</td>
<td>n = 6</td>
<td>n = 6</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>514 ± 35</td>
<td>623 ± 94</td>
<td>623 ± 94</td>
<td>623 ± 94</td>
<td>623 ± 94</td>
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<tr>
<td>RV mass, g</td>
<td>0.20 ± 0.04</td>
<td>0.20 ± 0.03</td>
<td>0.20 ± 0.03</td>
<td>0.20 ± 0.03</td>
<td>0.20 ± 0.03</td>
</tr>
<tr>
<td>LV + S mass, g</td>
<td>0.96 ± 0.19</td>
<td>0.98 ± 0.12</td>
<td>0.98 ± 0.12</td>
<td>0.98 ± 0.12</td>
<td>0.98 ± 0.12</td>
</tr>
</tbody>
</table>

Values are means ± SD. One of the 13 sham-operated animals was excluded from the study due to observed hematoma in the lungs starting at age 36 days. PCMRI, phase-contrast magnetic resonance imaging; CT, computer tomography; RV, right ventricle; LV + S, left ventricle and septum; MPA, main pulmonary artery. *At 20 days, equipment failure prevented flow measurements in one banded animal and two sham-operated animals.

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(1.0–3.0 cm on each side) was set to encompass the heart and large vessels.

**Image Analysis**

The arterial lumens were delineated with manual segmentation of the PCMRI magnitude images using custom software (40). Baseline correction was performed to account for eddy currents by using a first-order linear fit of velocity measurements among the reference regions of the no-flow calibration tube included in the images. The volumetric flow rate for each location was then calculated by integration of cross-sectional area and average velocity measurements at each voxel in the lumen for each frame in the cardiac cycle.

**Cardiac Output**

Total pulmonary flow, or CO, was calculated from PCMRI data as defined by the sum of mean RPA and LPA flows according to

\[
CO = RPA + LPA
\]  

(1)

**Right-Left Lung Flow Split**

Percent flow to the left lung was calculated from PCMRI data as defined by mean LPA flow divided by CO according to

\[
\text{Percent Left Lung Flow} = \frac{\text{LPA}}{\text{CO}} \times 100
\]  

(2)

Flow split (R:L split) was defined as “percent flow to the right lung: percent flow to the left lung.”

**Distensibility**

Percent distensibility of the pulmonary arteries was calculated as the measure of change in cross-sectional area between systole and diastole from PCMRI data using custom MATLAB (The MathWorks, Natick, MA) code according to

\[
D \equiv \frac{(A_s - A_d)/A_s \times 100}{\text{where } A_s \text{ is the vessel area at systole and } A_d \text{ is the vessel area at diastole.}}
\]  

(3)

**Shear Rate**

Assuming Poiseuille flow, the time-resolved shear rate at the inner surface of the vessel wall \( \gamma \) was calculated using MATLAB for the RPA and the LPA from PCMRI data according to

\[
\dot{\gamma} = 4Q/r^3
\]  

(4)

where \( Q \) is the instantaneous pulmonary flow and \( r \) is the corresponding instantaneous internal vessel radius, assuming a circular cross-sectional area. These time-averaged shear rates were then calculated as a surrogate for wall shear stress (\( \tau \)) according to

\[
\tau = \eta \dot{\gamma}
\]  

(5)

where \( \eta \) is the blood viscosity in rats at different ages.

**Echocardiography (Ultrasound)**

Echocardiography was used in the same animals as PCMRI experiments to measure heart rate and the diameters of the MPA and ascending aorta (Ao). After anesthesia, hair was removed from the chest area using a depilatory cream, Nair (Church and Dwight, Princeton, NJ). For age groups 20, 36, and 52 days, a Vivid 7 (GE Healthcare, Waukesha, WI) ultrasound scanner with the high-frequency (10–14 MHz) i13L transducer was used. In both cases, ultrasound transmission gel was applied to the transducer and spread over the chest. Transthoracic two-dimensional mode was used to obtain triplets of Ao and MPA diameter measurements at peak systole. Heart rate was calculated based on doppler measurements. All ultrasound measurements were conducted by the same experienced operator.

**Hemogram Assay**

Hemograms were analyzed on 250 \( \mu l \) of blood drawn retro-orbitally using heparinized capillary tubes (Chase Scientific Glass, Rockwood, TN) from each animal at both 22 and 160 days of age. The analysis consisted of the following parameters: white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

**Catheterization and Pressure Measurements**

Pulmonary pressure measurements were made in 160-day-old rats using fluoroscopy-guided catheterization. The animals were anesthetized with 3% isoflurane in 3 liters/min of oxygen, maintained with 1.5–2.0% isoflurane in 1.5–2.0 liters/min of oxygen. The jugular vein was cannulated using Jelco 24-gauge intravenous catheters (Smiths Medical, Dublin, OH). Hi-torque whisper guide wires (0.014-in. diameter) (Abbott Vascular, Redwood City, CA) and Prowler infusion catheters (0.75-mm diameter) (Cordis Neurovascular, Johnson & Johnson, Bridgewater, NJ) were used to gain access to the pulmonary arteries under a C-arm fluoroscopy scanner (OEC 9600, GE Healthcare) at 57 KVP and 2.1 mA·s. Radi pressure wires (Radi Medical Systems, St. Jude Medical, St. Paul, MN) were calibrated and utilized to make pulmonary blood pressure measurements at the level of the RPA and LPA in both groups of animals, and distal LPA were calibrated in the banded animals only. Distal pulmonary vascular resistance (PVR) was calculated for both the right and left lungs (distal to the band in the banded animals) assuming a negligible pulmonary venous pressure according to

\[
PVR = \frac{P}{Q}
\]  

(6)

where \( P \) is mean pulmonary arterial pressure as measured by Radi pressure wire and \( Q \) is mean pulmonary flow as measured by PCMRI. Heparin (0.1 ml/100 g body wt) was injected to induce anticoagulation before euthanization with 0.2 ml/100 g body wt of pentobarbital sodium (Lundbeck, Deerfield, IL).

**Arterial Casting**

Methods to provide contrast for the pulmonary arteries against the lung parenchyma in micro-CT imaging have been described previously (3, 28). Briefly, pulmonary arteries were infused with a radiopaque contrast agent in banded (\( n = 7 \)) and sham-operated (\( n = 6 \)) animals. The chest cavity was opened by a midline thoracotomy after euthanization, and the heart and lungs were exposed. Polylethylene (PE190) tubing (1.7-mm outer diameter, 1.19-mm inner diameter) (Becton Dickinson, Sparks, MD) cut at a length of 40 cm, with one end connected to an 18-G needle (Becton Dickinson) and the other blunted, was used as a catheter inserted into the RV. The catheter was advanced to the level of the MPA, where it was secured in place using 3-0 silk sutures. To allow drainage, a cut was made in the left atrial appendage of the heart, and 2 ml of diluted heparin sodium (5 U/ml) (APP Pharmaceuticals, Schaumburg, IL) were pumped at 2 ml/min using a continuous syringe pump (Harvard Apparatus, Holliston, MA) to flush the blood out of the pulmonary arteries. To ensure uniform filling, the animals were moved to an upright position, and freshly catalyzed silicone polymer casting material, MV-yellow Microfil (Flow Tech, Carver, MA), was pumped through the catheter at 2 ml/min until the frontline reached the level of the RV. The flow rate was then reduced to 0.1 ml/min as the polymer entered the pulmonary circulation. The pump was stopped when the polymer was visible uniformly on the lung surface. After complete polymerization of the
Microfil at 4°C for 24 h, the heart and lungs were harvested en bloc and fixed in 10% formalin (Fisher Scientific, Fairlawn, NJ).

**High-Resolution Micro-CT Imaging**

Fixed heart and lungs samples after arterial casting were imaged using two different micro-CT scanners (28). To validate the presence of the band around the LPA in the banded group, the fixed tissue samples were first evaluated under X-ray using an eXplore RS micro-CT scanner (GE Healthcare). The band was then removed to prevent metal artifacts in subsequent micro-CT images, and both lungs and the heart were imaged at 49-μm resolution using the eXplore RS scanner at 70 kVp, 50 μA, and 720 projections. To account for the smaller fields of view in the higher-resolution scans, the left, right, and auxiliary lobes from each sample were then separated and imaged individually at 12.5-μm resolution with a VivaCT 40 micro-CT system (SCANCO Medical AG, Southeastern, PA) at 45 kVp, 174 μA, and 2,000 projections.

**Vascular Tree Morphometry**

Vascular morphometry was assessed using the high-resolution (12.5 μm) micro-CT image data of each lung lobe. Using ITK-SNAP (41), pulmonary arteries >300 μm in lumen diameter were segmented in 3D. Analyze 10.0 (AnalyzeDirect, Overland Park, KS) was used to remove the segmented arteries from the image data to prevent erroneous skeletonizations. The resulting image containing vessels <300 μm in lumen diameter was thresholded and skeletonized using Analyze. The resulting tree structures depicting the arterial network were used to quantify vessels of different lumen sizes, and the information was compiled and analyzed using custom MATLAB code (28).

**Histology**

Histological assays were conducted to study the microscopic effects of chronic alteration in pulmonary flow. To prepare the samples for histology (n = 7 per experimental group), a similar procedure as arterial casting was used, except a mixture of barium sulfate in gelatin (Sigma Aldrich), instead of Microfil, was injected into the pulmonary arteries at 2 ml/min. The animals were kept at 4°C for at least 1 h to ensure solidification of the barium gelatin mixture before perfusion fixation of the lungs with 10% formalin via a tracheal tube for 2 min to ensure consistent filling. The lungs and the heart were then harvested en bloc and kept in 10% formalin for further fixation. After volume measurements of each lung individually using water displacement, sagittal cuts were made in each lung lobe, and the regions were embedded in paraffin for sectioning.

The slides were stained using the Movat stain for accentuated elastin fibers and observed under 400 magnification using a Leica DM2000 microscope (Leica Microsystems, Wetzlar, Germany). Automated alveolar counts and area measurements were conducted on a triplet of ×100 images per lung using ImageJ (1). For each image, the number of vessels <15 μm in lumen diameter were also counted manually. Percent wall thickness was calculated on ×400 images of five vessels of 50–150 μm in external diameter accompanying terminal or respiratory bronchioles per lung as

\[
\%WT = (2 \times \text{wall thickness/external diameter}) \times 100
\]  

(RV Hypertrophy)

After euthanasia and a midline thoracotomy, a separate group of animals were used for RV hypertrophy evaluations at 100 and 160 days of age (n = 6 per experimental group at each time point). The hearts were removed and cut at the level of the atrioventricular valves. The RV was separated from the LV + S, and their masses were measured. The ratio of RV mass to LV + S mass was used as an indication for RV hypertrophy.

**Statistical Analysis**

Continuous variables are reported as means (SD), as calculated using Excel (Microsoft, Redmond, WA). Intergroup differences were assessed using Student’s t-tests, paired in the case of dependent measurements, followed by corrections for multiple comparisons using the false discovery rate procedure (6). The critical significance level was defined as 0.05. Calculated means with standard deviations are reported, unless otherwise stated.

**RESULTS**

**Animals**

No significant differences in body weight were observed between banded and sham-operated animals throughout development (Table 1).
Right-left lung flow split changes gradually after banding. Before surgery, percent flow to the left lung was not significantly different between banded [31% (7%)] and sham-operated animals [26% (12%)] \((P = 0.29)\) (Fig. 2A). As early as 2 wk after banding, percent flow to the left lung was significantly reduced in the banded animals \((P < 0.0001)\) (Fig. 2A). In the banded animals, the flow split was reduced gradually from 69:31 (7%) at baseline to 84:16 (5%) at 36 days to 88:12 (4%) at 52 days of age and beyond. In the sham-operated controls, the flow split did not change significantly over time and remained constant at an average of 69:31 (4%).

CO and heart rate not affected. No significant differences were observed in CO between banded and sham-operated animals at any time point \((P > 0.35)\) (Fig. 2B). Both groups exhibited a sharp increase in CO until 52 days of age, followed by a slower plateau until 160 days. Similarly, there were no significant differences in average heart rate with banding (Table 1).

Elevated pulmonary arterial pressure in the proximal LPA. Chronic LPA stenosis resulted in a significant increase in mean pulmonary arterial pressure as measured by pressure wires at proximal LPA to 16 (3) mmHg in the banded animals compared with 12 (4) mmHg in sham-operated controls \((P < 0.05)\) (Fig. 3). Mean pulmonary arterial pressure measured in the RPA, however, did not show a significant difference between banded [14 (3) mmHg] and sham-operated animals [13 (4) mmHg] \((P = 0.48)\). In the banded animals, mean arterial pressure measured distal to the band in seven animals showed a significant drop to 5 (1) mmHg compared with the proximal LPA at 16 (3) mmHg \((P < 0.0001)\).

Pulmonary vascular resistance reduced in the right and elevated in the left lung. The average pulmonary vascular resistance (PVR) was calculated as mean flow divided by mean pulmonary arterial pressure. For the right lung, the average PVR was found to be 0.12 (0.04) mmHg·min⁻¹·ml⁻¹ [9,810 (2,900) dyn·s/cm⁵] in the banded animals compared with

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**Functional Effects of Chronic LPA Stenosis**

**Decreased LPA flow and increased RPA flow.** Aggregate curves for RPA and LPA flows at baseline and 160 days as measured by PCMRI are shown in Fig. 1, A and B. After LPA banding, mean flow to the left lung decreased significantly, whereas flow to the right lung increased significantly in the banded animals compared with sham-operated controls as early as 36 days of age \((P < 0.0001)\) for the LPA and \(P < 0.05\) for the RPA) (Fig. 1C). At 20 days, mean RPA and LPA flows were not significantly different between the two groups \((P = 0.67)\) for the RPA and \(P = 0.19\) for the LPA). Despite changes in flow with banding, absolute LPA flow increased significantly between neighboring time points until 52 days \((P < 0.01)\), whereas RPA flow increased significantly until 100 days of age \((P < 0.05)\) in both groups.
Structural Effects of Chronic LPA Stenosis

Smaller LPA and larger RPA immediately after banding. Banding of the LPA resulted in smaller LPA and larger RPA as shown in the sample PCMRI images in Fig. 4. At baseline, there were no significant differences in RPA and LPA sizes between banded and sham-operated animals \((P = 0.73\) for RPA; \(P = 0.61\) for LPA) as measured by PCMRI (Fig. 5A). Banding of the LPA at 21 days resulted in a significant reduction, as much as 50%, in average LPA cross-sectional area compared with sham-operated controls at all postoperative time points \((P < 0.001)\) (Fig. 5C). In both groups, LPA size grew continuously larger at each consecutive time point until 160 days of age \((P < 0.05)\). For the RPA, banded animals exhibited significantly higher average cross-sectional areas compared with sham-operated controls at all postoperative time points \((P < 0.05)\), except at 160 days \((P = 0.14)\). Growth trends in the RPA and LPA sizes were similar in both banded and sham-operated animals, with significant increases between all neighboring time points until 160 days \((P < 0.05)\). At all postoperative time points, distal LPA in the banded animals was significantly smaller compared with distal LPA in sham-operated controls \((P < 0.01)\).

Distensibility is reduced distal to the band. Percent distensibility of the distal LPA was significantly lower in the banded animals compared with sham-operated controls at 100 and 160 days of age \((P < 0.05)\). Percent distensibility calculations showed no significant differences between banded and sham-operated animals for either the RPA or the proximal LPA (Fig. 6A). No significant differences were observed in distensibility across time, except between 36 and 52 days in the banded LPAs \((P < 0.01)\).

Normal shear rates restored after banding despite changes in flow and size. Time-averaged shear rate calculations showed no significant differences between banded and sham-operated animals for both the RPA and LPA at all time points (Fig. 6B). For both vessels, changes in pulmonary flow resulted in
changes in cross-sectional areas such that normal levels of wall shear rate were restored.

**MPA and aortic diameters not affected.** Peak systolic MPA diameters showed no significant differences between banded and sham-operated animals ($P > 0.15$) (Table 1). Aortic diameters exhibited different growth patterns compared with the MPA but showed no significant differences between banded and sham-operated groups (Table 1).

**Lung volume reduced for the banded left lung and increased for the contralateral right lung.** At 160 days, inflated lung volume was significantly reduced for the left lung and significantly increased for the right lung in the banded animals compared with sham-operated controls ($P < 0.05$) (Fig. 7A). Total lung volume, however, was not significantly different between the two groups ($P = 0.68$).

**RV hypertrophy as early as 100 days.** The mass ratio of RV to LV + S was significantly higher in the banded animals [0.26 (0.02) at 100 days; 0.23 (0.02) at 160 days] compared with sham-operated controls [0.21 (0.02) at 100 days ($P < 0.01$); 0.21 (0.01) at 160 days ($P < 0.05$)] (Fig. 7B). There was an increase in LV + S mass in the banded animals from 100 days to 160 days (Table 1). This occurred in the absence of any significant differences in hematocrit levels between banded and sham-operated animals ($P > 0.24$).

**Differences in lung volume are associated with changes in alveolar size.** No significant differences were observed in alveolar counts per mm$^2$ of view field of Movat-stained histological sections in the right or left lungs of banded animals compared with sham-operated controls (Fig. 8; Table 2). Alveoli in the banded left lungs were smaller compared with those of sham-operated left lungs, whereas alveoli in the banded right lungs were larger compared with those of sham-operated right lungs. In light of the similarities in alveolar numbers between the two groups, differences in lung volume (Fig. 7A) can be explained by differences in alveolar size. No significant differences were observed in vessel counts ($<15\mu$m lumen diameter per mm$^2$ of view field) in the lungs of banded animals compared with sham-operated controls (Table 2).

**Lower wall thickness in both lungs.** Percent wall thickness (%WT) as defined by

$$\text{%WT} = \frac{2 \times \text{wall thickness/external diameter}}{\text{external diameter}} \times 100 \quad (8)$$

of 50- to 150-$\mu$m arteries was significantly lower in the right lung of banded animals compared with sham-operated controls ($P < 0.01$) (Fig. 9). In the left lung, there was a trend toward a significant reduction in %WT of these arteries in the banded animals compared with sham-operated controls ($P = 0.057$) (Table 2).
Underdevelopment of left lung vasculature. At 160 days, the banded left lung showed drastic vascular underdevelopment compared with the left lung of sham-operated controls as assessed by micro-CT (Fig. 10A). The banded left lung had a smaller and shorter central pulmonary artery, with underdeveloped stumps as side branches. The downstream pulmonary vasculature in the banded lung was found to be disorganized, the bronchial arteries were noticeably enlarged, and vascular connections had been made to the systemic circulation. The number of vessels with lumen diameters of 50–300 μm was much lower in the banded left lungs compared with sham-operated controls. Alveoli in the banded left lungs were smaller compared with sham-operated left lungs, whereas alveoli in the banded right lungs were larger compared with sham-operated right lungs. These sections were stained using the Movat stain and viewed at ×100.

DISCUSSION

In this study, we have quantitatively described the chronic effects of a partial pulmonary artery obstruction on pulmonary function and structure throughout development. Our PCMRI techniques (29) have enabled in vivo measurements of vessel size and pulmonary flow that indicate that pulmonary arterial underdevelopment results in lowered flow and smaller lumen sizes throughout development. We show, for the first time, that the wall shear autoregulatory phenomenon (18, 32, 43) that has been proposed as the biomechanical factor dictating vessel caliber in systemic arteries also holds in the case of the pulmonary arteries. More specifically, we have shown that both the RPA and LPA sizes adapt in response to changes in flow such that normal levels of wall shear are restored (Fig. 6B). These changes indicate that wall shear may act as a reliable biomechanical predictor of vessel caliber in response to changes in flow, regardless of baseline vessel size, flow, or age of the individual.

Our high-resolution image analysis techniques (28) in conjunction with conventional histological methods enabled, for the first time, a global evaluation of the structural changes in the lung vasculature that examined the entire proximal pulmonary arterial network (Fig. 10) and samples of the precapillary arterioles (Figs. 8 and 9). With this approach, we were able to demonstrate that, in the stenosed lung, the decreased flow leads to smaller proximal pulmonary arteries that are in a constant state of vasodilation and exhibit medial atrophy to maximally reduce total PVR. Despite this compensatory mechanism, pulmonary blood pressure rises proximally in the LPA, causing some degree of right ventricular hypertrophy. Distal to the stenosis, however, pulmonary blood pressure is significantly reduced, leading to an abnormal lung development process and

Table 2. Microscopic measurements of the right and left lungs in banded and sham-operated controls

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Alveoli per mm²</th>
<th>Vessels per mm²</th>
<th>Vessels per 100 Alveoli</th>
<th>Alveolar Size, μm²</th>
<th>%WT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L</td>
<td>R</td>
<td></td>
<td>L</td>
<td>R</td>
</tr>
<tr>
<td>Banded</td>
<td>119±24</td>
<td>131±34</td>
<td>⁹±2</td>
<td>7±2</td>
<td>8±4*</td>
</tr>
<tr>
<td>Sham-operated</td>
<td>122±38</td>
<td>131±46</td>
<td>⁹±3</td>
<td>8±0</td>
<td>8±1</td>
</tr>
</tbody>
</table>

Values are means ± SD. L, left; R, right; %WT, percent wall thickness in arteries of 50- to 150-μm external diameter. Significant difference between left and right lungs in the banded animals: *P < 0.01; †P < 0.05. §Significant difference between banded and sham-operated controls (P < 0.01). ‡Significant difference between banded and sham-operated controls (P = 0.06).
We have shown that, in neonates, banding leads to a reduction in the right lung and reduced external diameters in the left lung. Thickness for 50- to 100-μm arteries due to medial hypertrophy that banding in adult rats results in an increase in percent wall thickness in the right lung of banded animals compared with sham-operated controls (P < 0.01). In the left lung, there was a trend toward a significant reduction in percent wall thickness of these arteries in the banded animals compared with sham-operated controls (P = 0.057). These sections were stained using the Movat stain and viewed at ×400.

Fig. 9. Selected histological sections of arteries with 50- to 150-μm external diameters in the right lung (top) and the left lung (bottom) of sham-operated (left) and banded animals (right) at 160 days. Percent wall thickness was significantly reduced in the right lung of banded animals compared with sham-operated controls (P < 0.01). In the left lung, there was a trend toward a significant reduction in percent wall thickness of these arteries in the banded animals compared with sham-operated controls (P = 0.057). These sections were stained using the Movat stain and viewed at ×400.

We also show that the banding of the LPA in newborn rats leads to structural and hemodynamic responses that are different from those in adults. In particular, in contrast to the findings by Rabinovitch et al. who showed only a mild decrease in LPA lumen diameter 2 wk after LPA banding in adult rats (27), we describe a significant reduction in LPA size, as much as 50%, at all postoperative time points in neonates. Similarly, they observe no change in the size of the RPA, whereas we show a significant enlargement, as large as 15%, in the average RPA lumen size after banding. These differences suggest a higher sensitivity of the size of the central pulmonary arteries to changes in flow in neonates compared with adults. They show that banding in adult rats results in an increase in percent wall thickness for 50- to 100-μm arteries due to medial hypertrophy in the right lung and reduced external diameters in the left lung. We have shown that, in neonates, banding leads to a reduction in wall thickness that arises from chronic vasodilation of both lungs in accord with observations in patients with pulmonary stenosis and tetralogy of Fallot (35, 36). Wagenvoort et al. (36), in the absence of pulmonary pressure data, speculate that a reduction in pulse pressure may be a contributing factor to this medial atrophy. In our study, maximal dilatation to reduce distal pulmonary vascular resistance in both lungs may be another possible mechanism at work. The pulmonary arteries in the banded lung are maximally dilated to reduce distal PVR as much as possible to counterbalance the proximal resistance of the stenosis. On the contralateral side, the distal vasculature is again maximally dilated to normalize the proximal RPA pressure and to accommodate the increased pulmonary flow. The exact mechanism of this medial atrophy remains to be investigated, however, and further studies are warranted.

Rabinovitch et al. (27) also found reduced arterial density relative to the alveoli in both lungs, indicating that, in adults, vessels may shut down in response to altered flow, whereas alveoli are unaffected. In neonates, we observe no change in arterial density relative to the alveoli after banding, which may suggest that vessels, as well as alveoli, are equally underdeveloped in the younger animals such that the relative density is not affected, in accord with observations in infants with tetralogy of Fallot (13, 25). In both adults and neonates, however, LPA banding leads to RV hypertrophy, larger right lung volume, smaller left lung volume, and a decrease in right lung vascular resistance. Similarly, pulmonary artery pressure is increased significantly in the proximal LPA but not the RPA after banding in both adults and neonates under sedation. Under conditions of stress such as exercise or hypoxia, the pulmonary arterial pressure is likely to rise in the RPA after banding (33).

We also show that the structural effects of a partial LPA obstruction are different from those after a complete ligation in neonates. Haworth et al. (10–12) ligated the LPA of newborn pigs and observed abnormally small central pulmonary arteries in all animals but found smaller vessels in older animals. Their preventing the formation of new vessels. As a result, the number of vessels is reduced and a loss of vascular organization persists, both of which affect alveolar growth and lead to smaller alveoli and, therefore, smaller lung volumes. To compensate for these deleterious effects, the bronchial circulation is recruited, providing the pulmonary microvasculature at the precapillary level with oxygenated blood from the systemic circulation that ensures normal vascular development at the site of gas exchange. The recruitment of the bronchial circulation creates a shunt between the pulmonary and systemic circulations, leading to increased workload for the left ventricle and resulting in some degree of left ventricular hypertrophy over time. On the contralateral side, on the other hand, increased pulmonary flow leads to an enlargement of the proximal pulmonary arteries per shear stress autoregulatory phenomena. However, these vessels also exhibit medial atrophy since they are also in a constant state of maximal vasodilation to decrease pulmonary vascular resistance and normalize pulmonary pressure. Despite a normal RPA pressure, the increased flow may cause damage to some distal vessels and results in a mild reduction in the number of vessels.

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Fig. 10. Three-dimensional volume renderings of micro-CT images of sham-operated (left) compared with banded (right) animals. A: the left lung in the banded animals is smaller, with underdeveloped axial pulmonary artery branches, disorganized downstream pulmonary vasculature, enlarged bronchial arteries (arrows), and a smaller proximal LPA compared with sham-operated animals. B and C: the right lung in the banded animals exhibits a larger volume, a longer axial RPA, and sparse peripheral vasculature. D: number of vessels with lumen diameters of 50–300 µm in the sham (left) and banded (right) animals for the left lung and the right auxiliary lobes. The banded lungs have fewer vessels in both lobes compared with the sham-operated controls.
observed regression in vessel size after complete obstruction, therefore, contrasts with our findings of a continual yet impeded growth after partial obstruction. We find that, despite abnormally low perfusion levels and smaller LPAs, the banded lungs still continue to grow with growth spurts at normal times. This may indicate the presence of an adaptive growth response with reduced pulmonary flow that is entirely lost in the case of a complete obstruction. In both partial and total obstruction as shown here and by Haworth et al. (12), the obstructed left lung is smaller and the contralateral right lung is larger, resulting in a normal total lung volume. Even though we find that these changes in volume are associated with differences in the size of the alveoli in both lungs, Haworth et al. showed that, in the contralateral right lung, the differences in lung volume are due to an abnormal increase in alveolar number rather than a change in alveolar size. This indicates that, with any degree of pulmonary flow reduction in the left lung, moderate increases lead to larger alveoli, whereas severe increases in pulmonary flow increase the number of alveoli. As shown in our study and those by Haworth et al. in pigs (10) and Charan et al. in lambs (4), both partial and complete obstruction of the LPA in neonates lead to the recruitment of the systemic circulation. Others have made similar observations in adult dogs (21, 22, 31), rats (17, 38, 39), and mice (23, 37), indicating that, within a prolonged enough period of pulmonary flow reduction, the systemic circulation is recruited to supply the obstructed lung. This recruitment of the systemic circulation may account for the increased density of small arteries observed in the banded left lungs in our study. In addition, the recruitment of the bronchial circulation and the increased bronchial flow lead to an increased burden on the LV as the oxygenated blood from the aorta is shunted back to the lungs (20, 38). As the shunting bronchial flow increases, the LV exhibits some degree of hypertrophy that is reflected in an increased mass of the LV + S in the banded animals from 100 days to 160 days (Table 1).

We have shown that the distal LPA in the banded animals is significantly smaller at all time points. As such, no evidence of poststenotic dilatation was observed after LPA banding, as others have seen in the great vessels in patients with a variety of CHDs (30). This can be attributed to the absence of turbulent flow patterns in rats with considerably smaller Reynolds numbers compared with humans. The distal LPA is not only smaller than normal in the banded animals, but also experiences significantly lower mean pulmonary arterial pressures and, consequently, exhibits reduced distensibility compared with the proximal LPA at all time points. This may point to an initial compensatory vasodilation of the distal pulmonary arteries in the banded lungs such that the intraluminal pressure and percent distensibility are close to normal in the earlier stages of development, indicating the presence of a limited window of vascular plasticity, during which interventions may reverse vascular remodeling and result in normal hemodynamics and after which the structural changes are irreversible (26).

Clinical Implications and Future Directions

We show that, after partial pulmonary arterial obstruction in neonatal rats, the flow split between the two lungs change gradually from 69:31 at baseline to 84:16 at 36 days to 88:12 at 52 days and beyond. In humans, the normal flow split has been measured as 54:46 previously (5, 34) and is different from rats due to the presence of an auxiliary cardiac lobe in the rodents. Our findings indicate that the hemodynamic and structural abnormalities observed are due to an equivalent flow split change of 54:46 at baseline to 76:24 to 82:18 in humans. The present study thus demonstrates that, in the presence of a fixed obstruction in the pulmonary arteries of young patients with an eventual 82:18 flow split, there are severe consequences on the development of both lungs, suggesting that intervention is necessary to optimize left lung growth and minimize right lung vascular pathology. It is imperative to conduct repeated studies with decreasing severity of the obstruction to determine at which degree of stenosis normal vascular growth can occur and intervention can be forgone without pathological effects. Further studies are also needed to determine whether and when such vascular abnormalities are reversible to explain the clinical observations that only a limited number of treated patients show signs of hypoplasia reversal after intervention (2).

We have also shown that, in the presence of a chronic pulmonary artery stenosis, the resistance of the obstruction may still be significant relative to the downstream PVR, suggesting that relief of a stenosis may still be beneficial in reducing the workload on the heart even after vascular remodeling in older patients. This is particularly significant in lesions requiring Fontan circuitry in which global lung development may be compromised (16).

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DISCLOSURES

No conflicts of interest, financial or otherwise are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


