Quantitative characterization of postnatal growth trends in proximal pulmonary arteries in rats by phase-contrast magnetic resonance imaging

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Razavi H, Zarafshar SY, Sawada H, Taylor CA, Feinstein JA. Quantitative characterization of postnatal growth trends in proximal pulmonary arteries in rats by phase-contrast magnetic resonance imaging. Am J Physiol Lung Cell Mol Physiol 301: L368–L379, 2011.—Malformations of the pulmonary arteries can increase right heart workload and result in morbidity, heart failure, and death. With the increased use of murine models to study these malformations, there is a pressing need for an accurate and noninvasive experimental technique that is capable of characterizing pulmonary arterial hemodynamics in these animals. We describe the growth trends of pulmonary arteries in 13 male Sprague-Dawley rats at 20, 36, 52, 100, and 160 days of age with the introduction of phase-contrast MRI as such a technique. PCMRI results correlated closely with cardiac output measurements by ultrasound echocardiography and with fluorescent microspheres in right-left lung flow split (flow partition). Mean flow, average cross-sectional area, distensibility, and shear rates for the right and left pulmonary arteries (RPA and LPA) were calculated. The RPA was larger and received more flow at all times than the LPA ($P < 0.0001$). Right-left flow split did not change significantly with age, and arterial distensibility was not significantly different between RPA and LPA, except at 160 days ($P < 0.01$). Shear rates were much higher for the LPA than the RPA ($P < 0.0001$) throughout development. The RPA and LPA showed different structure-function relationships but obeyed similar allometric scaling laws, with scaling exponents comparable to those of the main pulmonary artery. This study is the first to quantitatively describe changes in RPA and LPA flows and sizes with development and to apply phase-contrast MRI techniques to pulmonary arteries in rats.

hemodynamics; lungs; wall shear; structure-function relationships; allometric scaling power laws

PULMONARY VASCULAR DISEASE (PVD), a diverse category of disorders affecting the blood vessels in the lungs, has a broad spectrum of clinical, pathophysiological, and histological consequences. A major category of PVD includes structural malformations in the pulmonary arteries that are either congenital, arising from structural heart defects and/or genetic disorders such as Alagille and Williams syndromes, or acquired after surgical interventions, most frequently following repair of tetralogy of Fallot. Whether these malformations are congenital or acquired, they are known to disturb normal pulmonary hemodynamics and lead to lung hypoplasia, impaired vascular growth, ventilation-perfusion mismatching, and, in severe cases, right ventricular failure and death. Despite these severe consequences, very little is known about the optimal surgical timing and strategies for restoration of normal anatomy and physiology in patients with pulmonary arterial malformations. Additionally, there are no quantitative data on the condition of diseased pulmonary arteries and their hemodynamic state as the patient grows from infancy to adulthood.

In light of the difficulties associated with conducting longitudinal studies in newborn patients, animal models are the only alternate means for the study of pulmonary arterial malformations. In particular, murine models have been most frequently used, mainly because of their relative ease of use and similarity in their lung development to humans (40). The ability to quantitatively characterize the effects of pulmonary arterial disease in these animal models, however, first requires a thorough understanding of normal growth in the absence of disease. Previous studies of normal development in the lung have exclusively focused on anatomic changes in the alveoli and histological microstructure of airways and pulmonary vessels. Morphometric changes in postnatal lung development have been described extensively in humans (6, 9, 11, 14, 30, 32, 40, 41), rats (4, 5, 7, 20, 24, 25), mice (1), pigs (26), and sheep (12). In contrast to the considerable number of such studies, to the best of our knowledge, there are no studies quantifying the developmental changes of right and left pulmonary arterial (RPA and LPA) hemodynamics. For example, no studies have been conducted to determine how blood flow to the two lungs changes during normal development, despite the fact that lung perfusion scans from magnetic resonance (MR) or scintigraphy are the primary clinical means for diagnosing pulmonary vascular abnormalities. Given the interest in the description of lung development, it is surprising that no information has been gathered on the normal growth patterns of RPA and LPA hemodynamics. This paucity of information is primarily due to the absence of an accurate, robust, and noninvasive experimental technique that is easy to use for the in vivo investigation of pulmonary blood flow in murine models.

In recent years, phase-contrast MRI (PCMRI) has been a powerful noninvasive technique to measure blood flow in limited parts of the anatomy in small animals. In rats, in particular, PCMRI has been applied to study blood flow only in the abdominal aorta (16), the cardiac chambers and the aortic outflow tract (38), the coronary arteries (21), and the right ventricular outflow tract (33). To the best of our knowledge, no previous studies have used this technique in the assessment of RPA and LPA flows, partly because of the unique challenges of low proton density and cardiac and respiratory motion artifacts involved with MRI in the lungs. In this study, we demonstrate the successful application of PCMRI to the proximal RPA and LPA in rats to reveal a quantitative description of changes in the in vivo hemodynamics of these vessels at different stages of postnatal develop-
ment. In addition, we present a comprehensive validation of PCMRI flow measurements in the RPA and LPA in animals over a wide range of ages (20–160 days) against two different experimental techniques: 1) ultrasound echocardiography and 2) fluorescent microspheres. With this new technique, we quantitatively describe RPA and LPA growth trends and highlight the similarities and differences between these two vessels over time.

MATERIALS AND METHODS

PCMRI measurements were used to determine 1) cardiac output (CO) and heart rate, 2) RPA and LPA blood flow and size, 3) right-left lung flow split, 4) arterial distensibility, 5) arterial wall shear rate, and 6) allometric scaling of pulmonary arterial flow and size with body mass. PCMRI measurements of CO were validated using ultrasound echocardiography, while right-left lung flow splits were validated using fluorescent microsphere techniques.

Animals

All imaging and animal care procedures were approved by Stanford University’s Institutional Animal Care and Use Committee. Pulmonary hemodynamics were studied longitudinally in 13 male Sprague-Dawley rats at different stages of development, 20, 36, 52, 100, and 160 days of age, when approximate average body weights of 50, 150, 300, 500, and 600 g, respectively, were reached (Table 1). The animals were obtained from Charles River Laboratories (Wilmington, MA) at 19 days of age and housed three per cage until 36 days of age, when they were separated into individual cages. Animals were given water and regular rat chow ad libitum.

PCMRI: Image Acquisition

For longitudinal measurement of RPA and LPA blood flow in vivo, the rats were imaged in the supine position on a 7-T magnet (Agilent Technologies, Santa Clara, CA) with Excite version 12.0 clinical interface (GE Healthcare, Waukesha, WI) using one of two in-house quadrature transmit/receive radio-frequency birdcage coils with inner diameter of 5.25 or 7.5 cm. The rats were anesthetized using 3% isoflurane in oxygen (3 l/min) and maintained at 1.5–2% isoflurane in oxygen (2 l/min). Gadolinium contrast (0.1 ml/100 g body wt; Magnevist, Bayer Healthcare Pharmaceuticals, Wayne, NJ) was injected intravenously through the tail vein into the 20- and 36-day-old rats before the scan for improved signal-to-noise ratio of localizer scans. The rat’s ECG, as well as respiration, was monitored during imaging and utilized for gating (SA Instruments, Stony Brook, NY). The ECG signal was generated using two subcutaneous electrodes placed on the chest, at approximately axis II, while the respiration signal was created using a pneumatic pillow (SA Instruments) taped to the abdomen. Images were only acquired during the expiration phase of the respiration cycle. In addition, the animal’s surface body temperature was monitored using a thermometer underneath the animal and maintained at 34–37°C via feedback to a warm air blower. To provide areas of zero velocity around the pulmonary vessels for calibration, a helical tube filled with 1% copper sulfate in agar gel (Aldrich, St. Louis, MO) was wrapped around the animal’s chest.

Temporally and spatially resolved in vivo throughplane blood flow velocity measurements were made at the proximal RPA and LPA, about halfway between the main pulmonary arterial (MPA) bifurcation and the first side branch of each vessel. To ensure correct prescription of the cross-sectional slice for both locations, at least two sets of long-axis localizer scans (2-dimensional spoiled gradient echo with flow compensation) of each vessel segment were completed. The cross-sectional PCMRI slice was prescribed perpendicular to all longitudinal localizer scans (Fig. 1). For velocity measurements, a two-dimensional fast gradient echo, sequential, flow-compensated, phase-contrast sequence was used. The imaging parameters were as follows: minimum repetition time = 3.2–4.6 ms, echo time = 1.5–2.1 ms, flip angle = 20°, views per segment = 1 and velocity-encoding parameter = 150–200 cm/s, acquisition matrix = 256 × 256, number of excitations = 4, and slice thickness = 1 mm. The field of view and consequent resolution were adjusted according to the animal’s age group to account for larger animal sizes in the older age groups and smaller vessels in the younger age groups (Table 2). Care was taken during image prescription to ensure that the signal from the no-flow calibration tube never aliased over anatomies of interest (Fig. 1). In addition, the number of frames per cardiac cycle was maximized depending on the animal’s heart rate and the minimum repetition time, ranging from 17 to 39 frames per cardiac cycle. A main field shim volume was set to encompass the heart and large vessels.

Image Analysis

The arterial lumen of the RPA and LPA was delineated with manual segmentation of the PCMRI magnitude images using custom-purposed software (36). Baseline correction was performed to account for eddy currents by a first-order linear fit of velocity measurements among the 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 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 during the systolic phase of the cardiac cycle (RPA_{systolic} and LPA_{systolic}) according to

\[ CO = RPA_{systolic} + LPA_{systolic} \]

Right-Left Lung Flow Split

Percent flow to the left lung was calculated from PCMRI data as defined by mean LPA flow divided by the sum of mean RPA and LPA flows according to

### Table 1. Number of animals and average body weights for each experimental group

<table>
<thead>
<tr>
<th>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>PCMRI and echocardiography</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of animals</td>
<td>13</td>
<td>13</td>
<td>13</td>
<td>13</td>
<td>11*</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>42 (4)</td>
<td>153 (16)</td>
<td>292 (23)</td>
<td>523 (41)</td>
<td>606 (61)</td>
</tr>
<tr>
<td>Fluorescent microspheres</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of animals</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>46 (6)</td>
<td>112 (11)</td>
<td>240 (12)</td>
<td>490 (79)</td>
<td>589 (43)</td>
</tr>
</tbody>
</table>

Values are means (SD). The same animals were used for phase-contrast MRI (PCMRI) and echocardiography (ultrasound) studies at all time points. Fluorescent microsphere experiments were performed on a different group of animals for each time point. *There was 1 unexplained mortality at 101 days and 1 missed data acquisition due to equipment failure.
percent left lung flow = \frac{LPA}{RPA+LPA} \times 100 \quad (2)

Right-left flow 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 using custom MATLAB (MathWorks, Natick, MA) code according to

\[ D = \left( \frac{A_s - A_d}{A_s} \right) \times 100 \quad (3) \]

where \( A_s \) is vessel area at systole and \( A_d \) is vessel area at diastole.

**Shear Rate**

With the assumption of Poiseuille flow, the time-resolved shear rate at the inner surface of the vessel wall (\( \dot{\gamma} \)) was calculated using MATLAB for the RPA and LPA according to

\[ \dot{\gamma} = \frac{4Q}{\pi r^3} \quad (4) \]

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

\[ \tau = \eta \dot{\gamma} \quad (5) \]

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

**Echocardiography**

To validate PCMRI measurements of total pulmonary flow, echocardiography (ultrasound) was used in the same animals to measure CO using five distinct instances of velocity-time integral (VTI) measurements of the MPA according to

\[ CO = area_{max} \times VTI \times heart rate \quad (6) \]

where \( area_{max} \) was calculated assuming a circular cross section using an average of three instances of systolic diameter measurements. The rats at all ages were anesthetized using 3% isoflurane in oxygen (3 l/min) and maintained at 1.5–2% isoflurane in oxygen (2 l/min). A depilatory cream (Nair, Church and Dwight, Princeton, NJ) was used to remove hair from the chest area. For the 20-, 36-, and 52-day-old groups, a Vivid 7 (GE Healthcare, Waukesha, WI) ultrasound scanner with a high-frequency (10–14 MHz) i13L transducer was used. For the two oldest (100- and 160-day-old) groups, a Vevo 2100 scanner (Visual Sonics, Toronto, ON, Canada) with the high-frequency (13–24 MHz) MS-250 transducer with its larger focal length was used to ensure deep penetration of the sound waves to the level of the MPA in the larger animals. In both cases, ultrasound transmission gel was applied to the transducer and spread over the chest. Transthoracic two-dimensional and pulsed-wave Doppler modes were used to obtain the triplet of MPA diameter measurements and the five VTI measurements.
Fluorescent Microspheres

As a validation step for PCMRI measurements, fluorescent microspheres were used to determine the relative blood flow to the left and right lungs at different stages of development. All microsphere procedures were performed with reference to the protocols developed previously by Sasaki et al. (27, 28). At each time point, different groups of animals were used for the PCMRI and microsphere studies (Table 1). Because of the terminal nature of the procedure, no longitudinal studies of flow splits were possible using this technique, and different groups of animals were used at each time point. The animals were obtained from Charles River Laboratories 2 days before the experiment and housed three per cage in the 20-, 36-, and 52-day-old groups.

With 3% isoflurane anesthesia, the rat’s jugular vein was cannulated using a 22- or 24-gauge intravenous catheter (Jelco, Smiths Medical, Dublin, OH). In each experiment, heparin (0.1 ml/100 g body wt) was injected to induce anticoagulation. Then 0.2 ml (~200,000 beads) of a well-mixed suspension of 15-μm-diameter crimson or orange fluorescent polystyrene microspheres (Invitrogen, Molecular Probes, Eugene, OR) was mixed with 0.8 ml of blood, drawn from the catheter, and injected into the vein. The microspheres were allowed to circulate and lodge in the pulmonary capillary bed for ≥15 min before the animals were euthanized using pentobarbital sodium (0.2 ml/100 g body wt; Lundbeck, Deerfield, IL).

After the animals were euthanized, the chest cavity was opened, the heart and lungs were harvested en bloc, and the left and right lungs were transferred to separate 50-ml polyethylene tubes. The lung tissues were digested for 72 h in 4 mol/l ethanolic potassium hydroxide (EMD Chemicals, Darmstadt, Germany) and 5 g of Tween 80 (MP Biomedicals, Solon, OH) per liter of 100% ethanol (Fisher Scientific, Fairlaw, NJ). To enhance digestion, the tubes were placed in a 50°C water bath for 72 h and manually shaken after 24 and 48 h. After complete digestion of the tissue, the samples were centrifuged for 30 min at 3,200 rpm, and the supernatant was carefully removed. Two rounds of wash steps were performed in which the pellet was completely resuspended in 1% Triton X-100 (Sigma Aldrich, St. Louis, MO), and the samples were again placed in a 50°C water bath overnight. Finally, two rounds of centrifugation and resuspension in phosphate buffer [0.01 mol/l; potassium phosphate monobasic and dibasic (Sigma Aldrich) in distilled water] were performed as the final rinse steps. Then the samples were centrifuged, and the supernatant was carefully removed, with care taken to prevent disturbance of the pellet. Finally, 3 ml of 2-ethoxyethyl acetate (Sigma Aldrich) were added to the pellet to extract the fluorescent dye from the microspheres. Tubes were then vortexed and allowed to stand overnight. Fluorescence levels in each lung sample were detected using a plate reader (SpectraMax M5e, Molecular Devices, Sunnyvale, CA). An excitation wavelength of 493–607 nm and an emission wavelength of 526–650 nm were used. The concentration of microspheres (microspheres/ml of solvent) was calculated from standard curves that were previously generated from serial dilutions of known concentrations of microspheres dissolved in 2-ethoxyethyl acetate.

Allometric Scaling

Allometric scaling laws, also known as power laws, describe changes in physiology and anatomy in terms of different scaling exponents (b) of body mass (M) according to

\[ Y = Y_0 M^b \]  

where Y is the parameter of interest and \( Y_0 \) is a normalization constant (3). To define allometric scaling relationships in the pulmonary arteries at different stages of development, regression lines of log-log plots of CO, mean RPA and LPA flows, MPA systolic area, and RPA and LPA average cross-sectional areas vs. body mass were used. The scaling exponent b was defined as the slope of regression lines. A 95% confidence interval (CI) for b, as well as correlation coefficients \( R^2 \) for the regression line was also calculated.

Statistics

Continuous variables are reported as means and standard deviations (SDs), as calculated using Excel (Microsoft, Redmond, WA). Intergroup differences were assessed in Excel using Student’s t-tests, paired in the case of dependent measurements, followed by corrections for multiple comparisons using the false discovery rate procedure (10). One-way ANOVA was used in JMP 8.0.2 (SAS Institute) in the case of multiple independent groups. The critical significance level \( \alpha \) was defined as 0.05 in all statistical tests. All data graphics also show calculated means with SDs, unless otherwise stated.

RESULTS

Animal Growth

Animals from PCMRI/ultrasound echocardiography and microsphere experiments showed a consistent increase in body weight with age (Table 1). The same 13 animals were followed longitudinally for all PCMRI and ultrasound experiments. There was one unexplained mortality at 101 days of age and another missed measurement at 160 days of age due to equipment failure.

Heart Rate and Cardiac Cycle

Experiments were conducted as the rats grew from 20 to 36 to 52 to 100 and then to 160 days of age (Fig. 2). The average heart rate decreased with age [452 beats/min (SD 32) at 20 days, 434 beats/min (SD 35) at 36 days, 406 beats/min (SD 32) at 52 days, 364 beats/min (SD 28) at 100 days, and 365 beats/min (SD 29) at 160 days]. Correspondingly, the cardiac cycle period was longer in the older animals, as shown by the aggregate pulmonary flow and area waveforms (Fig. 2, see Fig. 6).

Cardiac Output

PCMRI. PCMRI was used to calculate total pulmonary flow as defined by the sum of mean systolic RPA and LPA flows (Eq. 1). All pair-wise comparisons across time showed a significant difference \((P < 0.05)\). The average CO changed over time as follows: 29.7 ml/min (SD 6.6) at 20 days, 105.6 ml/min (SD 21.8) at 36 days, 168.6 ml/min (SD 27.0) at 52 days, 208.0 ml/min (SD 34.8) at 100 days, and 238.8 ml/min (SD 46.7) at 160 days. The rate of increase in CO was highest between 20 and 36 days but slowed during the subsequent time intervals (Fig. 3A). The sudden decrease in CO growth velocity occurred at 52 days of age, but CO continued to increase until 160 days of age.

Ultrasound echocardiography. Similar trends were observed in CO as measured by ultrasound echocardiography on the MPA. All time points showed a significant difference in CO compared with all other time points \((P < 0.05)\), with the exception of measurements at 100 and 160 days, which exhibited no significant differences \((P = 0.77; \text{Fig. 3A})\). The average CO as measured by echocardiography was as follows: 51.6
ml/min (SD 15.0) at 20 days, 127.1 ml/min (SD 34.4) at 36 days, 167.5 ml/min (SD 36.8) at 52 days, 223.0 ml/min (SD 42.0) at 100 days, and 216.5 ml/min (SD 46.2) at 160 days. As with PCMRI, the growth rate in CO was highest between 20 and 36 days but was similarly shown to decrease during the subsequent time intervals.

There was an excellent correlation between PCMRI and ultrasound echocardiography for measuring total pulmonary flow, with regression lines for individual animals between the two techniques showing a mean slope of 1.02 ± 0.06 (95% CI = 0.91–1.14; Fig. 3B). However, a much higher degree of variability, as depicted by higher SDs, was observed in ultrasound than PCMRI results, especially among the younger animals at 20 and 36 days of age.

**Right-Left Lung Flow Split**

**PCMRI.** Relative flow to the left lung from PCMRI was defined as mean LPA flow divided by the sum of mean RPA and LPA flows (Eq. 2). Flow split values obtained using PCMRI did not show a significant change with age (P = 0.37–0.92), with the left lung receiving an average of 32% (SD 2) of CO, rendering a right-left flow split of 68:32 (Fig. 4).

**Fluorescent microspheres.** Percent flow to the left lung was also measured using fluorescent microspheres as defined by left lung fluorescence divided by the sum of left and right lung fluorescence values. Flow split values calculated from fluorescent microsphere experiments also indicated no significant change over time (P = 0.52–0.93), with the left lung receiving an average of 36% (SD 3%) of total pulmonary flow, with a right-left flow split of 64:36 (Fig. 4).

There was a maximum of 5.3% and a minimum of 2.5% difference in percent flow to the left lung at a given time point between the average values obtained from the two techniques (Fig. 4). The ratio of the average flow split values obtained from microspheres to those obtained from PCMRI was 1.1–1.2, with higher percent flow to the left lung measured by microspheres than by PCMRI. Both techniques showed no significant differences in right-left flow splits with age, suggesting that the partitioning of left and right pulmonary vascular resistance remains constant during development. Variability in the measurement of percent flow to the left lung among animals of the same age was similar for the two techniques.

**RPA and LPA Flows**

Resampled aggregate RPA and LPA flows from all animals within each age group are shown in Fig. 2. RPA flow was consistently higher than LPA flow at all points of the cardiac cycle for all age groups. As expected, peak flow occurred at the same point in the cardiac cycle for both lungs, indicating similar flow patterns between RPA and LPA.

![Fig. 2. Aggregate flow waveforms for all animals at each experimental time point: 20 days (A), 36 days (B), 52 days (C), 100 days (D), and 160 days (E). Waveforms for each group were resampled to yield a sampling rate equivalent to the lowest number of cardiac frames in each age group and scaled to the group’s average heart rate. As expected, peak flows coincide for the RPA and LPA at all time points, and the average period for the cardiac cycle increases as the animals age. Error bars, SE.](http://ajplung.physiology.org/)
Beginning as early as 20 days of age and at each subsequent time point, mean RPA flow was statistically higher than mean LPA flow \( (P < 0.0001; \text{Fig. 5A}) \). Mean RPA flow was statistically different at each time point \( (P < 0.05) \), except between 100 and 160 days \( (P = 0.54) \). For LPA flow, all time points were statistically different from one another \( (P < 0.05) \). Mean RPA flow increased with age as follows: 13.0 ml/min (SD 2.6) at 20 days, 45.3 ml/min (SD 9.4) at 36 days, 67.8 ml/min (SD 11.9) at 52 days, 81.8 ml/min (SD 17.7) at 100 days, and 87.5 ml/min (SD 19.1) at 160 days. Similarly, mean LPA flow increased with age as follows: 5.3 ml/min (SD 2.1) at 20 days, 21.2 ml/min (SD 5.1) at 36 days, 33.6 ml/min (SD 8.9) at 52 days, 39.9 ml/min (SD 6.7) at 100 days, and 45.7 ml/min (SD 10.3) at 160 days. Both vessels showed the fastest rate of increase in mean pulmonary flow between 20 and 36 days, and their growth slowed in the subsequent time intervals. The rate of increase was higher for RPA than LPA flow at all time intervals, except between 100 and 160 days of age. The rate of increase dropped substantially at 52 days for RPA and LPA flows. Mean pulmonary RPA and LPA flows with respect to age, in days, were modeled using fitted exponential curves \( \text{Fig. 5B} \) as

\[
\begin{align*}
\text{RPA flow (ml/min)} &= 36.1 \ln(\text{age (days)}) - 86.5 \quad (8) \\
\text{LPA flow (ml/min)} &= 19.2 \ln(\text{age (days)}) - 48.4 \quad (9)
\end{align*}
\]

**RPA and LPA Sizes**

The aggregate time-resolved waveforms of RPA and LPA cross-sectional areas are shown in \text{Fig. 6} as the rats grew from 20 to 36 to 52 to 100 and then to 160 days of age. Cross-sectional area was consistently larger for the RPA than the LPA throughout the cardiac cycle at all time points. The timing of peak cross-sectional area coincides for both lungs but occurs later in the cardiac cycle than does the timing of peak flow \( \text{Fig. 2} \), as expected in physiological flows.

RPA average cross-sectional area was statistically higher than LPA average cross-sectional area \( (P < 0.0001) \) at all time points \( \text{Fig. 7A} \). For the RPA and LPA, average cross-sectional area was statistically different at each time point compared with all other time points \( (P < 0.05) \). RPA average area increased with age as follows: 1.3 mm\(^2\) (SD 0.2) at 20 days, 3.2 mm\(^2\) (SD 0.6) at 36 days, 4.5 mm\(^2\) (SD 0.8) at 52 days, 5.8 mm\(^2\) (SD 0.9) at 100 days, and 6.6 mm\(^2\) (SD 1.3) at 160 days. LPA average area increased with age as follows: 0.5 mm\(^2\) (SD 0.1) at 20 days, 1.3 mm\(^2\) (SD 0.3) at 36 days, 1.9 mm\(^2\) (SD 0.3) at 52 days, 2.4 mm\(^2\) (SD 0.4) at 100 days, and 2.6 mm\(^2\) (SD 0.5) at 160 days. Both vessels exhibited the fastest average growth rate between the two earliest time points. The RPA grew at a higher rate than the LPA throughout the cardiac cycle (\text{Fig. 7A}). As with mean flows, average cross-sectional areas of the RPA and LPA showed a significant drop in their growth velocity at 52 days. Average RPA and LPA areas with respect to age, in days, were modeled using fitted exponential curves \( \text{Fig. 7B} \) as

\[
\begin{align*}
\text{RPA Area (mm}\(^2\)) &= 2.5 \ln(\text{age (days)}) - 6.0 \quad (10) \\
\text{LPA Area (mm}\(^2\)) &= 1.0 \ln(\text{age (days)}) - 2.2 \quad (11)
\end{align*}
\]
Structure-function relationships. The RPA and LPA exhibit linear structure-function relationships, as defined by the dependency between vessel size and mean flow through the vessel (Fig. 8). Slopes of the size-flow lines for individual animals were significantly higher for the RPA than the LPA (0.072 vs. 0.058 mm²·min⁻¹·ml⁻¹, P < 0.001), indicating different growth trends between the RPA and LPA in rats. That is, the LPA of an older animal has a smaller cross-sectional area than the RPA of a younger animal that receives approximately the same amount of blood flow (Fig. 8).

Distensibility

Percent distensibility calculations showed no significant differences between the RPA and the LPA at ≤100 days of age, with an average distensibility of 53% (SD 4%) for the RPA and 58% (SD 2%) for the LPA. At 160 days, LPA distensibility was significantly higher than RPA distensibility (62% vs. 55%, P < 0.01). Arterial distensibility calculations for the LPA showed no significant differences among different time points (Fig. 9A), while RPA distensibility was significantly smaller at 20 days than all other time points (P < 0.05).

Shear Rate

Time-averaged shear rate of the RPA was significantly different from that of the LPA at all time points (P < 0.0001): 741 s⁻¹ (SD 158) for the RPA and 1,392 s⁻¹ (SD 219) for the LPA (Fig. 9B). Average shear rate decreased over time for the RPA and LPA in a similar manner, with statistically significant differences between neighboring time points only between 52 and 100 days of age (P < 0.05).

Allometric Scaling

Allometric scaling exponents were calculated for flow (Fig. 10A) and cross-sectional area (Fig. 10B) of the MPA, RPA, and LPA. For flow, the power b was 0.776 (95% CI = 0.723–0.829) for the MPA, 0.714 (95% CI = 0.655–0.773) for the RPA, and 0.820 (95% CI = 0.739–0.901) for the LPA. For cross-sectional area, b was calculated as 0.588 (95% CI = 0.537–0.638) for the MPA, 0.594 (95% CI = 0.551–0.638) for the RPA, and 0.587 (95% CI = 0.540–0.633) for the LPA.

DISCUSSION

In this study, we have used PCMRI to quantitatively characterize changes in pulmonary arterial flow and size longitudinally in the postnatal development of rats. We have introduced PCMRI as a technique for investigating the proximal pulmonary arterial hemodynamics and anatomy in small animals and have enabled future investigations of hemodynamic effects of pulmonary vascular disease by establishing how pulmonary flow and size change normally during postnatal development in the absence of disease.

PCMRI is a reliable and noninvasive technique for measuring pulmonary arterial blood flow and obtaining time-resolved and spatially resolved blood flow waveforms that allow calculation of parameters such as the pulmonary artery acceleration times, which are used for disease classification. We conducted in vivo validation studies of PCMRI with ultrasound echocardiography and fluorescent microspheres. Echocardiography was used to validate total pulmonary arterial flow, and fluorescent microspheres were used to validate percent flow to the left lung. With ultrasound, we found an excellent correlation between echocardiographic CO measurements and the sum of mean systolic RPA and LPA flows from PCMRI measured on the same day on the same animals. CO measurements with echocardiography represent systolic flow, as diameter measurements are made at the time of maximal cross-sectional area. Furthermore, VTI measurements are made at the peak of the velocity profile, which has been shown to be not flat (13), rendering these flow measurements sensitive to the location of VTI sampling volume. Our validation studies are in excellent accord with the correlation studies of Urbioniene et al. (33) of cardiac index using echocardiography and MR in the MPA of 42-day-old rats. A correlation slope of 0.96 between echocardiography and MR for rats of a single age group was found by Urbioniene et al. compared with a slope of 1.02 in our study of...
rats of different sizes at different stages of development. A wider variability among animals was observed in flow measurements using echocardiography than PCMRI, a finding not reported in the previous study. This higher variability may stem from ultrasound being a more user-dependent imaging modality than MRI for flow measurements and may account for the absence of significant differences in ultrasound measurements of CO between 100 and 160 days that were detected with PCMRI (Fig. 3A). It is important to note that because of such user-dependencies, echocardiographic measurements of CO are not considered as accurate as other techniques such as dye-dilution or direct Fick methods. In our study, we were constrained to a noninvasive technique for serial measurements and were limited by the invasiveness of the more accurate techniques. In particular, gaining repeated access to the pulmonary arteries of the same rats at different stages of development proved difficult using the invasive techniques. In light of other validation studies between echocardiography and direct Fick methods (15), however, we strongly believe in the validity of our results, but we also recognize that future studies would be beneficial to validate CO measurements of PCMRI against the “gold standard.”

For right-left flow splits, a maximum difference of 5.3% in the average percent flow to the left lung between PCMRI and fluorescent microspheres was observed at any time point. This small difference is considered clinically insignificant and can be explained by an underestimation by PCMRI and/or an overestimation by microspheres. With PCMRI, RPA and LPA slices were imaged during the same acquisition with an identical spatial resolution. The LPA, being inherently the smaller vessel, has, as a result, fewer voxels in cross section than the RPA, leading to a possibility for partial volume effects in the LPA and a slight underestimation of LPA flow and peak velocity (18, 23). On the other hand, fluorescent microspheres may be overestimating percent flow to the left lung, since mixing of these particles with the blood may be incomplete in a system with low-Reynolds-number flows, as in the rat pulmonary arteries. Partial mixing can result in preferential accumulation of the microspheres in the left lung and an overestimation of percent flow to the left lung (17, 28). Despite differences in body weights between the animals studied using PCMRI and those studied using microspheres, at 36 and 52 days of age, most likely due to different housing conditions, both experimental techniques show that flow split remains constant over time. Therefore, these two techniques can be reliably considered equivalent as long as experiments are conducted consistently. However, other experimental techniques, such as those employing radio-labeled albumin macroaggregates, could have also been used to validate PCMRI flow split results. These techniques, while enabling in vivo measurements of flow splits serially, pose radiation hazards for the experimenters and do not accurately define lung boundaries.

Fig. 6. Resampled aggregate cross-sectional area waveforms for all animals at 20 days (A), 36 days (B), 52 days (C), 100 days (D), and 160 days (E). Waveforms for each group were resampled to yield a sampling rate equivalent to the lowest number of cardiac frames in each age group and scaled to the group’s average heart rate. As expected, peak cross-sectional areas coincide for the RPA and LPA at all time points but occur later in the cardiac cycle than peak flows. Error bars, SE.
between the adjacent left lungs and the right cardiac lobe in rats.

PCMRI and fluorescent microspheres indicate that the right-left lung flow split does not change during normal development in rats. We have shown with PCMRI that the average right-left lung flow split is 68:32 throughout development. This is in accord with data from humans that show no significant differences in flow split between children and adults; 56:43 and 53:47, respectively (8), and an average of 54:46 in 21- to 51-yr-old adults (29). The left lung in humans thus receives a higher percentage of the CO than the left lung in rats. This difference can be explained by the different lung anatomy between rats and humans: in rats, the LPA supplies blood to one large left lung, while the RPA supplies three smaller right lobes and a right cardiac lobe (auxiliary lobe), which receives blood from the RPA but is physically located on the left. At 160 days, we found that the inflated volumes were 6.6 ml (SD 0.5) for the right lung and 3.5 ml (SD 0.2) for the left lung. The right lung, as such, accounted for 65% and the left lung for 35% of the total lung volume, with ratios very close to our flow split values in rats of 68:32. In humans, the right lung is slightly larger and has three lobes, while the left lung has two lobes. According to previous studies, the lung volume ratio in adult humans is ~53:47 (31), similar to flow split ratios of 53:47 reported by Cheng et al. (8). As such, the difference in flow splits between humans and rats may be explained by the difference in the relative sizes of right and left lungs. In both species, however, right-left flow splits have now been shown to remain constant with age. With this study, we have confirmed, in rats of five different ages, the results obtained previously in humans in a comparison between two age groups of children vs. adults (8, 29). These results provide evidence that the downstream pulmonary vascular resistance in both lungs decreases consistently over time in both species to account for the increased pulmonary flow. This occurs, despite the observed differences in proximal pulmonary arterial flow and geometry between the right and the left lung.

We have shown that the RPA and LPA experience an initial phase of fast growth followed by a slower growth starting at 52 days of age. Despite the similarity in the timing of their initial growth spurt, we have demonstrated that these two vessels exhibit different growth rates throughout development. In particular, the RPA grows at a faster rate than the LPA at all time periods considered here, with the growth velocity in RPA flow and size as high as three times the LPA growth velocities. This can be attributed to the larger volume of the right lung than the left lung in rats, as mentioned above and in previous investigations (33). In addition, we have shown that the RPA and LPA grow according to different structure-function relationships during development. While the strong correlations between flow and area for each of these vessels in individual animals point to feedback mechanisms that dictate vessel growth.
caliber based on blood flow, we found that the vessel size for a given pulmonary flow in the RPA differs from that in the LPA. In particular, the LPA in an older animal, which receives the same amount of blood flow as the RPA in a younger animal, does not exhibit the same cross-sectional area. This implies that, in addition to flow, other genetic, biochemical, and/or biomechanical factors determine pulmonary arterial caliber during development. In particular, biomechanical effects such as strain and wall shear stress are thought to play an important role in the structure, function, and metabolism of the lung and the pulmonary arteries (22, 37). As such, we investigated differences in distensibility between the RPA and the LPA and showed that distensibility was not significantly different at all time points. When considering shear rate as a surrogate for wall shear stress, however, we found that the RPA and LPA undergo substantially different levels of time-averaged shear rates throughout development. In particular, we discovered that the average shear rate of the LPA was approximately twice that of the RPA. This drastic contrast between the levels of wall shear stress and, consequently, the biomechanical forces experienced by the two vessels, in addition to the difference in metabolic demands of the two lungs, could explain the difference in structure-function relationships between the RPA and the LPA.

Despite differences in RPA and LPA growth patterns, we have shown that both vessels continue to grow in size and blood flow beyond the accepted age of adulthood and sexual maturity of 56–84 days (2). A large number of previous studies have been conducted in animals at much younger ages that were inaccurately considered adults. The responses to disease or treatment, for example, in these studies might have been different if older animals had been used. There is, therefore, a pressing need to establish consistency among the ages of rat models used in the study of pulmonary vascular disease as a way to enable cross-

![Fig. 9. Biomechanical properties of the RPA and LPA throughout development. A: arterial distensibility shows no significant differences between the RPA and LPA, except at the last time point (P < 0.01). LPA distensibility shows no significant changes over time. RPA distensibility, however, is significantly different at 20 days compared with all other time points (P < 0.02). B: time-averaged shear rates are significantly different between the RPA and LPA (P < 0.0001) at all time points. RPA and LPA show similar trends in the decrease of shear rate over time, with significant differences (P < 0.05) between neighboring time points only between 52 and 100 days.](image)

![Fig. 10. Allometric scaling laws applied to the MPA, as well as the RPA and LPA. Pulmonary flow (A) and area (B) are plotted on log-log scales against body mass. Scaling exponents for all 3 vessels are similar for flow and area, as indicated by parallel regression lines (A and B). The 95% CIs are for slopes of regression lines indicating scaling exponents.](image)
comparisons among different studies. To this end, we have established mathematical models (Eq. 8–11) that describe the growth trends of the RPA and LPA in rats as they grow from an early postnatal age to adulthood. These models can accurately predict blood flow through the pulmonary arteries and estimate the size of these vessels at any given age for rats.

To the best of our knowledge, this study is the first to report an allometric scaling relationship of arterial flow and size in the pulmonary circulation. As expected, we have shown a decrease in heart rate and an increase in CO with age to account for the increasing body weight, larger blood volume, and increasing metabolic demand in the older animals. These changes across age are in excellent agreement with published allometric scaling laws across species (Eq. 7), which predict that CO increases with a theoretical exponent \( b = 0.75 \) with body mass across species. Our data indicate \( b = 0.776 \) for CO, exactly as observed previously by White et al. (35). This value is also in close support of the theoretical cross-species allometric scaling laws, even though animals of different ages within a single species were considered here. Moreover, the scaling exponent we calculated for RPA and LPA flows was similar to that for MPA flow but has different normalization constants (Fig. 10), reflecting the relatively higher MPA flow than RPA and LPA flows. In addition to pulmonary flow, arterial geometries also obeyed allometric scaling laws. For the MPA, the cross-sectional area increased with body weight according to \( b = 0.588 \). For the ascending aorta, previous investigations (19, 34) report an increase in diameter according to \( b = 0.36 \) or, equivalently, an increase in cross-sectional area according to \( b = 0.72 \) (observed) and \( b = 0.75 \) (theoretical) across species. The difference between our calculated power law coefficients for the MPA and that of the aorta reported previously may indicate fundamental differences in vascular design between the systemic and pulmonary circulations. In addition, the scaling exponents we calculated for RPA and LPA sizes were similar to that for the MPA, but with different normalization constants.

Conclusions

We have quantitatively described the growth patterns of the proximal pulmonary arteries in rats during development. With the normal course of development established, it is now possible to apply similar approaches to devise a quantitative stratification on the timing and strategies of interventions that can effectively restore normal pulmonary vascular anatomy and physiology in patients with pulmonary arterial malformations. It is important to note, however, that the study of early postnatal development, before 20 days of age in rats, would enable a better understanding of the effects of early alveolarization and microvascular maturation on proximal pulmonary artery hemodynamics and elucidate the effects of congenital malformations. We have, additionally, highlighted the similarities and differences between RPA and LPA flows and have shown that the two proximal pulmonary arteries exhibit unexpected differences in their growth patterns and structure-function relationships. We have offered possible biomechanical explanations for these differences but believe that it is now imperative to shed light on the genetic and molecular mechanisms that translate these biomechanical effects to distinct growth patterns for the two vessels. These differences point to an inherent asymmetry between the right and left lungs and emphasize that, in future investigations of pulmonary vascular disease, care should be taken in selecting the lung that is subjected to interventions, sampling, or treatment.

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DISCLOSURES

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