

# Pulmonary vascular remodeling in isolated mouse lungs: Effects on pulsatile pressure–flow relationships

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## Abstract

Chronic hypoxia causes pulmonary vasoconstriction and pulmonary hypertension, which lead to pulmonary vascular remodeling and right ventricular hypertrophy. To determine the effects of hypoxia-induced pulmonary vascular remodeling on pulmonary vascular impedance, which is the right ventricular afterload, we exposed C57BL6 mice to 0 (control), 10 and 15 days of hypobaric hypoxia ( $n = 6$ , each) and measured pulmonary vascular resistance (PVR) and impedance ex vivo. Chronic hypoxia led to increased pulmonary artery pressures for flow rates between 1 and 5 ml/min ( $P < 0.01$ ), and increased PVR, 0-Hz pulmonary vascular impedance and the index of wave reflection ( $P < 0.05$ ) as well as a more negative impedance phase angle for low frequencies ( $P < 0.05$ ). The increases in resistance and 0-Hz impedance correlated with increased muscularization of small arterioles measured with quantitative immunohistochemistry ( $P < 0.01$ ). The increases in wave reflection and decreases in phase angle are likely due to increased proximal artery stiffness. These results confirm that chronic hypoxia causes significant changes in steady and pulsatile pressure–flow relationships in mouse lungs and does so via structural remodeling. They also provide important baseline data for experiments with genetically engineered mice, with which molecular mechanisms of pulmonary vascular remodeling can be investigated.

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**Keywords:** Pulmonary vascular resistance; Pulmonary vascular impedance; Muscularization; Chronic hypoxia; Pulmonary hypertension; Right ventricular afterload

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## 1. Introduction

Chronic exposure to hypoxia, which initially causes pulmonary hypertension via hypoxic pulmonary vasoconstriction (Rabinovitch et al., 1979; Yu et al., 1998), causes pulmonary hypertension and pulmonary vascular remodeling. Hypoxia-induced pulmonary vascular remodeling is characterized by smooth muscle cell (SMC) hypertrophy and hyperplasia and increased extracellular matrix protein deposition (Hislop and Reid, 1976;

Rabinovitch et al., 1979). While somewhat controversial (Stenmark and McMurtry, 2005), arteriolar SMC encroachment into the lumen is thought to be one of the ways that pulmonary vascular remodeling increases pulmonary vascular resistance (PVR) (Rabinovitch et al., 1979). Increased extracellular matrix deposition, which has been shown to stiffen pulmonary arteries (Kobs et al., 2005), may be an important factor in increased pulmonary vascular impedance (Nichols and O'Rourke, 2005). Understanding the effects of pulmonary vascular remodeling on pulmonary vascular impedance, which is the right ventricular afterload, is critical to understanding the progression of ventricular dysfunction in chronic hypoxia-induced pulmonary hypertension.

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The pulmonary vascular response to chronic hypoxia has been widely studied in calves (Zuckerman et al., 1991), pigs (Fike and Kaplowitz, 1994, 1996; Fike et al., 2002), rats (Hislop and Reid, 1976; Rabinovitch et al., 1979, 1981; Adnot et al., 1991; Le Cras et al., 1996; Liu, 1996; Herget et al., 2003) and mice (Steudel et al., 1998; Eddahibi et al., 2000; Quinlan et al., 2000; Fagan et al., 2001; Zhao et al., 2001; Beppu et al., 2004; Hasegawa et al., 2004; Kobs et al., 2005). Despite increasing recognition that impedance, not resistance, determines right ventricular energy requirements (Grant and Lieber, 1996; Huez et al., 2004), most studies in small animals still measure only steady pulmonary vascular pressure–flow relationships and do not investigate changes in pulsatile pressure–flow relationships with pulmonary vascular remodeling.

To address this knowledge gap, we investigated the effects of chronic hypoxia-induced pulmonary hypertension on steady and pulsatile pressure–flow relationships in the lungs of C57BL6 mice. We hypothesized that pulmonary vascular remodeling would increase PVR and impedance, and that increased muscularization in the small arteries and arterioles of mouse lungs would be correlated with increased PVR and impedance.

## 2. Methods and materials

### 2.1. Animal handling

Eighteen 8–12-week-old C57BL6/J mice,  $19.4 \pm 2.4$  g weight were used in this study (Jackson Laboratory, Bar Harbor, ME). Mice were subjected to either 0, 10 or 15 days ( $n = 6$ , each group) of hypobaric hypoxia in the University of Wisconsin-Madison Biotron facility. All groups had equal numbers of male and female mice. The hypoxic groups were exposed to a barometric pressure of 380 mmHg, which produces the equivalent of 10% inspired  $O_2$ . The pressure was returned to atmospheric conditions (760 mmHg) no more than 30 min/day for regular animal care and maintenance. Mice were euthanized with an intraperitoneal injection of 150 mg/kg pentobarbital solution. All protocols and procedures were approved by the University of Wisconsin Institutional Animal Care and Use Committee.

### 2.2. Isolated lung preparation

The isolated, ventilated, perfused lung preparation was used as previously described (Tuchscherer et al., 2006). Briefly, after euthanasia, the trachea was cannulated for ventilation and the main pulmonary artery and left atrium were cannulated for perfusion inflow and outflow, respectively. Room air was used for ventilation; steady flow of heated perfusate was driven by a syringe pump (Cole Palmer Instrument Company, Vernon Hills,

IL); pulsatile flow was driven by a high-frequency oscillatory piston pump (EnduraTEC Systems, Bose Corporation, Minnetonka, MN) operated in parallel with the syringe pump. Flow exited the left atrial cannula into small diameter tubing, which then exited to the atmosphere at the same elevation as the left atrial cannula. Pulmonary artery pressure ( $P_{PA}$ ) and left atrial pressure ( $P_{LA}$ ) were measured by pressure transducers (P75, Hugo Sachs Elektronik); pulmonary vascular flow rate ( $Q$ ) was measured by an in-line flowmeter (Transonic Systems, Inc., Ithaca, NY). All pressures and flows were monitored by continuous display on a laptop computer.

### 2.3. Initial measurements

To obtain initial steady flow rate measurements of  $P_{PA}$ ,  $P_{LA}$  and  $Q$ , the lungs were perfused at 1 ml/min. This perfusion rate was based on previously reported flow rates for isolated perfused mouse lungs (von Bethmann et al., 1998). Data were recorded while keeping lungs inflated at the end inspiratory pressure of 10 cmH<sub>2</sub>O to prevent edema and to normalize the effects of airway pressure and volume on  $P_{PA}$ ,  $P_{LA}$  and  $Q$  (Milnor, 1989).

### 2.4. Pulsatile flow rate measurements

After initial measurements, the steady flow rate was gradually increased to 3 ml/min and sinusoidal flow rates of the form  $Q = 3 + 2 \sin(2\pi ft)$  ml/min were generated for frequencies of  $f = 1, 2, 5, 10, 15$  and 20 Hz. This frequency range was chosen to include the physiological heart rate for mice ( $\sim 10$  Hz).  $P_{PA}$ ,  $P_{LA}$  and  $Q$  were recorded at 200 Hz. As above, lungs were kept inflated at end inspiratory pressure during data collection. After the sinusoidal flow rates measurements were performed, the steady flow rate was gradually reduced to 0.5 ml/min and normal ventilation resumed.

### 2.5. Steady flow rate measurements

After the last pulsatile flow rate measurement, at least 1 min of steady flow at 0.5 ml/min was imposed, and then flow rate was increased to 1 ml/min. Steady flow rate was then increased from 1 to 5 ml/min in 1 ml/min steps at 10 s intervals while  $P_{PA}$ ,  $P_{LA}$  and  $Q$  were recorded. Lungs were inflated to end inspiratory pressure during data collection as above. The steady flow rate was then returned to 0.5 ml/min and normal ventilation resumed.

### 2.6. Muscularization measurements

After all pressure–flow data were collected, the ventilation and perfusion were stopped and the lungs

were removed from the isolated lung system. The lungs were filled with freezing compound OCT through the trachea, and the entire lung was frozen in 2-methyl butane cooled by liquid nitrogen. Sections with a thickness of  $7\mu\text{m}$  were cut from each lung at  $-20^\circ\text{C}$  on a cryostat. Serial sections were obtained on two slides. Sections on the first slide were immunoreacted with factor VIII primary antibody (rabbit polyclonal, Dako Cytomations, Carpinteria, CA) diluted 1:8000 in PBS. Sections on the second slide were immunoreacted with  $\alpha$ -smooth muscle actin primary antibody ( $\alpha$ -SMC, mouse monoclonal) diluted 1:300 in PBS using the DAKO animal research kit (ARK, Dako Cytomations). All sections were then incubated with secondary antibody conjugated to horseradish peroxidase (goat anti-rabbit, 1:500, Jackson ImmunoResearch Laboratories, West Grove, PA). DAB chromogen (Dako Cytomations) was used as a substrate, yielding a brown labeling product. All sections were counterstained with hematoxylin to identify cell nuclei. The primary antibody was replaced with non-immune serum for negative control slides.

Histological specimens were imaged on an inverted microscope (TE-2000, Nikon, Melville, NY). Spot camera and MetaVue software (Optical Analysis Systems, Nashua NH) were used for image capture and quantitative image analysis. Five fields of view (FOV) were captured from one histological section of each lung at  $20\times$  magnification; in each FOV, the inner diameter of vessels identified by positive factor VIII staining (in a serial section) were measured with quantitative image analysis tools. For all vessels of average diameter less than  $80\mu\text{m}$ , the vessel was categorized as non-muscularized (NM), partially muscularized (PM) or fully muscularized (FM). NM vessels had no  $\alpha$ -SMC-positive cells, PM vessels had less than 75% positive cells around the wall circumference and FM vessels had more than 75% positive cells, following the methods used in prior studies (Fagan et al., 1999a,b; Quinlan et al., 2000; Zhao et al., 2001; Marcos et al., 2003). The percentage of vessels that were NM, PM or FM was computed for each mouse lung; mean values for a given condition (0-, 10- or 15-day) were computed from the values for each lung at that condition. Measurements were not obtained for two lungs (one 0-day and one 15-day) because of poor fixation.

### 2.7. Calculations

PVR was calculated as  $\Delta P/Q$  where  $\Delta P = P_{\text{PA}} - P_{\text{LA}}$ . Pulmonary vascular impedance magnitude ( $Z$ ) and pulmonary vascular impedance phase ( $\theta$ ) were calculated from one full sinusoidal cycle of  $\Delta P$  and  $Q$  at each imposed sinusoidal flow rate frequency. The 0 Hz (DC) and fundamental frequency values were found for each imposed sinusoidal flow rate frequency. Input resistance

$Z_0$ , characteristic impedance  $Z_c$  and index of wave reflection  $R_w$ , were calculated from the impedance as previously described (Tuchscherer et al., 2006).

### 2.8. Statistics

Data were analyzed using one-way ANOVA and Levene's test, with  $P$ -values less than 0.05 considered significant. Analyses of correlation between the arteriolar muscularization and PVR were performed using non-parametric Spearman's rank correlation coefficient. The regression analysis coefficient of determination ( $R^2$ ) was reported to reflect the linearity of the correlative relationship; Spearman's correlation coefficient ( $r_s$ ) and its associated  $P$  value (if significant) were reported to reflect the trends in the data. For each significant test, a general two-sided significance level of 5% was applied. All statistical analyses were performed using SAS software (SAS Institute Inc., Cary, NC) version 8.1. All data are presented in terms of means  $\pm$  standard deviation. All hemodynamic measurements reported are for  $n = 6$  for each group.

## 3. Results

### 3.1. Initial measurements

The 0-day group had a mean  $P_{\text{PA}} = 9.8 \pm 0.8$  mmHg at 1 ml/min flow rate.  $P_{\text{PA}}$  was significantly increased in both the 10- and 15-day groups ( $P < 0.01$ ) but there was no difference between the 10- and 15-day groups (Fig. 1). These initial measurements of pressure were higher than those measured with steady perfusion after pulsatile flow (see Fig. 2 at 1 ml/min), which demonstrates the preconditioning effects of the intervening pulsatile flow perfusion.

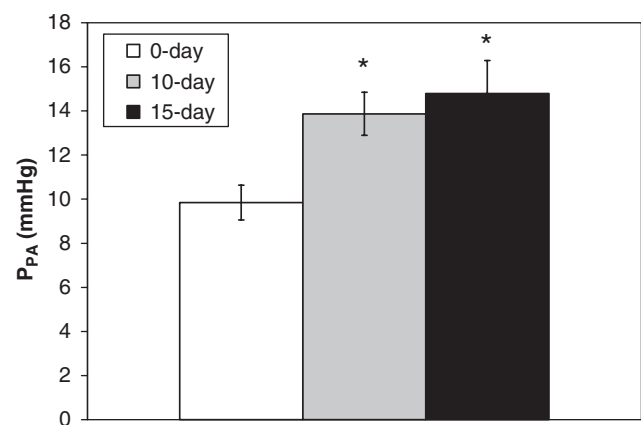


Fig. 1. Mean pulmonary artery pressure  $P_{\text{PA}}$  at 1 ml/min for 0-, 10- and 15-day groups. \* $P < 0.05$  vs. 0-day.

3.2. Steady flow rate measurements

$P_{PA}$  and  $P_{LA}$  both increased with flow rate from 1 to 5 ml/min (Fig. 2).  $P_{LA}$  increased linearly based on the Poiseuille-type resistance in the outflow tubing;  $P_{PA}$  increased similarly with flow rate in the control and hypoxic groups such that  $\Delta P$  was nearly constant with flow under all conditions. After chronic hypoxia,  $P_{PA}$  and  $\Delta P$  were higher than before hypoxia for all flow rates

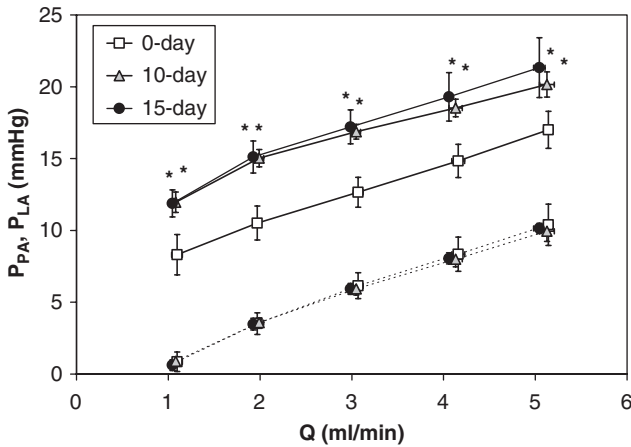


Fig. 2. Mean pulmonary artery pressure  $P_{PA}$  (solid lines) and left atrial pressure  $P_{LA}$  (dashed lines) versus flow rate for steady flow rate perfusion for 0-, 10- and 15-day groups. \* $P < 0.01$  vs. 0-day.

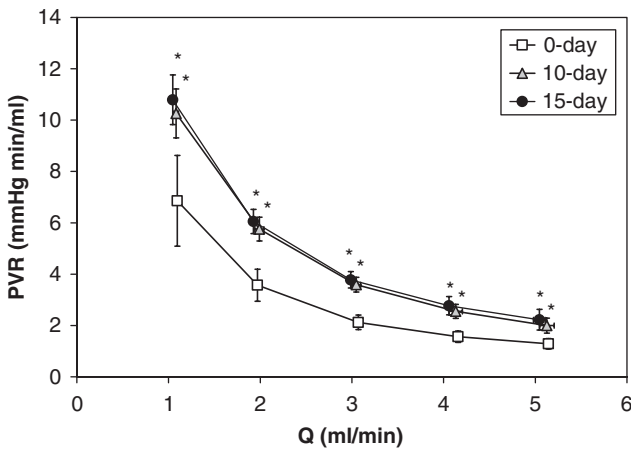


Fig. 3. Pulmonary vascular resistance versus flow rate for steady flow rate perfusion for 0-, 10- and 15-day groups. \* $P < 0.05$  vs. 0-day.

Table 1

Pulmonary vascular impedance at 0 Hz ( $Z_0$ ), characteristic impedance ( $Z_c$ ), and reflected wave coefficient ( $R_w$ ) for the 0-, 10- and 15-day groups

	0-day	10-day	15-day
$Z_0$ (mmHg min/ml)	$2.0 \pm 0.3$	$3.4 \pm 0.2^a$	$3.5 \pm 0.4^a$
$Z_c$ (mmHg min/ml)	$0.36 \pm 0.14$	$0.27 \pm 0.07$	$0.28 \pm 0.09$
$R_w$	$0.70 \pm 0.08$	$0.85 \pm 0.03^a$	$0.85 \pm 0.04^a$

<sup>a</sup> $P < 0.05$  vs. 0-day.

tested ( $P < 0.01$ ). There were no significant differences between the 10- and 15-day groups at any flow rate.

PVR was  $6.9 \pm 1.8$  mmHg min/ml at 1 ml/min before hypoxia and decreased significantly as flow rate increased from 1 to 5 ml/min ( $P < 0.05$ ) (Fig. 3). After hypoxia, PVR also decreased with increasing flow rate ( $P < 0.01$ ); PVR at each flow rate was significantly higher than at the same flow rate before hypoxia. There were no significant differences in PVR between the 10- and 15-day groups at any flow rate.

3.3. Pulsatile flow rate measurements

Before hypoxia, average 0 Hz impedance modulus, or input resistance ( $Z_0$ ) was  $2.0 \pm 0.3$  mmHg min/ml and increased significantly after chronic hypoxia ( $P < 0.01$ , Table 1 and Fig. 4). Impedance magnitudes at frequencies from 1 to 20 Hz were much lower than those at 0 Hz for all conditions and showed different trends as a function of frequency after chronic hypoxia. The impedance phase was significantly more negative after hypoxia at 1 and 2 Hz ( $P < 0.05$ ); there were no differences above 2 Hz (Fig. 4).

Some significant differences in impedance at non-zero frequencies were evident after hypoxia; we replotted the impedance magnitude to highlight these differences (Fig. 5). Before hypoxia at low frequencies (1–5 Hz),  $Z$  showed little variation but as frequency increased (5–20 Hz),  $Z$  tended to increase and then decrease. After hypoxia,  $Z$  tended to be lower than 0-day between 5 and 20 Hz. Both hypoxic groups displayed similar behavior in impedance magnitude and phase as functions of frequency.

Characteristic impedance ( $Z_c$ ), an indicator of proximal arterial elastance and inertial effects, was  $0.36 \pm 0.14$  mmHg ml/min before hypoxia and decreased slightly but not significantly after 10 and 15 days of chronic hypoxia (Table 1). Mean wave reflection index ( $R_w$ ), an indicator of pulse pressure wave reflections, was  $0.70 \pm 0.08$  before hypoxia and increased significantly with hypoxia ( $P < 0.01$ ) (Table 1).

3.4. Muscularization measurements and correlations

Qualitatively, the muscularization of small vessels increased with chronic hypoxia (Fig. 6). The quantitative

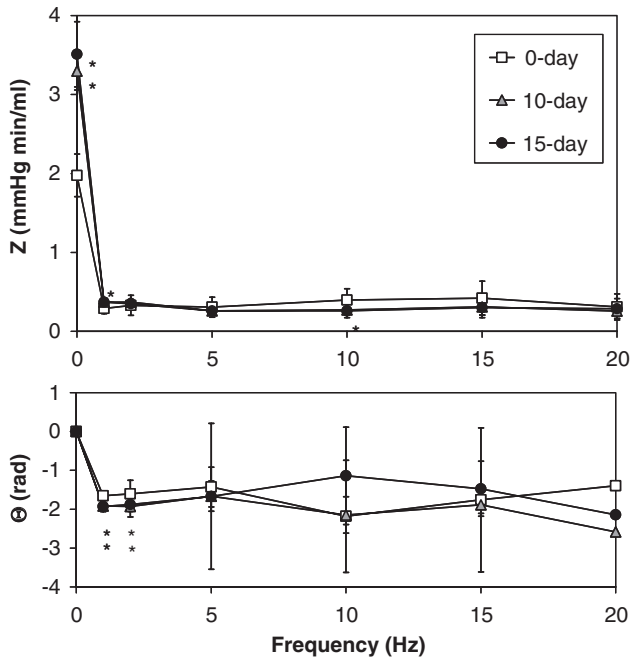


Fig. 4. Pulmonary vascular impedance magnitude and phase versus frequency for 0-, 10- and 15-day groups. \* $P < 0.05$  vs. 0-day.

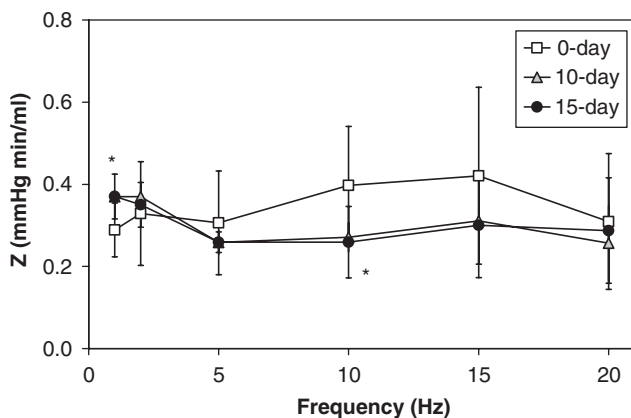


Fig. 5. Pulmonary vascular impedance magnitude versus frequency (1–20 Hz only) for 0-, 10- and 15-day groups. \* $P < 0.05$  vs. 0-day.

analysis confirmed a decrease in NM vessels after 10 and 15 days ( $P < 0.01$ ), increase in PM vessels after 10 days ( $P < 0.05$ ) and increase in FM vessels after 15 days ( $P < 0.05$ ). There were no significant differences between the 10- and 15-day groups (Fig. 7).

The percentage of FM vessels correlated with PVR measured at 1 ml/min ( $R^2 = 0.25$ ;  $r_s = 0.50$ ;  $P < 0.05$ ). Since the percentage of PM vessels increased at 10 days and the percentage of FM vessels increased at 15 days, we also investigated the correlation between the sum of partially and FM vessels (for a given mouse) with PVR. The correlation between all muscularized vessels and PVR at 1 ml/min was also significant ( $R^2 = 0.45$ ;  $r_s = 0.65$ ;  $P < 0.01$ ) (Fig. 8). Note, analogous results are obtained if  $Z_0$  is correlated with the percentage of

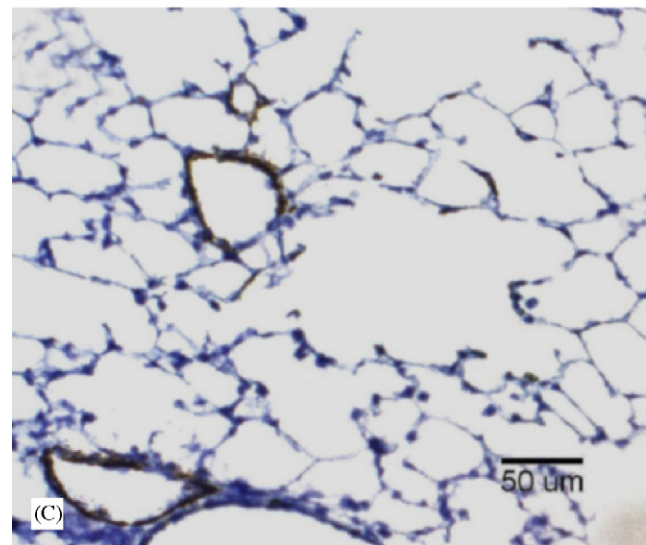
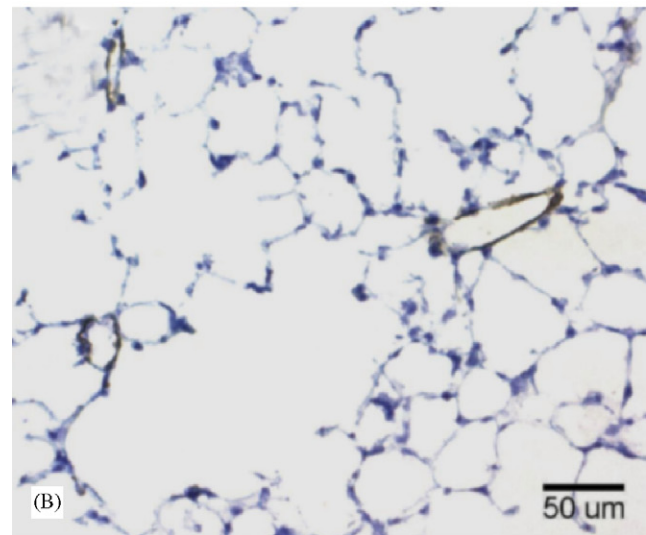
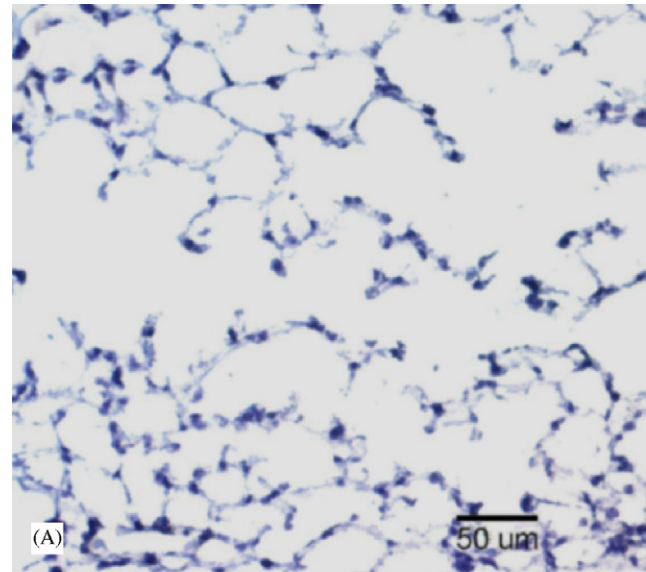


Fig. 6. Representative sections of mouse lung stained with  $\alpha$ -smooth muscle actin (brown) and counterstained with hematoxylin (blue) to identify non-, partially and fully muscularized vessels. 0 days (A), 10 days (B) and 15 days (C).

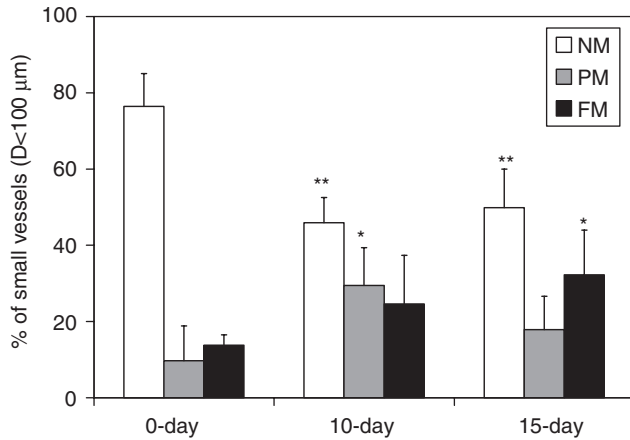


Fig. 7. Percent of non-(NM), partially (PM) or fully (FM) muscularized small vessels in 0-, 10- and 15-day lungs;  $n = 5, 6$  and  $5$ , respectively. \* $P < 0.05$  vs. 0-day; \*\* $P < 0.01$  vs. 0-day.

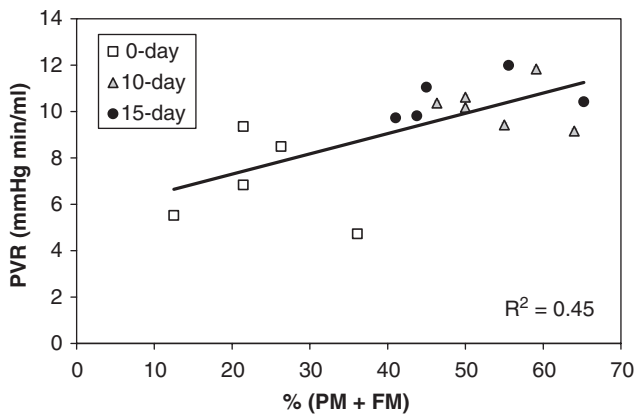


Fig. 8. Pulmonary vascular resistance (PVR) measured at 1 ml/min versus sum of partially and fully muscularized vessel for all conditions and all lungs for which histological data were obtained ( $n = 16$ ). Spearman's correlation coefficient  $r_s = 0.65$ ;  $P < 0.01$ .

muscularized vessels or if PVR is correlated with the percentage of NM vessels.

#### 4. Discussion

Our major findings are that steady and pulsatile pressure–flow rate relationships in isolated mouse lungs were significantly altered by chronic hypoxia. Specifically, PVR and two metrics of pulmonary vascular impedance were increased ( $Z_0$  and  $R_w$ ) and the impedance phase at low frequencies became more negative. The increases in PVR and  $Z_0$  were likely caused by increased small vessel muscularization or vessel drop-out; the increase in  $R_w$  and decrease in impedance phase were likely caused increases in proximal artery stiffness previously demonstrated with isolated vessel experiments (Kobs et al., 2005).

#### 4.1. Steady pressure–flow relationships

The initial measurements of  $P_{PA}$  at 1 ml/min for the 0-day group were similar to previous results in isolated mouse lungs (Archer et al., 1999; Fagan et al., 1999a, b; Parker et al., 1999). In the hypoxic groups, there was a 40–50% increase  $P_{PA}$  compared to the normoxic group. This increase in  $P_{PA}$  was measured with normoxic ventilation at least 60 min after the mice were removed from the hypoxic environment, strongly suggesting that pulmonary vascular remodeling occurred. That is, increases in blood viscosity are known to occur with hypoxia (West, 1990) but since the lungs were perfused with cell-free perfusate, increased viscosity is not responsible for the elevated pressures measured here. Similarly, hypoxic pulmonary vasoconstriction is not likely to be responsible for the increased pressures since the data were collected under normoxic conditions. However, some vasoconstriction may have persisted and affected the results.

When steady flow perfusion followed pulsatile perfusion, the resistance of the lungs decreased on average. Just as mechanical preconditioning is required prior to isolated vessel tests, this finding suggests that hemodynamic preconditioning is required prior to isolated lung tests and that ventilation is not sufficient.

All groups showed similar pressure responses to increasing steady flow rates. Other studies have shown increases in  $P_{PA}$  and  $P_{LA}$  with increasing flow rate in the isolated lung model (Parker et al., 1999; Weissmann et al., 2004; Tuchscherer et al., 2006) similar to these. While the increase in  $P_{LA}$  with flow can be assumed to be linear due to Poiseuille flow in the outlet tube, the increase in  $P_{PA}$  was well fit by a power law function ( $R^2 > 0.99$  for all conditions), which has been proposed to model the effects of exercise (i.e., increasing cardiac output) on  $P_{PA}$  (Reeves et al., 2005).

#### 4.2. Pulsatile pressure–flow rate relationships

The impedance spectra in all groups followed the classical pattern of a high 0-Hz value followed by a local minimum and oscillations at higher frequencies. The  $Z_0$  increase in the hypoxic groups was consistent with and comparable to the increase in PVR.  $Z_c$ , which is typically interpreted as the ratio of proximal arterial stiffness to fluid inertia (Milnor, 1989), did not increase significantly with hypoxia and in fact tended to decrease. Since our prior work clearly demonstrates an increase in proximal artery stiffness in this mouse type exposed to these conditions—via increased collagen and elastin deposition (Kobs et al., 2005)—the effects of increased stiffness must have been balanced by increased effects of inertia. Increases in proximal artery diameter and length could have had this effect. Our prior work supports the former: whereas the inner diameter of the main

pulmonary artery of 0-day mice was approximately 700  $\mu\text{m}$  at normal pulmonary artery pressures, the inner diameter of 10- and 15-day hypoxic mice was 800–850  $\mu\text{m}$  at hypertensive pressures (Kobs et al., 2005). Comparable increases in diameter due to hypertension are likely to have occurred in the secondary and tertiary pulmonary arteries as well. Increases in pulmonary arterial length with hypoxia have also been reported previously in rats (Molthen et al., 2004).

$R_w$  was significantly larger after hypoxia, which has also been reported in primary pulmonary hypertension patients (Weinberg et al., 2004). Increases in  $R_w$  suggest increasing wave reflections or speed of wave reflections, and can be caused by increases in arterial stiffness in vascular networks (Milnor, 1989). A more negative phase angle, as found here with hypoxia, can be interpreted similarly (Milnor, 1989). In the systemic circulation, increased wave reflection speed is known to detrimentally affect left ventricular function (Nichols and O'Rourke, 2005). The effects of wave reflection—and this degree of wave reflection in particular—on right ventricular function are not known.

#### 4.3. Muscularization and structure–function relationships

The increased muscularization of pulmonary vessels in chronically hypoxic mice has been well documented (Steudel et al., 1998; Fagan et al., 1999a, b; Quinlan et al., 2000; Levi et al., 2001; Zhao et al., 2001; Marcos et al., 2003; Pascaud et al., 2003). As noted above, proliferation of SMCs and subsequent narrowing of vessels is thought to be one of the ways that hypoxia-induced pulmonary remodeling increases resistance in the vasculature (Rabinovitch et al., 1979). This theory is supported by the significant correlation between muscularization and PVR. However, since we did not eliminate SMC activity (with a calcium-free perfusate, rho-kinase inhibition or papaverine, for example), we cannot rule out increased SMC tone and residual hypoxia-induced vasoconstriction as mechanisms by which PVR increases. Furthermore, since we did not measure luminal diameters throughout the pulmonary arterial tree, we cannot rule out small vessel drop-out, which would also increase PVR independent of muscularization.

#### 4.4. Experimental considerations

Typically, in vivo measurements of pulmonary vascular impedance are made based on the response to the normal heart beat. The normal heart beat generates a large flow magnitude at the fundamental frequency and smaller amplitudes of flow at harmonics of that frequency. In this study, approximately physiological flow magnitudes were generated at each frequency tested, from 1 to 20 Hz. If the pulmonary vasculature

were a linear system, the response to these two different modes of excitation would be identical. However, in a non-linear system, one cannot assume that the impedance resulting from these two modes of excitation is the same. Thus, while the measurements obtained here are robust and repeatable, they may not be straightforwardly comparable to those obtained in response to a normal heart beat, e.g., to those obtained in vivo. Since technology is now available to measure pulmonary vascular pressures and flows in mice in vivo at nearly physiological heart rates, the effects of non-linearities can be explored in future experiments. Our goal in these experiments was not to mimic the in vivo situation but instead to quantify the effects of hypoxia-induced pulmonary vascular remodeling on the frequency dependent pulmonary vascular impedance with well-controlled and repeatable ex vivo perfusion experiments. As long as identical methods are used, these data are easily comparable to impedance measurements in other strains of mice, including transgenics and knockouts.

#### 4.5. Implications

The technical difficulties inherent in measuring the dynamic effects of pulmonary vascular remodeling in lungs as small as mouse lungs at physiologically relevant frequencies are offset by the availability of exciting and important transgenic and knockout strains of mice, which can be used to investigate the role of genetic defects in pulmonary vascular disease. For example, experiments in genetically engineered mice have shown that gene defects in natriuretic peptides (Zhao et al., 1999), plasminogen activators (Levi et al., 2001), bone morphogenetic proteins (Beppu et al., 2004), and endothelial nitric oxide synthase (Steudel et al., 1998; Fagan et al., 1999a, b; Quinlan et al., 2000; Zhao et al., 2001) modulate arteriolar muscularization and extracellular matrix protein deposition. The effects of these genes on pulmonary vascular function—that is, on steady and pulsatile pressure–flow relationships—remain poorly understood. The data presented here provide important baseline data for determining the role of specific genes in pulmonary hemodynamics through pulsatile pressure–flow experiments with lungs from genetically engineered mice.

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