SYSTEMIC LOBAR SHUNTING INDUCES ADVANCED PULMONARY VASCULOPATHY

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Objectives: We characterized the morphology and vasomotor responses of a localized, high-flow model of pulmonary hypertension.

Methods: An end-to-side anastomosis was created between the left lower lobe pulmonary artery and the aorta in 23 piglets. Control animals had a thoracotomy alone or did not have an operation. Eight weeks later, hemodynamic measurements were made. Then shunted and/or nonshunted lobes were removed for determination of vascular resistance and compliance by occlusion techniques under conditions of normoxia, hypoxia (F_{IO_2} = 0.03), and inspired nitric oxide administration. Quantitative histologic studies of vessel morphology were performed.

Results: Eighty-three percent of animals having a shunt survived to final study. Aortic pressure, main pulmonary artery and wedge pressures, cardiac output, blood gases, and weight gain were not different between control pigs and those receiving a shunt. Six of 9 shunted lobes demonstrated systemic levels of pulmonary hypertension in vivo. Arterial resistance was higher (24.3 ± 12.0 vs 1.3 ± 0.2 mm Hg · mL^{-1} · s^{-1}, P = .04) and arterial compliance was lower (0.05 ± 0.01 vs 0.16 ± 0.03 mL/mm Hg, P = .02) in shunted compared with nonshunted lobes. Hypoxic vasoconstriction was blunted in shunted lobes compared with nonshunted lobes (31% ± 13% vs 452% ± 107% change in arterial resistance, during hypoxia, P < .001). Vasodilation to inspired nitric oxide was evident only in shunted lobes (34% ± 6% vs 1.8% ± 8.2% change in arterial resistance during administration of inspired nitric oxide, P = .008). Neointimal and medial proliferation was found in shunted lobes with approximately a 10-fold increase in wall/luminal area ratio.

Conclusions: An aorta–lobar pulmonary artery shunt produces striking vasculopathy. The development of severe pulmonary hypertension within a short time frame, low mortality, and localized nature of the vasculopathy make this model highly attractive for investigation of mechanisms that underlie pulmonary hypertension. (J Thorac Cardiovasc Surg 2000; 120:88-98)

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A variety of congenital heart defects associated with increased pulmonary blood flow may be complicated by the development of pulmonary hypertension. Pulmonary hypertension adversely affects the prognosis of affected patients[1] and significantly complicates surgical repair.[2-4] The severity of vascular remodeling appears to depend on the proportionate increase in pulmonary blood flow and the pressure at which it is delivered. Various experimental systemic-pulmonary connections have been devised to model this clinical problem. Most models produce modest degrees of pulmonary hypertension and early changes in vascular morphology.[5-7] Furthermore, many are associated with significant postoperative mortality.[5,8,9] A model of

increased pulmonary flow, particularly localized to one lobe of the lung, that is not associated with systemic alterations, such as high-output congestive heart failure, pulmonary edema, or slowed growth, would be highly useful in investigations of mechanisms that underlie and sustain the vasculopathy of pulmonary hypertension. In such a model, gene expression, as well as protein and product formation, could be compared in hypertensive versus normotensive lung tissue. Furthermore, this model would also allow us to test the effects of interventions commonly used clinically, such as inhaled nitric oxide (iNO), on the distribution of resistance and compliance in lungs with pulmonary vasculopathy. The following report delineates physiologic, histologic, and angiographic characteristics of an aorta–left lower lobe pulmonary artery shunt in piglets. The shunt produced systemic levels of pulmonary hypertension over an 8-week period with little associated clinical morbidity or mortality. Our studies demonstrated profound remodeling and altered vasomotion of the shunted pulmonary circulation.

Materials and methods

Animals used. Thirty-one weanling infant pigs were used in this study. An aorta–left lower lobe pulmonary artery connection was constructed in 23 piglets. Four piglets then had a sham operation and another 4 had no operation. These 8 piglets comprised the control group. The surgical protocol and postoperative care were in compliance with the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health (NIH publication 85-23, revised 1985) and approved by the Animal Studies Committee of the Zablocki Veterans Administration Hospital and the Medical College of Wisconsin.

Operative methods. Initial sedation was achieved with acepromazine (1.5 mg/kg) and ketamine (30 mg/kg) injected intramuscularly. An ear vein was cannulated, fentanyl 2 mg/kg (intravenous) was administered, and the animal was intubated and its lungs ventilated with a halothane mixture to achieve general anesthesia. A left thoracotomy was performed through a mid thoracic intercostal space just inferior to the scapular tip. The aorta was mobilized and the overlying hemiazygos vein was divided. The left lower lobe pulmonary artery was separated posteriorly from the adjacent lower lobe bronchus. The interlobar fissure was completed by cautery dissection. The lingular branch of the exposed pulmonary artery was divided to provide additional pulmonary artery length for subsequent anastomosis. The descending pulmonary artery was suture ligated just distal to the upper lobe branches and divided. The distal lumen was then sewn end to side to the descending thoracic aorta (Fig 1). Patency was confirmed by a palpable thrill throughout the left lower lobe. The pleural space was evacuated and the chest was closed. Animals were extubated in 1 to 2 hours. Intramuscular cefazolin (25 mg/kg) and furosemide (1 mg/kg every other day) were given over 5 days. Furosemide was administered prophylactically, in part according to the protocol of Rendas, Lennox, and Reid[5] and not on the basis of clinical impressions of pulmonary edema.

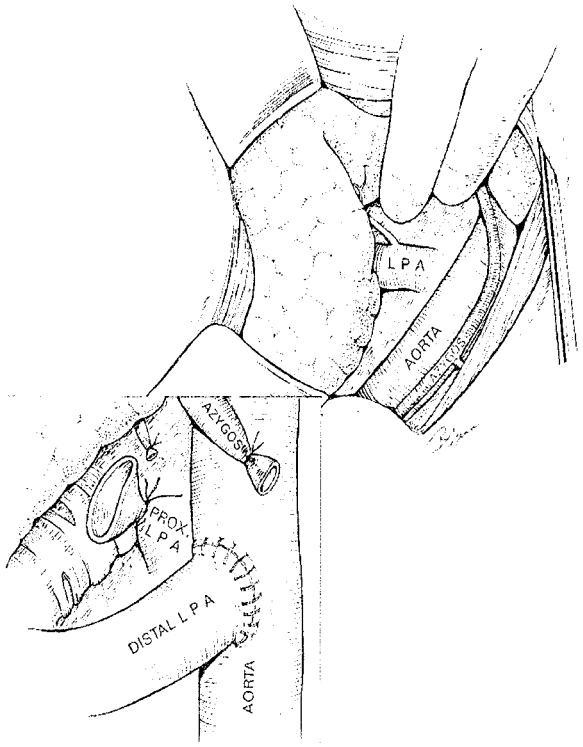

Fig 1. Schema of surgical anastomosis of left lower lobe pulmonary artery and aorta. The *inset* shows the ligated proximal left pulmonary artery and azygos veins and end-to-side connection of the distal left pulmonary artery and aorta. *LPA,* Left pulmonary artery.

Assessment of cardiopulmonary and general data. Seven to 8 weeks after the initial procedure, animals were sedated with acepromazine (1.5 mg/kg) and ketamine (30 mg/kg) intramuscularly. Intravenous pentobarbital (5 mg/kg) was administered to achieve general anesthesia. A cervical tracheostomy tube was placed for mechanical ventilation (tidal volume 10 mL/kg) and the carotid artery was cannulated for arterial pressure monitoring. A thermodilution catheter (Swan-Ganz, Baxter Healthcare Corp, Edwards Div, Santa Ana, Calif) was floated into the right pulmonary artery via a jugular vein. The thermodilution method was used to determine right ventricular output. At least 3 serial determinations of cardiac output were made, and these were consistently within a 10% range. Left atrial pressure was estimated by the pulmonary artery wedge pressure. Total pulmonary vascular resistance was determined for the delivered right ventricular output by means of the standard equation: mean pulmonary artery pressure minus wedge pressure divided by cardiac output ([Pa – Pa wedge]/CO).

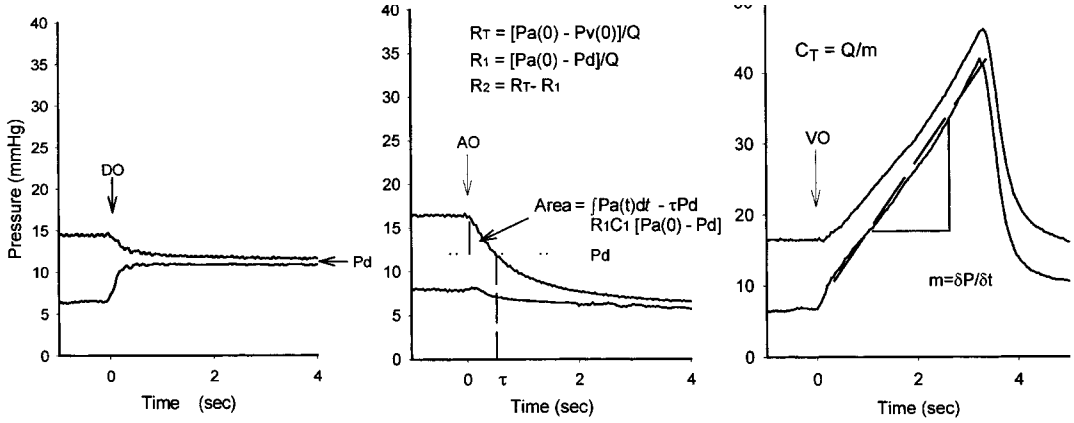

Fig 2. The *left panel* shows a typical double occlusion curve. Simultaneous occlusion of the arterial and venous outflows at t = 0 *(DO)* results in asymptotic approximation of the arterial *(Pa)* and venous *(Pv)* pressures to new steady levels *(Pd).* The *middle panel* depicts a typical arterial occlusion *(AO),* which is followed by decay of arterial and venous pressures. The area beneath the arterial decay curve between t = 0 and t = τ (defined by time at which arterial pressure reaches Pd) is calculated. In the *right panel,* typical venous occlusion *(VO)* curves are depicted. The slope, *m,* of the venous pressure is used to determine total pulmonary vascular compliance. R_T, Total resistance; *Pa(0)* and *Pv(0),* arterial and venous pressures, respectively, at the moment of occlusion; *Q,* flow; R_1, arterial resistance; R_2 venous resistance; C_1, arterial compliance; C_T, total compliance.

An extended left thoracotomy with rib resection exposed the shunted left lower lobe. Careful dissection was necessary to delineate the aorta–left lower lobe pulmonary artery anastomosis. A Transonic flow probe (Transonic Systems, Inc, Ithaca, NY) was placed around the pulmonary artery for blood flow measurement. Direct needle puncture of the left lower lobe pulmonary artery was used for pressure determination. Samples from shunted or nonshunted lobes were collected for wet/dry weight ratio measurements.

Vascular occlusion methods. The lower lobes from animals with and without a shunt were removed and perfused as described by Nelin and associates.[10] In brief, the left or right lower lobe was separated by completing the interlobar fissure and dividing adhesions. Adequate cuffs of the pulmonary artery, bronchus, and left atrium were mobilized for subsequent division and cannulation. Heparin (1500 units/kg) and 10% dextran (15 mL/kg) were given intra-arterially, and the piglet was exsanguinated. The autologous blood (hematocrit value 30 ± 1.5 mL/dL) was used to prime the perfusion system. The lung was excised in an expanded state with the pulmonary artery clamped to prevent air entry into the vasculature. Rigid cannulas were placed into the bronchus, pulmonary artery, and left atrium. The lobe was suspended in a heated, humidified perfusion chamber. The perfusion system consisted of a reservoir, Masterflex roller pump (Cole-Parmer Instrument Company, Vernon Hills, Ill), heat exchanger, and angiographic dye injector. The venous effluent drained into a reservoir the height of which could be adjusted to set the venous pressure. The pulmonary arterial, venous, and airway pressures (Pa, Pv, PA, respectively) were continuously recorded. Ventilation was begun with a baseline gas mixture containing approximately 6% CO_2, 15% O_2, and the balance N_2 with a Harvard piston-type respirator (Harvard Apparatus Co, Inc, S Natick, Mass), with tidal volumes of 100 to 200 mL at a rate of 10 breaths/min and an expiratory pressure of 3 mm Hg. A series of vascular occlusion maneuvers were performed with flows ranging between 1 and 5 mL/s and respiration held at end expiration. The double occlusion maneuver was performed by simultaneously occluding the arterial inflow and venous outflow cannulas until the arterial and venous pressures reached new steady levels, Pd, a reflection of the microvascular capillary pressure.[11] The flow was restarted and arterial inflow was occluded until the arterial and venous pressures reached a new steady level. Finally, after flow had been restarted, the venous outflow was occluded while the inflow continued into the lobar arteries at the previous constant rate. Representative plots of pressure versus time during the 3 vascular occlusion maneuvers are shown in Fig 2. By means of a 3-compliance 2-resistor model of the pulmonary vascular bed as described by Audi, Dawson, and Linehan,[11] the arterial or upstream resistance (R_1), the venous or downstream resistance (R_2), and the arterial, capillary, venous, and total compliance (C_1, C_2, C_3, and C_T) can be derived by the following equations:

$$A = \int_0^{\tau} Pa(t)\,dt - P_d\tau \quad (\tau = \text{elapsed time at which PaAO = Pd}) \qquad \text{(Eq 1)}$$

$$R_1C_1 = A/(Pa[0] - Pd) \qquad \text{(Eq 2)}$$

$$R_1 = Pa(0) - Pd/Q \qquad \text{(Eq 3)}$$

$$R_2 = R_T - R_1 \qquad \text{(Eq 4)}$$

$$C_T = Q/m \qquad \text{(Eq 5)}$$

$$C_2 = C_T - R_TC_1/R_2 \qquad \text{(Eq 6)}$$

$$C_3 = C_T - C_1 - C_2 \qquad \text{(Eq 7)}$$

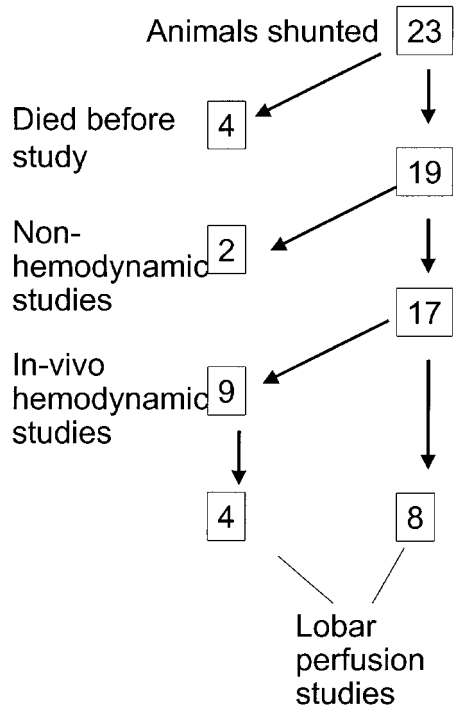

Fig 3. Flow diagram showing the numbers of lobes used for hemodynamic studies.

Area (A) (Eq 1) is defined by the difference between the arterial occlusion (AO) pressure-time curve and Pd (Fig 2). Pa(0) and Pv(0) are the arterial and venous pressures at the moment of occlusion. Q is flow, m is slope of the venous occlusion pressure-time curve, and R_T is total resistance. After these measurements were obtained, the lung was ventilated with a hypoxic gas mixture (F_{IO_2} = 0.03; P_{O_2} approximately 20 mm Hg) and measurements were repeated. In 8 shunted lobes and 5 nonshunted lobes, iNO (30 ppm) was added to the ventilatory circuit and occlusions were again performed.

Histologic methods. After in vivo cardiopulmonary hemodynamic measurements were obtained, a thoracotomy was performed and the lungs were removed for perfusion with neutral formalin and/or immersion-fixation. After fixation for more than 2 days, representative samples were obtained from proximal, middle, and distal sections of both shunted and nonshunted lungs. Sections were embedded in paraffin, sectioned, and stained with a Movat pentachrome stain.[12] Digital images were captured at magnifications of 200× or 400× with Image 1 software (version 4.0, Universal Imaging Corporation, West Chester, Pa). All histomorphometric measurements were made with Image Tool Program V 1.27 (developed at the University of Texas Health Sciences Center at San Antonio) and as outlined by Miano and colleagues.[13] This method enabled accurate area calculation independent of the degree of vessel distention. An image of 1-mm slide micrometer was used to calibrate all measurements. Distances between lumen, internal elastic lamina, and external elastic lamina of short and long axes were measured in 30 randomly chosen pulmonary arteries of shunted lobes and 30 arteries of similar size from nonshunted lobes. Two independent observers carefully traced the perimeter of the endoluminal border, the internal elastic lamina, and the external elastic lamina.

Statistical methods. Resistance and compliance data from nonshunted and shunted lobes and general characteristics of pigs (eg, hematocrit, cardiac output, weight) were compared by means of unpaired *t* tests (when data were normally distributed) or Mann-Whitney rank sum tests when tests for normal distribution failed. Data from the same lobes studied under normoxic or hypoxic conditions were compared by means of paired *t* tests or Mann-Whitney rank sum tests when data were not normally distributed. Comparative data are expressed as means plus or minus standard error from the mean. A flow diagram delineates the number of in vivo and lobar perfusion studies performed (Fig 3).

Results

General observations. Four postoperative deaths occurred in 23 pigs receiving a shunt. Cause of death was determined by postmortem examination in these 4 animals. Three deaths were due to congestive heart failure that developed within 2 weeks of the operation, and one was related to bilateral pneumonia that developed clinically nearly 8 weeks after operation. Congestive heart failure appeared to be associated with large shunt size and preoperative anemia.

Postoperatively, pigs that received a shunt and those having a sham operation grew equally well (Table I). Both right and left lungs were well expanded at the time of reoperation, although there were typically adhesions between the left upper and left lower lobes in animals with a shunt. No gross evidence of hemorrhage or edema was noted within the shunted lobe. The aortopulmonary anastomosis was patent in all cases, although in some it was significantly narrowed by intimal hyperplasia. The shunted lower lobe artery was dilated and thickened. Distal pulmonary artery thrombus was present in the left lobe of 1 animal. In 10 cases no thrill was evident across the anastomosis, indicating the presence of systemic levels of pulmonary hypertension within the lobe (ie, no pressure gradient to create a thrill).

Table I compares physiologic parameters between control animals without a shunt and animals with an aorta–left lower lobe pulmonary artery shunt. Pulmonary artery pressures and cardiac outputs of animals with a shunt were obtained from the right-sided or nonshunted pulmonary circulation. Systemic arterial and pulmonary arterial pressures, as well as cardiac out-

Table I. *Physiologic comparison of animals with and without a shunt*

	Pa (mm Hg)	*PCWP* (mm Hg)	*CO* (L/min)	*PVR* (mm Hg · L^{-1} · min^{-1})	*Ao* (mm Hg)	*Hct* (%)	*pH*	Pao_2 (mm Hg)	*BW* (kg)	*W/D ratio* (lung)
Shunted	17 ± 1	4.0 ± 0.6	2.1 ± 0.2	6.6 ± 0.6	78 ± 4	30 ± 2	7.46 ± 0.01	135 ± 9	17 ± 1	
Nonshunted	19 ± 1	4.8 ± 0.6	2.9 ± 0.6	5.9 ± 1.2	81 ± 10	33 ± 3	7.45 ± 0.01	127 ± 9	16 ± 2	4.6 ± 0.7
Left lobe	75 ± 8									5.1 ± 1.0

Pulmonary artery pressures of animals having a shunt represent values obtained from the nonshunted main pulmonary artery. Left lobe pulmonary artery pressures are the pressures measured directly from the lobar pulmonary artery distal to the shunt. *Pa*, Pulmonary artery pressure; *PCWP*, pulmonary capillary wedge pressure; *CO*, cardiac output; *PVR*, pulmonary vascular resistance; *Ao*, mean aortic pressure; *Hct*, hematocrit; Pao_2, arterial oxygen tension; *BW*, body weight; *W/D ratio*, wet/dry weight ratio.

Table II. *Distribution resistance and compliance in shunted and nonshunted lobes*

	Arterial resistance (mm Hg · mL^{-1} · s^{-1})	*Venous resistance* (mm Hg · mL^{-1} · s^{-1})	*Arterial compliance* (mL/mm Hg)	*Capillary compliance* (mL/mm Hg)	*Venous compliance* (mL/mm Hg)
Nonshunted					
Normoxia (9)	1.26 ± 0.20	1.52 ± 0.34	0.16 ± 0.03	0.69 ± 0.17	0.17 ± 0.04
Hypoxia (9)	6.16 ± 1.00* (P = .002)	2.70 ± 0.55	0.13 ± 0.02	0.24 ± 0.09* (P = .03)	0.27 ± 0.05
iNO (5)	1.52 ± 0.30	1.50 ± 0.50	0.25 ± 0.16	0.96 ± 0.58	0.34 ± 0.17
Shunted					
Normoxia (12)	24.3 ± 12.0‡ (P < .001)	4.2 ± 1.6	0.05 ± 0.01† (P = .005)	0.63 ± 0.20	0.15 ± 0.03
Hypoxia (12)	26.0 ± 12.0§ (P = .04)	4.6 ± 1.6	0.07 ± 0.03	0.42 ± 0.09	0.14 ± 0.03
iNO (8)	20.4 ± 12.0	2.8 ± 1.4	0.05 ± 0.02	0.69 ± 0.17	0.13 ± 0.02

iNO, Inspired nitric oxide.
*Paired *t* test compared with normoxia.
†Unpaired *t* test, nonshunted to shunted.
‡Rank sum test, shunted versus nonshunted.
§Rank sum test, normoxia versus hypoxia.

puts, were similar between groups, indicating the shunt did not produce overt hemodynamic effects outside the left lower lobe. There were no significant differences in acid-base status, oxygenation, hematocrit, wet/dry lung weights, or peak airway pressures (8-15 mm Hg in all cases).

In vivo hemodynamic assessment of the shunted left lower lobe. Direct hemodynamic measurements of the shunted lobe were made in 9 animals. Shunted lobar pressure was equivalent to simultaneously recorded aortic pressure in 6 pigs. Two animals with a persistent bruit had elevated lobar pulmonary artery/systemic (aortic) pressure ratios of 0.75 and 0.50. One animal with a strong bruit had pulmonary artery pressure only slightly greater than left atrial pressure. Shunt flow to 3 hypertensive lobes averaged 0.14 ± 0.05 L/min, a value significantly lower than that of 4 nonshunted lobes, which averaged 1.0 ± 0.3 L/min (P < .001).

Vascular occlusion studies. Vascular occlusion studies were performed in 9 nonshunted lobes (4 from right lower lobes of animals having a shunt and 5 from left or right lower lobes of animals not having a shunt). Data from nonshunted lobes are compared with 12 shunted left lower lobes. Averaged resistance and compliance data during normoxic and hypoxic ventilation and after administration of iNO are given in Table II. Arterial resistance was markedly increased and arterial compliance was significantly decreased in shunted compared with nonshunted lobes. Although shunted lobes generally had increased arterial resistance, the degree of increase was variable (Fig 4). Lobes without a bruit had profoundly increased levels of arterial resistance and had more arterial remodeling, with thickening of the lobar artery and its branches. Animals with a bruit across the anastomosis had a more modest increase in arterial resistance, and the

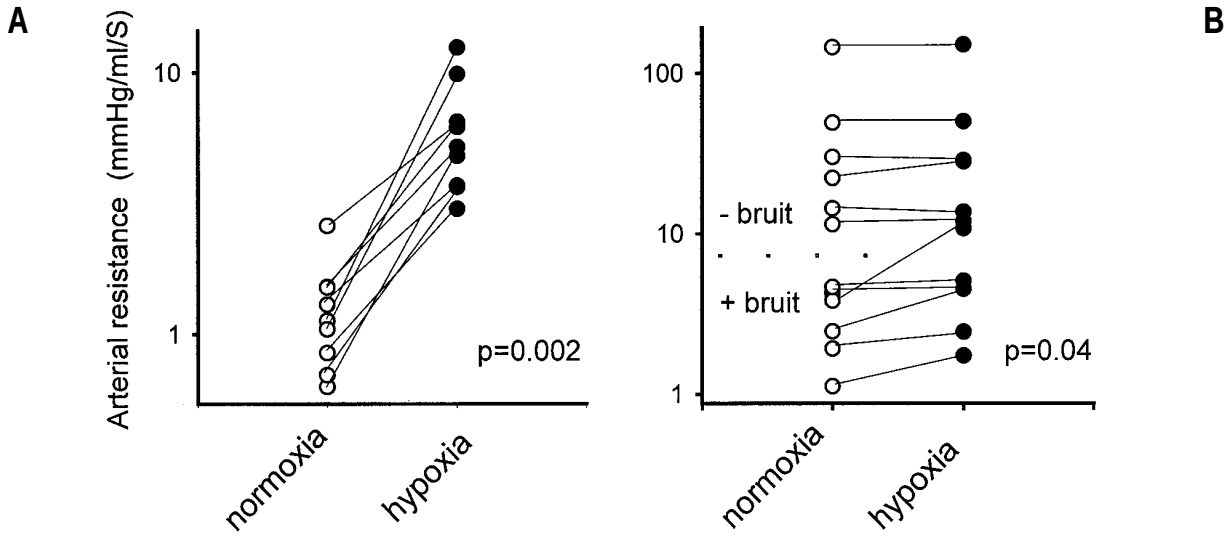

Fig 4. Individual values for arterial resistance under normoxic and hypoxic (FIO_2 = 0.03) conditions in nonshunted (n = 9) **(A)** and shunted (n = 12) **(B)** lobes are shown. Resistance data are plotted on a semi-log scale in nonshunted and shunted lobes, but the magnitude of the scales is different. Data obtained under normoxic conditions appear in *open circles*, and those acquired during hypoxia are represented as *closed circles. P* values in the figure compare normoxic to hypoxic data in the same lobes (paired *t* test). Normoxic resistance values were higher in shunted than nonshunted lobes (*P* = .048; unpaired *t* test).

lobar artery and branches appeared less thickened on gross examination. During hypoxic ventilation, arterial resistance of shunted lobes increased an average of 34% ± 46% (*P* = .04), whereas nonshunted lobes demonstrated a greater hypoxic vasoconstriction, with an average increase in arterial resistance of 450% ± 320% (*P* < .001 shunted lobes vs nonshunted lobes). The arterial compliance was reduced in all but one of the shunted lobes, and arterial compliance was unaffected by hypoxia in both groups (Table II). Capillary and venous compliance and venous resistance (C_2, C_3, and R_2) were similar between shunted and nonshunted lobes. Hypoxia decreased capillary compliance in nonshunted lobes but not in shunted lobes (Table II). Eight shunted and 5 nonshunted lobes underwent hemodynamic studies during administration of iNO. Vascular occlusions demonstrated a reduction in arterial resistance in shunted lobes during administration of iNO (*P* = .008), but no effect on venous resistance or compliance values was observed. In nonshunted lobes, no iNO effect on resistance or compliance values was demonstrated. In 2 shunted lobes with a marked increase in arterial resistance (R_1 = 49 and 145 mm Hg · s^{-1} · mL^{-1}, respectively) increasing iNO produced a progressive reduction in arterial perfusion pressure at concentrations up to 1000 ppm (data not shown). There was no effect of iNO on perfusion pressure beyond 30 ppm iNO in 2 nonshunted lobes and 1 shunted lobe with low arterial resistance.

The difference in hypoxic response between shunted and nonshunted lobes is well illustrated by comparing double occlusion curves during normoxia and hypoxia (Fig 5). In the nonshunted lobe, hypoxia resulted in a marked increase in perfusion pressure mostly as a result of an increase in arterial resistance. Perfusion pressure during normoxia in the shunted lobe was greater than in the nonshunted lobe even at lower flow, but the perfusion pressure response to hypoxia was small and arterial resistance was minimally changed. Another notable finding was that arterial and venous perfusion pressures in the shunted lobe did not reach a common asymptote during double occlusion. This finding was evident in 6 shunted lobes with high arterial resistance (>10 mm Hg · s^{-1} · mL^{-1}), where arterial and venous double occlusion pressures differed by 2.5 to 28 mm Hg. This difference, referred to as the "closing pressure gradient," averaged 7 mm Hg in these lobes. In contrast, low-resistance lobes, both shunted and nonshunted, usually approached a common double occlusion pressure.

Angiographic studies performed during lobar perfusion yielded images of a highly remodeled pulmonary

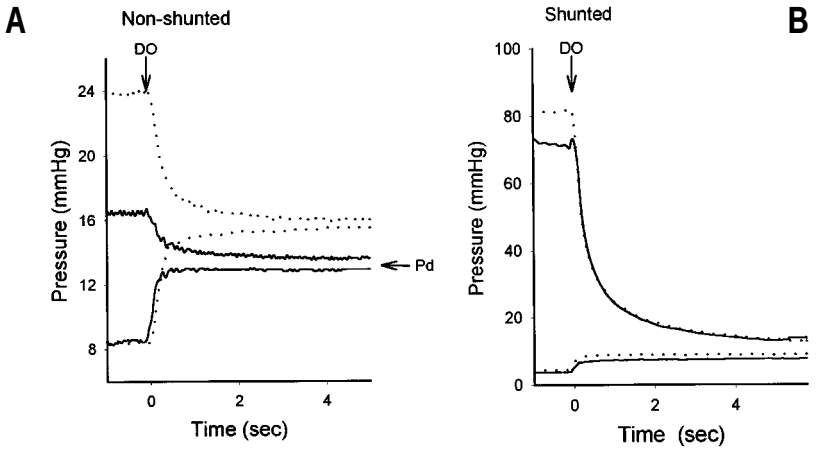

Fig 5. Representative double occlusion experiments in nonshunted **(A)** and shunted **(B)** lobes during ventilation with normoxic *(solid lines)* and hypoxic *(dotted lines)* gases. *DO,* Double occlusion.

vascular tree in shunted lobes (Fig 6). Characteristically, the main lobar pulmonary artery was dilated with luminal irregularity. Narrowing of the orifices of branch vessels was common. Second- and third-order vessels were narrowed compared with those of control animals.

Histomorphometric data. The shunted vasculature was extensively remodeled at all levels of the arterial circulation. Representative images from nonshunted and shunted lung sections are shown in Fig 7. In some sections from shunted lungs, structures that were almost certainly smaller arteries had entirely obliterated lumina; these were not included in the analysis. Analysis of 30 vessels from nonshunted and 30 vessels from shunted lungs was undertaken. Vessel diameters and areas bounded by the external elastic lamina of nonshunted and shunted vessels were similar (293 ± 46 mm for nonshunted vessel diameter vs 284 ± 60 mm for shunted vessel diameter). In contrast, average wall/lumen area ratios were increased by approximately an order of magnitude in shunted compared with nonshunted vessels. Increased ratios were noted across a wide range of pulmonary arterial sizes (Fig 8, *A*). Thickening of both intima and media in shunted lungs contributed to the larger wall area ratios (Fig 8, *B*).

Discussion

In this swine model, blood was delivered at high flow under systemic pressure to the limited vascular volume of the left lower lobe. By this process, sheer stress and transluminal pressure on the vessel wall were increased. In turn, vascular remodeling in the form of intimal and medial growth ensued. Direct aorta–lower lobe pulmonary artery anastomosis offered several advantages. Avoidance of a synthetic conduit eliminated the problem of thrombotic occlusion of the anastomosis. A second benefit was derived by the more limited flow through the aorta-lobar shunt, which was readily tolerated, with clinical heart failure developing in only 3 animals. In fact, right ventricular output and pulmonary artery wedge pressures were not different between animals with and without a shunt, further underscoring the reduced systemic physiologic effects of aorta-lobar shunting. In earlier central shunting studies, higher incidences of conduit thrombosis, congestive heart failure, and late mortality were encountered.[5,8,14] Finally, the aorta-lobar pulmonary connection resulted in advanced pulmonary hypertension with increased arterial resistance, decreased arterial compliance, and clear neointimal and medial changes in shunted lobes. Dammann, Baker, and Muller[15] characterized a similar model of lobar hypertension. They noted subintimal and medial hemorrhage and disruption of the elastic laminae immediately after the shunt procedure. A proliferative response in the intima and media progressively developed over 9 weeks, and systemic levels of pulmonary hypertension were obtained. Schnader and coworkers[16] performed hemodynamic and histologic studies of a systemic artery–lobar artery shunt in sheep. Shunted lobar pressures measured in 2 animals were near systemic levels. Neointimal lesions were observed and their prevalence increased over the observation period from 2 months to 1½ years. Our results confirm and extend these observations. We also investigated changes in the distribution of vascular resistance and compliance and noted

the effects of hypoxia and iNO on shunted lobes. We noted that shunt flow was markedly decreased in hypertensive lobes. In contrast, the studies reported by Schnader's group[16] showed that shunt flow remained greater than control lobar flows, and this was associated with an increase in cardiac output and wedge pressure not observed in our animals that received a shunt. The use of young animals whose immature pulmonary vasculature is more prone to proliferative changes may be responsible for the advanced arteriopathy and the secondary reduction in lobar flow in our shunted lobes. Although shunted lobar blood flow was not measured, we assume that it was initially very high, as evidenced by a vigorous thrill within the lobe and the development of heart failure in 3 animals.

Other models of flow-induced pulmonary vasculopathy have produced lesser degrees of pulmonary hypertension. Central shunts between the aorta and the main pulmonary artery produce pulmonary artery pressures between one third and one half of systemic values.[5-7,17] In these models, shunt ligation immediately results in normalization of pulmonary vascular resistance.[6,17] Pulmonary hypertension has been a salient feature of preparations in which the aortopulmonary connection is performed early in development. In recent experiments, we found that central shunts in neonatal piglets produced greater levels of pulmonary hypertension than in animals receiving a shunt at 4 to 6 weeks of age.[8,18] In utero placement of aortopulmonary shunts by Reddy,[14,19] Wong,[9] and their associates resulted in pulmonary/systemic pressure ratios of approximately 0.75 at 1 month of age. Pulmonary vascular resistance was moderately increased, and early histologic changes of vessel wall thickening and reduced arterial density were evident.

Not all animals in our experiments had systemic levels of pulmonary hypertension. In fact, there were 2 sets of vascular pathology in shunted lobes. The first set included 10 shunted lobes with systemic levels of pulmonary hypertension. Six of these shunted lobes were shown by vascular occlusion studies to have marked elevation of arterial resistance (>10 mm Hg $\cdot$ s^{-1} $\cdot$ mL^{-1}). They had thicker and stiffer arteries on gross inspection. Attempts to increase perfusion of these lobes beyond 100 mL/min resulted in supraphysiologic arterial pressure, while iNO continued to reduce perfusion pressure at concentrations as high as 1000 ppm. The double occlusion maneuver resulted in a closing pressure gradient. The mechanism responsible for the closing pressure gradient phenomenon awaits elucidation.

The second set of shunted lobes (n = 8) had less marked pulmonary hypertension and a persistent anastomotic bruit. In the 6 lobes studied by vascular occlusion maneuvers, arterial resistance was less than 5 mm Hg $\cdot$ s^{-1} $\cdot$ mL^{-1}. The pulmonary artery vasculature was less thickened than in lobes with high arterial resistance but was still grossly abnormal compared with that of controls. Increased tone was evident in these lobes, based on the fact that they relaxed in response to iNO. The closing pressure gradient was small or nonexistent during double occlusion, implying hemodynamic continuity across the microcirculation.

The factors responsible for maintaining low pulmonary artery pressures in some shunted lobes are not clear. Every effort was made to construct the shunts by a regimented and consistent method. Postoperative recovery was generally rapid and without incident in both sets of animals receiving a shunt. Anastomoses in all cases were patent and the flow across them seemed uncompromised. Inherent vasoactive factors could be responsible for the variable development of pulmonary hyperten-

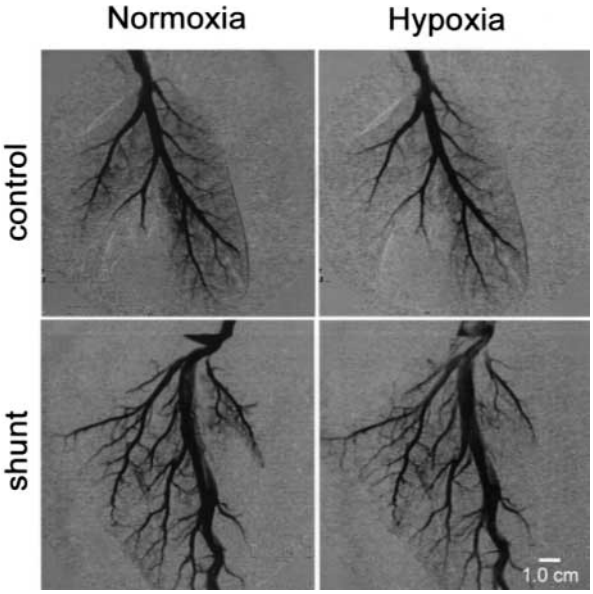

Fig 6. Angiograms of nonshunted and shunted lobes under normoxic and hypoxic conditions.[25] Images from nonshunted lobes demonstrate a gradual decrease in arterial diameter with decreasing vessel size and extensive "arborization" of terminal vessels. The diameter of small vessels decreases under hypoxic conditions with an apparent increase in the size of the lobar pulmonary artery (consistent with hypoxic vasoconstriction of microvessels). In contrast, images from shunted lobes demonstrated rapid tapering of large arteries with loss of small peripheral runoff vessels (consistent with histologic data) and little change during ventilation with hypoxic gas.

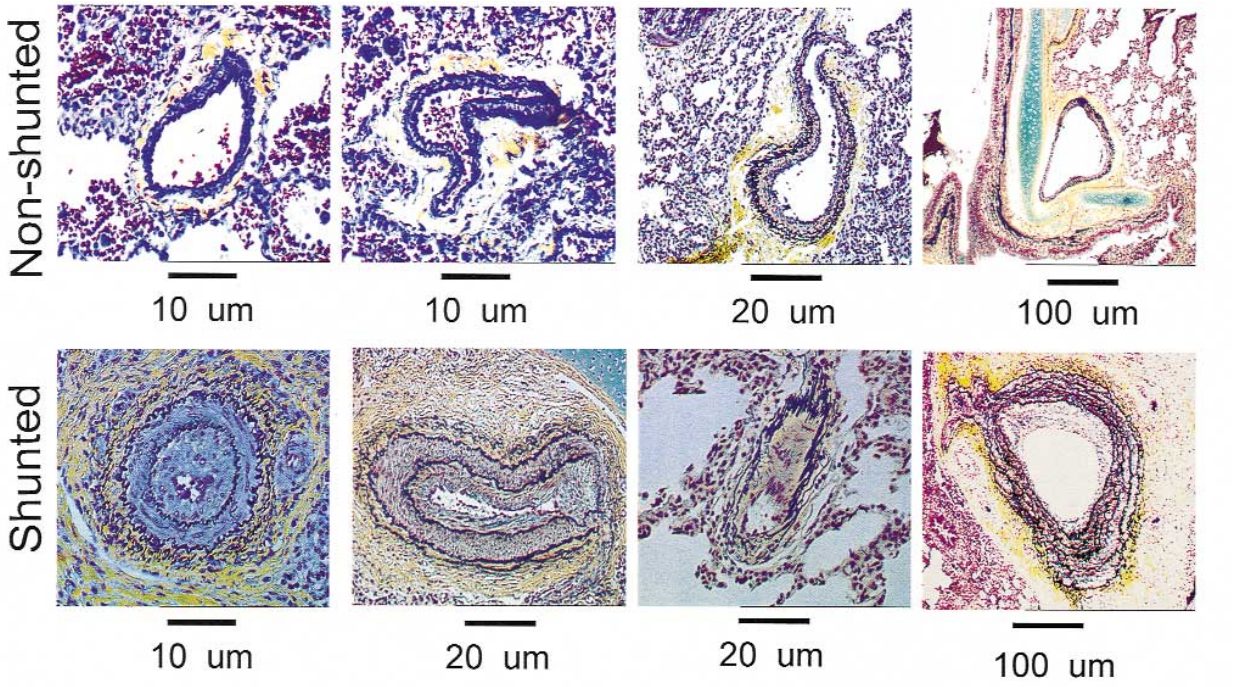

Fig 7. Representative histologic images from shunted and nonshunted lungs. Sections were fixed and stained with a pentachrome stain to facilitate identification of features, including internal and external elastic lamina and neointimal tissue. Small pulmonary arteries from nonshunted lungs (less than 500 μm in diameter) were thin walled and consistently without structures other than blood cells inside the internal elastic lamina. In contrast, arteries of similar size from shunted lungs exhibited thickening of medial structures, as well as neointimal proliferative changes. Arterial lumina were narrowed, and in some cases of arteries less than 20 μm in diameter, all openings were obliterated; these vessels were not included in the analysis.

sion. Differences in baseline vascular tone, nitric oxide production, and differential expression of factors modulating vasomotor tone or proliferative propensity could contribute to the observed spectrum of pulmonary hemodynamic and morphologic responses.

Botney[20] has underscored the importance of pulmonary hemodynamics with respect to vascular remodeling, particularly the neointimal formation associated with primary pulmonary hypertension and severe forms of secondary pulmonary hypertension. In monocrotaline-treated rats, neointimal lesions occurred in conjunction with medial hyperplasia when increased pulmonary blood flow was imposed by pneumonectomy.[21] Either monocrotaline or increased flow alone resulted only in medial hypertrophy. Botney hypothesized that neointimal formation was initiated by injury (eg, monocrotaline) and then amplified by the stress of increased flow. In our aorta–lobar pulmonary artery shunt model, advanced neointimal and medial changes were produced. Consistent with Botney's "two hit" model,[21] we postulate that the markedly increased pulmonary flow delivered at increased pressure served both to initiate intimal damage and then to propagate the remodeling process by increased sheer stress and normal (transluminal) stress. The relative contributions of sheer and normal stress with respect to vascular remodeling and pulmonary hypertension are currently undetermined, but they may be elucidated by longitudinal measurements of shunt flow and pressure over time.

Although shunted lobes displayed a wide range of arterial resistance values, they shared 2 common features. The first common finding among shunted lobes was the blunted vasoconstrictor response to hypoxia. Lobes with an arterial resistance of more than 5 mm Hg $\cdot$ s^{-1} $\cdot$ mL^{-1} had no discernible change in arterial resistance during hypoxia, whereas lobes with an arterial resistance of less than 5 mm Hg $\cdot$ s^{-1} $\cdot$ mL^{-1} demonstrated a modest increase in arterial resistance (Fig 4). De Canniere and associates[6] previously noted this phenomenon in vivo in piglets subjected to a central aortopulmonary shunt. Second, shunted lobes had a lower

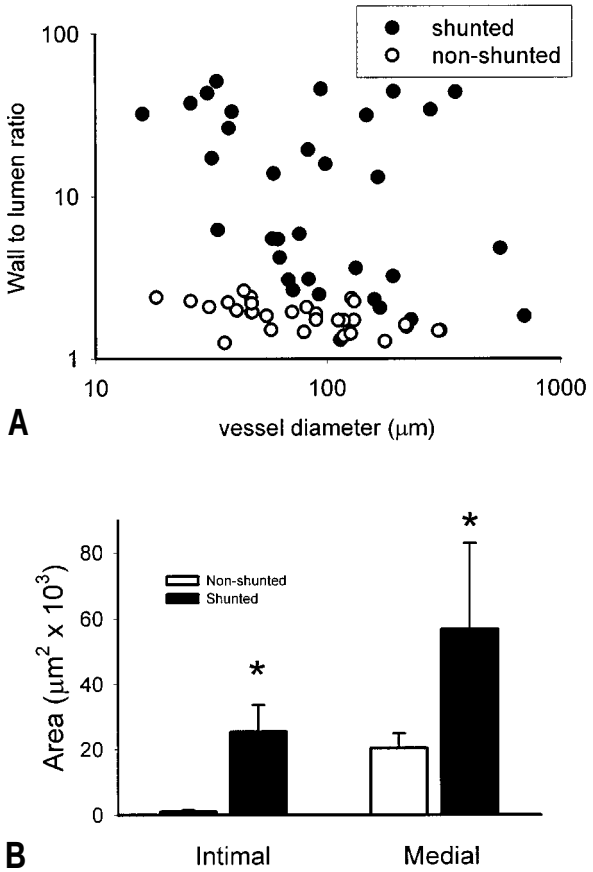

Fig 8. A, Individual values for wall/lumen area ratios from 30 arteries of nonshunted *(open circles)* and 30 shunted *(closed circles)* lungs. Wall/lumen values are plotted on a semi-log scale *(y-axis)* against the shortest distance between the external elastic lamina on the *x-axis.* Over the size range of vessels studied, wall/lumen ratios were greater in shunted than nonshunted lungs with minimal overlap between the two. **B,** Distribution of the proliferative changes of the pulmonary arteries. Vessels from shunted lobes had larger medial and intimal areas than their counterparts from nonshunted lobes. *Significant differences between the shunted and nonshunted areas (both the intimal and medial areas of the pulmonary arteries were greater in samples from shunted than nonshunted lungs).

arterial compliance than nonshunted lobes. In our earlier studies with central aortopulmonary shunts, pulmonary hypertension did not develop and arterial resistance was unchanged; however, the arterial compliance was significantly reduced.[22] Notably, these animals were studied only 4 weeks after surgery, and the central shunt allowed dissipation of the increased flow throughout the entire lung as opposed to one lobe, as in the current experiment. Assuming the remodeling process is progressive, one may deduce that a reduction in arterial compliance is the first vasomotor response to increased flow. With greater, more prolonged sheer stress, increased arterial resistance develops after the reduction in arterial compliance.

The effects of iNO were also tested on nonshunted lobes and shunted lobes with and without pulmonary hypertension. Use of iNO consistently reduced perfusion pressure and decreased arterial resistance in shunted lobes. The effect was similar in shunted lobes with both high and low arterial resistances. Infants and children with left-to-right shunts have demonstrated similar pulmonary vascular responses to iNO.[23] Our finding of a progressive response to very high concentrations of iNO in lobes with extensive vascular remodeling is new and provocative. Previous clinical and experimental studies have shown a maximal iNO effect at 40 ppm.[24] The lobes with a higher arterial resistance in this experiment probably have more advanced arteriopathy than in previous clinical studies. Such thick-walled vessels would pose a greater diffusion distance to alveolar nitric oxide. Alternatively, the resistance vessels may be further upstream due to the remodeling process, and remote from alveolar nitric oxide. These findings and deductions need to be further evaluated in this model.

In conclusion, this investigation characterizes a model of increased pulmonary blood flow confined to a single lung lobe. In most lobes, systemic levels of pulmonary hypertension developed, whereas others had more modest increases in resistance. Increased pulmonary blood flow also produced significant changes in pulmonary vascular geometry and vasomotor tone. The nonshunted pulmonary circulation had hemodynamics similar to the hemodynamics of control animals having a sham operation. Given these conditions, data obtained from remaining lobes of animals receiving a shunt could serve as paired data with shunted lobes. This model of lobar pulmonary hypertension may serve well in investigations directed at the underlying mechanisms responsible for pulmonary vasculopathy caused by increased blood flow. Because the aorta-lobar shunt produced advanced lesions over a short time, potential therapies aimed at slowing the progression of vascular pathology may be studied effectively.

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