0031-3998/09/6605-0539
PEDIATRIC RESEARCH

Vol. 66, No. 5, 2009
Printed in U.S.A.

Oxygen Concentration and Pulmonary Hemodynamics in Newborn Lambs With Pulmonary Hypertension

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ABSTRACT: The effect of oxygen concentration on lowering pulmonary vascular resistance (PVR) during resuscitation in a model of persistent pulmonary hypertension of the newborn (PPHN) is not known. PPHN was induced in fetal lambs by ductal ligation 9 d before delivery. After delivery by cesarean section, resuscitation of PPHN lambs with 21%, 50%, or 100% O_2 ($n = 6$ each) for 30 min produced similar decreases in PVR. Lambs were then ventilated with 50% O_2 for 60 min and exposed to inhaled nitric oxide (iNO, 20 ppm). Initial resuscitation with 100% O_2 significantly impaired the subsequent response to iNO compared with 21% O_2 (42 ± 9% *vs* 22 ± 4% decrease from baseline PVR). Finally, each lamb was randomly and sequentially ventilated with 10%, 21%, 50%, or 100% O_2. PVR decreased with increased concentrations of inhaled O_2 up to 50%, there being no additional decrease in PVR with 100% O_2. When PVR was correlated with Pao_2, the maximal change in PVR was achieved at Pao_2 values <60 mm Hg. We conclude that resuscitation with 100% O_2 does not enhance pulmonary vasodilation compared with 21% and 50% O_2, but impairs the subsequent response to iNO in PPHN lambs. Hypoxia increases PVR but hyperoxia does not confer significant additional pulmonary vasodilation in lambs with PPHN. (***Pediatr Res* 66: 539–544, 2009**)

The Neonatal Resuscitation Program currently recommends resuscitation of term newborn infants with 100% O_2 (1). However, as 21% O_2 has been associated with reduced neonatal mortality (2), some suggest that it may be appropriate for initial resuscitation of term infants (3). Although there is increasing availability of oxygen blenders for neonatal resuscitation (4), few studies have addressed the effect of resuscitation with an intermediate range of oxygen in the delivery room. We recently reported that ventilation of normal term lambs for 30 min at birth (referred to as "resuscitation") with 50% and 100% O_2 resulted in greater decrease in pulmonary vascular resistance (PVR) compared with 21% O_2 (5).

As oxygen mediates reduction of high fetal PVR at birth (1,6), 100% O_2 is often recommended as the first line of resuscitation for circumstances associated with persistent pulmonary hypertension of the newborn (PPHN) (7). However, it is not always possible to anticipate conditions that might lead to *in utero* pulmonary hypertension (8,9). If PPHN infants are resuscitated at birth with 21% O_2, PVR may remain high, resulting in severe PPHN and altered response to inhaled nitric oxide (iNO).

Once identified, babies with PPHN are traditionally managed with mechanical ventilation with high FiO_2 (fraction of inspired oxygen) and iNO. Hyperoxic ventilation may increase production of reactive oxygen species (ROS), which in turn impair pulmonary vasodilation (10,11). However, little is known about the optimal FiO_2 or Pao_2 that maximizes pulmonary vasodilation in PPHN (12). We recently reported that ventilation with 100% O_2 increases contractile responses of pulmonary arteries (PA) to norepinephrine from both control (13) and PPHN lambs (14), and blunts vascular responses to iNO in newborn lambs with acute pharmacologically induced PPHN (5). The effect of resuscitation with high FiO_2 at birth on subsequent vasodilation to iNO in PPHN associated with vascular remodeling is not known.

This study examines the impact of FiO_2 during resuscitation on the initial decrease in PVR at birth in lambs with PPHN induced by antenatal ductal ligation. We hypothesized that ventilation with 100% O_2 would lead to a more rapid decline in PVR at birth in PPHN lambs compared with 21% or 50% O_2 but would impair subsequent pulmonary vasodilation to iNO. Finally, at 2–3 h of age, when PVR had decreased from high fetal values, we studied the effect of change in FiO_2 on PVR. We hypothesized that higher FiO_2 and Pao_2 would result in lower PVR in control and PPHN lambs.

METHODS

The Institutional Animal Care and Use [1]Committee at Buffalo approved this study. Time-dated pregnant ewes were obtained from the Swartz family farm, Attica, NY. After 12 h of fasting, ewes were anesthetized with i.v. thiopental (20 mg/kg) and inhaled isoflurane (2%). Epidural preservative-free morphine (6 mg) and transdermal fentanyl patches (100 μg/h) provided postoperative analgesia. Fetal ductal ligation was performed at 126–128 d gestational age (term ~ 145 d) to induce PPHN as previously described (14) and fetus was replaced in the uterus and allowed to recover for 9 d. The study consisted of three different postnatal time periods and protocols.

Effect of resuscitation with 21%, 50%, or 100% O_2 for 30 min on pulmonary hemodynamics at birth. Eighteen PPHN lambs and four control twins were partially exteriorized by cesarean section. While still on placental circulation, catheters were placed in the jugular vein, carotid artery, PA and left atrium, and an ultrasonic flow transducer around the main PA. Fetal

Received May 29, 2009; accepted July 24, 2009.
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Supported by the American Academy of Pediatrics/Neonatal Resuscitation Program (S.L.) and Grant HL-54705 from the National Heart, Lung, and Blood Institute (R.H.S.).

Abbreviations: ABG, arterial blood gas; **BP,** blood pressure; **FiO_2,** fraction of inspired oxygen; **PPHN,** persistent pulmonary hypertension of the newborn; **PA,** pulmonary artery; **Q_p**, pulmonary blood flow; **PVR,** pulmonary vascular resistance; **ROS,** reactive oxygen species

carotid arterial blood gases (ABG) were obtained. The umbilical cord was clamped and cut. The lamb was weighed and ventilated with PIP 30, PEEP 5 cmH_2O, rate 40/min. The four control (non-PPHN) lambs were resuscitated with 21% O_2. For the PPHN lambs, the O_2 concentration was randomly set at 21%, 50%, or 100% ($n = 6$ each). The ductus was tied during instrumentation before delivery in control lambs. Analgesia for the lamb was provided with a ketamine bolus (3 mg/kg) followed by an infusion of 20–100 μg/kg/min ketamine and 2 μg/kg/min of diazepam. One dose of pancuronium (0.1 mg/kg) was administered to all lambs at birth. Dextrose 10% solution with 25 mEq NaCl, 20 mEq KCl, and 10 mEq $NaHCO_3$/L were administered at 120 mL/kg/d. ABGs were drawn every 5 min for 30 min, and ventilator adjustments were made to maintain $Paco_2$ between 35 and 50 mm Hg. PVR was calculated using the following equation: PVR (mm Hg·mL^{-1}·min·kg body weight) = (mean PA pressure − left atrial pressure in mm Hg) 47 pulmonary blood flow corrected for body weight (Q_p, mL·min^{-1}·kg^{-1}).

Response to inhaled no. After 30 min of ventilation at 21%, 50%, or 100% O_2, PPHN lambs in all the three groups were ventilated with 50% O_2 for 60 min. This concentration of oxygen was chosen because preliminary studies in PPHN lambs ventilated with 50% O_2 for 24 h demonstrated similar survival with lower risk of oxygen toxicity compared with 100% O_2. During this period, hypotension [mean systemic blood pressure (BP) <40 mm Hg] was treated with a transfusion of cord blood (10 mL/kg). When systemic BP was >40 mm Hg and PVR was stable, iNO was administered at 20 ppm for 10 min. The percent decrease in PVR after iNO was calculated.

Effect of varying FiO_2 on PVR at 2–3 h of age. Approximately 30 min after discontinuing iNO, and when PVR was stable, PPHN lambs were ventilated randomly and sequentially with 21%, 50%, and 100% O_2 (two periods of 20 min each per lamb) and 10% O_2 for 10 min. Eighteen control lambs without PPHN (twin lambs from other studies) with gestational age between 135 and 142 d were similarly ventilated. A shorter duration of ventilation with 10% O_2 was chosen as PPHN lambs did not tolerate prolonged hypoxic ventilation. Two to three ABGs and PVR were obtained after 8–10 min of ventilation with each FiO_2. Lambs were euthanized with pentobarbital (100 mg/kg) at the end of the study.

Statistical analysis was performed with ANOVA. The change points in Pao_2 and PVR were determined by Markov chain Monte Carlo (MCMC) procedure using SAS 9.2 software (SAS, Cary, NC).

RESULTS

Thirty-four fetal lambs from time-dated pregnant ewes underwent antenatal fetal ductal ligation to induce PPHN. Eight premature deliveries after surgery, three still births, and four cases of severe hydrops were excluded. One lamb assigned to the 50% O_2 group was hypotensive, acidotic, died at 60 min of life, and was excluded. There were no significant differences in birth weight and fetal ABG between the three groups of PPHN lambs (Table 1).

After delivery, all lambs with PPHN were hypoxemic compared with controls. Control lambs resuscitated with 21% O_2 increased Pao_2 significantly better than PPHN lambs by 30 min of age. Resuscitation of PPHN lambs with 100% O_2 for 30 min significantly increased Pao_2 compared with both fetal levels and lambs ventilated with 21% O_2 for 30 min (Table 1).

Effect of resuscitation with 21%, 50%, and 100% O_2 for 30 min on pulmonary hemodynamics at birth. PA pressure was greater at all time points in lambs with PPHN compared with controls. PA pressure significantly decreased after 30 min of ventilation with 21%, 50%, or 100% O_2 in all groups. There were no differences in PA pressures between the three PPHN groups (Fig. 1).

During fetal life, PPHN lambs had low Q_p compared with controls but increased significantly after ventilation (Fig. 2). The increase in Q_p with ventilation in PPHN lambs was significantly less compared with controls, but there were no differences between the three groups of PPHN lambs.

Left atrial pressure was similar in all groups of lambs and increased significantly compared with fetal values in PPHN lambs ventilated with 50% and 100% O_2. Systemic BP increased after delivery in all groups. There were no differences between control and PPHN lambs (data not shown).

PVR was significantly higher in PPHN lambs compared with controls (Fig. 3). Ventilation decreased PVR in all groups of lambs. However, ventilation with different FiO_2 produced similar decreases in PVR in lambs with PPHN. After 30 min of ventilation, PVR remained significantly higher in all PPHN lambs compared with controls.

Systemic blood pressure increased after delivery in all groups of lambs. There were no differences between control and PPHN lambs (Fig. 4).

Response to iNO. After 30 min of ventilation with 21%, 50%, or 100% O_2, all the three groups of PPHN lambs were ventilated with 50% O_2 for 60 min. After 90 min of ventilation (30 min with 21, 50, or 100% followed by 60 min with 50% O_2), there were no significant differences in PVR among the three groups of PPHN lambs (baseline PVR in Fig. 5A).

Table 1. *Birth weight and arterial blood gases before delivery and after 30 min of ventilation with 21% O_2 in control lambs and 21, 50, or 100% O_2 in PPHN lambs*

	Control lambs	PPHN lambs		
	21% O_2 ($n = 4$)	21% O_2 ($n = 6$)	50% O_2 ($n = 6$)	100% O_2 ($n = 6$)
Birth weight (kg)	3.2 ± 0.2	2.7 ± 0.3	3 ± 0.1	3.2 ± 0.2
Gestational age (d), median (range)	136 (135–137)	135.5 (135–137)	136 (135–137)	135 (135–137)
Fetal arterial blood gases				
pH	7.31 ± 0.02	7.32 ± 0.02	7.24 ± 0.07	7.25 ± 0.05
$Paco_2$ (mm Hg)	55 ± 2	55 ± 4	60 ± 4	59 ± 3
Pao_2 (mm Hg)	18 ± 2	20 ± 1	20 ± 2	20 ± 3
Base excess (mEq/L)	−0.2 ± 0.3	1 ± 1	−2 ± 2	−1 ± 1
Arterial blood gases after 30 min of ventilation				
pH	7.37 ± 0.02*	7.36 ± 0.03	7.36 ± 0.03*	7.34 ± 0.04
$Paco_2$ (mm Hg)	42 ± 2*	44 ± 3*	39 ± 3*	47 ± 5
Pao_2 (mm Hg)	57 ± 6*	23 ± 2†	36 ± 8†	40 ± 5*†‡
Base excess (mEq/L)	0.2 ± 1	−3 ± 1	−4 ± 1	−3 ± 1
Tail pulse oximeter Spo_2 (%)	90 ± 3	61 ± 8†	78 ± 9†	83 ± 5‡

Data shown as mean ± SEM.

* $p < 0.05$ compared with corresponding fetal gas.

† $p < 0.05$ compared with non-PPHN control lambs.

‡ $p < 0.05$ compared with 21% O_2 PPHN lambs.

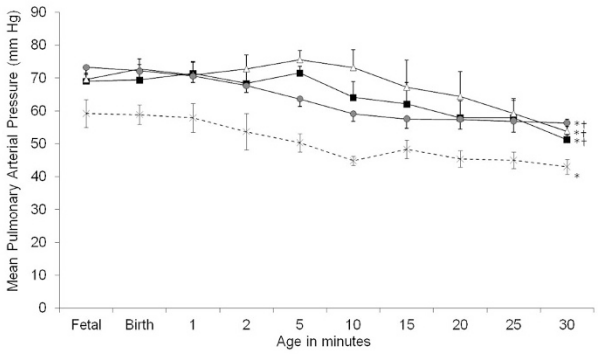

Figure 1. Changes in mean main pulmonary arterial pressure (mean ± SEM) during fetal life, at birth, and during the first 30 min of life in PPHN lambs ventilated with 21% (*black squares*), 50% (*white triangles*), or 100% O_2 (*gray circles*) ($n = 6$ each). Similar changes in PA pressure in four control twin lambs without PPHN ventilated with air (*dashed line*) are shown for comparison. (*$p < 0.05$ by ANOVA over time; $p < 0.05$ compared with control by repeated measures ANOVA).

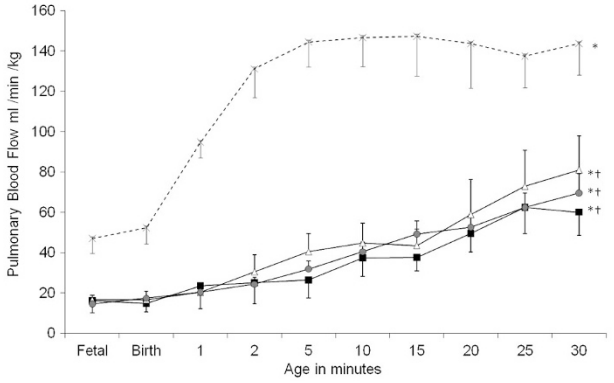

Figure 2. Changes in the blood flow in the main pulmonary artery (mean ± SEM) during fetal life, at birth, and during the first 30 min of life in PPHN lambs ventilated with 21% (*black squares*), 50% (*white triangles*), or 100% O_2 (*gray circles*). Similar changes in Q_P in four control twin lambs without PPHN ventilated with air (*dashed line*) are shown for comparison. (*$p < 0.05$ by ANOVA over time; $p < 0.05$ compared with control by repeated measures ANOVA).

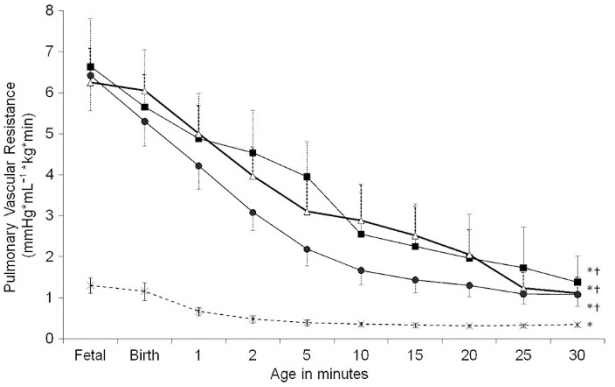

Figure 3. Changes in pulmonary vascular resistance (mean ± SEM) during fetal life, at birth, and during the first 30 min of life in PPHN lambs ventilated with 21% (*black squares*), 50% (*white triangles*), or 100% O_2 (*gray circles*) ($n = 6$ each). Similar changes in PVR in four control twin lambs without PPHN ventilated with air are shown for comparison (*dashed line*). (*$p < 0.05$ by ANOVA over time; $p < 0.05$ compared with control by repeated measures ANOVA).

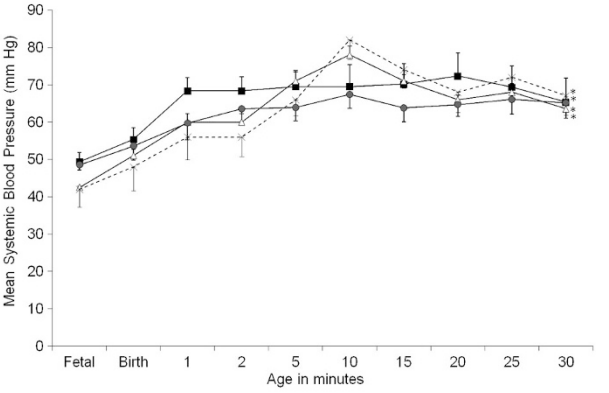

Figure 4. Changes in mean systemic arterial pressure (mean ± SEM) during fetal life, at birth, and during the first 30 min of life in lambs with PPHN ventilated with 21% (*black squares*), 50% (*white triangles*), or 100% O_2 (*gray circles*) ($n = 6$ each). Similar changes in systemic arterial pressure in four control twin lambs without PPHN ventilated with air (*dashed line*) are shown for comparison. (*$p < 0.05$ compared with fetal values by ANOVA).

Inhaled NO (20 ppm with 50% O_2) for 10 min significantly reduced PVR in all groups of lambs. Changes in Pao_2 after iNO are shown in Figure 5B. The decrease in PVR, expressed as a percent of baseline, was significantly greater in PPHN lambs resuscitated with 21% compared with 100% O_2 (42 ± 9% *vs* 22 ± 4%; Fig. 5C). However, PVR values did not differ among the three groups of lambs after exposure to iNO (Fig. 5A).

Effect of varying FiO_2 on pulmonary hemodynamics after the initial decrease in PVR at birth. PPHN lambs were ventilated with 50%O_2 for 30 min after cessation of iNO. When PVR was stable, they were randomly ventilated with 10%, 21%, 50%, and 100% O_2 to examine the response of PVR to varying oxygen concentrations. Six PPHN lambs were unstable (bradycardia and/or cardiac arrest) during 10% O_2 ventilation and required chest compressions and/or epinephrine and were excluded from the study. ABG values, PA pressure, and PVR at each FiO_2 in control and PPHN lambs are shown in Table 2. Pao_2 values significantly increased with increasing FiO_2 in control and PPHN lambs. PA pressure and PVR decreased with increase in O_2 from 10 to 21% and from 21 to 50% in both control and PPHN lambs. There was no further decrease in PA pressure or PVR after an increase from 50 to 100% O_2 (Fig. 6A).

Figure 6B is a scatter plot of Pao_2 and PVR in control and PPHN lambs. Data points from control lambs corresponded to a two-line regression model with one change point (Pao_2 value at which the slope of the regression line changes) at 52.5 ± 1.7 mm Hg (Fig. 6B). The PVR values were significantly higher in PPHN lambs (especially with low Pao_2 values, note change in *y* axis). These data points corresponded to a three-line regression model with two change point Pao_2 values at 13.9 ± 1 and 59.6 ± 15.3 mm Hg. PVR values were plotted against a clinically relevant range of oxygen saturation from 80 to 100% (Fig. 6C).

DISCUSSION

PPHN is a syndrome of failed pulmonary circulatory adaptation at birth, resulting in high PVR and neonatal respiratory

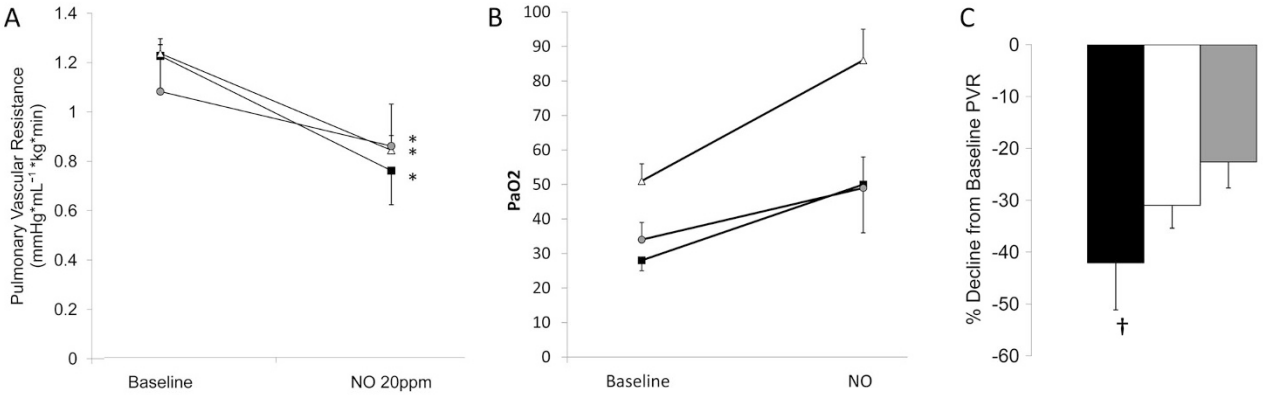

Figure 5. Changes in PVR (*A*) and Pao_2 (*B*) at 90 min of age (baseline) and after 10 min of ventilation with 20 ppm of iNO. Lambs with PPHN were ventilated with 21% (*black squares*), 50% (*white triangles*), or 100% O_2 (*gray circles*) for 30 min at birth and all the groups were ventilated with 50% O_2 for 60 min before and during exposure to NO. (*$p < 0.05$ compared with baseline PVR). The percent fall in PVR with inhaled NO is shown in *C* (*black bar*, 21%; *white bar*, 50%; and *gray bar*, 100% O_2 at birth). (†$p < 0.05$ compared with 100% O_2).

Table 2. *Changes in arterial blood gases and mean pulmonary arterial pressure (PAP) and pulmonary vascular resistance (PVR in mmHg·mL^{-1}·min·kg) after 10–20 min ventilation of 2- to 3-h-old control and PPHN lambs with 10%, 21%, 50%, and 100% O_2 in a random sequence*

	Control lambs (n = 18)				PPHN lambs (n = 12)			
O_2 (%)	pH	Po_2 (mm Hg)	PAP (mm Hg)	PVR	pH	Po_2 (mm Hg)	PAP (mm Hg)	PVR
10	7.39 ± 0.02	17 ± 1	63 ± 2	0.49 ± 0.04	7.28 ± 0.03*	14 ± 1	63 ± 4	3.0 ± 0.5*
21	7.40 ± 0.01	53 ± 1†	42 ± 1†	0.28 ± 0.01†	7.31 ± 0.03*	25 ± 3*	55 ± 3*†	1.6 ± 0.2*†
50	7.34 ± 0.03	148 ± 15†‡	35 ± 1†‡	0.20 ± 0.13†‡	7.33 ± 0.01	42 ± 3*†‡	48 ± 1*†‡	1.0 ± 0.1*†‡
100	7.37 ± 0.03	358 ± 25†‡§	37 ± 2‡†	0.21 ± 0.01†‡	7.33 ± 0.02	67 ± 7*†‡§	56 ± 2*†§	1.0 ± 0.1*†‡

Data are shown as mean ± SEM.
* $p < 0.05$ compared with corresponding control value.
† $p < 0.05$ compared with 10% O_2.
‡ $p < 0.05$ compared with 21% O_2.
§ $p < 0.05$ compared with 50% O_2.

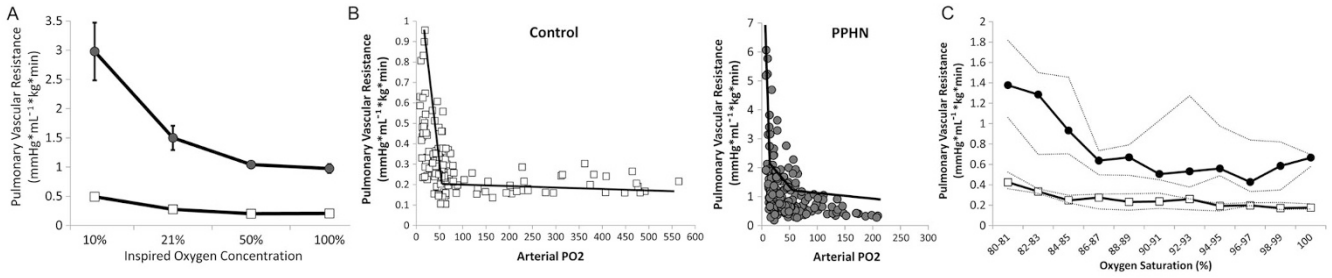

Figure 6. Changes in PVR in 2- to 3-h-old control and PPHN lambs with changing inspired oxygen concentration (*A*). PVR in control (*white squares*) and PPHN lambs (*black circles*) significantly decrease with increasing oxygen concentration from 10 to 21 to 50% O_2. However, PVR does not decrease further with increase in oxygen from 50 to 100% O_2 in both control and PPHN lambs. Scatter-plot showing PVR and Pao_2 in control and PPHN lambs (*B*). The two regression lines for the control lambs correspond to PVR = 0.2 − 0.00667 (Po_2 − 52.5) and PVR = 0.24 − 0.00013 (Po_2 − 52.5), where 52.5 is the mean change point. The three regression lines for the PPHN lambs correspond to PVR = 1.41–0.5495 (Po_2 − 13.9), PVR = 1.41–0.0152 (Po_2 − 13.9), and PVR = 0.7206–0.00286 (Po_2 − 59.6) where 13.9 and 59.6 are the means of the two change points. (*C*) Median and interquartile range (*dashed lines*) of PVR plotted against a clinically relevant range of oxygen saturations (80–100%) in control (*white squares*) and PPHN (*black circles*) lambs.

failure (12). Recent studies suggest that resuscitation with 21% O_2 is just as successful as resuscitation with 100% O_2 in term neonates (2). Theoretically, the use of supplemental oxygen during resuscitation may result in a more rapid increase in Q_p (1) and improve brain oxygenation (15) in a neonate with PPHN. Also, the optimal FiO_2 and Pao_2 in the management of neonates with PPHN is not known (12). This is the first study to examine the effect of varying FiO_2 on PVR in an animal model of *in utero* pulmonary hypertension with vascular remodeling. We studied this effect at birth, when fetal PVR is high, and at 2–3 h of age, after the initial decrease in PVR with postnatal ventilation.

In term lambs with normal pulmonary vasculature, we recently reported that 100% O_2 decreased PVR more rapidly and to a greater magnitude than 21% O_2 (5). As PPHN has been reported to affect as many as 10% of neonates with

respiratory failure (9), we extended these studies to determine the effectiveness of 21%, 50%, and 100% O_2 during resuscitation in reducing PVR in a well established lamb model of PPHN with remodeled pulmonary circulation (14,16).

In lambs with PPHN, PVR decreased from high fetal values without any significant increase in Pa_{O_2} after resuscitation with 21% and 50% O_2 (Fig. 3) probably secondary to ventilation and several fold increase in alveolar oxygen levels. Contrary to our hypothesis, 100% O_2 did not enhance the decrease in PA pressure and PVR, or increase Q_p compared with 21% or 50% O_2 (Figs. 1–3). The differences in transitional responses to varying FiO_2 relative to control lambs could be explained by three possible mechanisms. Control fetal ovine PA smooth muscle cells respond to an acute increase in Po_2 by decreasing intracellular calcium concentration, resulting in vasodilation (17), whereas PA smooth muscle cells isolated from lambs with PPHN after ductal ligation do not (18). Decreased PA NO synthase (NOS) function and activity in PPHN lambs (16,19,20) could also be a contributory factor. Lastly, this model of PPHN is associated with increased oxidative stress, even with fetal Po_2 levels (21). Ventilation with 100% O_2 would be expected to increase in alveolar oxygen tension at birth by 20- to 25-fold and promote further ROS formation to enhance vasoconstrictive effects on the neonatal pulmonary circulation (11,22,23). These effects of ROS on pulmonary circulation may be mediated by the formation of isoprostanes (24). In fact, we have previously shown that 24 h exposure to 100% O_2 significantly increases lung isoprostane levels in PPHN lambs (14).

The primary aim of PPHN therapy is selective pulmonary vasodilation (25). Inhaled NO is a rapid, potent, and selective pulmonary vasodilator that has been shown to reduce PVR in ductal ligation lambs and improve oxygenation and reduce ECMO use in human infants with PPHN (26–29). Although iNO significantly decreased PVR in all groups of lambs in this study, prior exposure to 100% O_2 decreased the magnitude of response to iNO (Fig. 6). We previously reported that resuscitation with 100% O_2 similarly blunted subsequent pulmonary vascular responses to iNO in normal lambs with acute pulmonary hypertension induced by pharmacological infusion of a thromboxane analog (5).

Why does prior exposure to 100% O_2 reduce the effectiveness of iNO? More than a decade ago, Sanderud *et al.* (30) suspected that ROS-mediated inactivation of NO contributes to pulmonary vasoconstriction. We speculate that exposure to 100% O_2 increases superoxide anions resulting in rapid inactivation of NO (31) through formation of peroxynitrite (14,24). We recently reported an increase in superoxide anion and peroxynitrite production after 24 h ventilation of lambs with PPHN with 100% O_2 and iNO (14,32). Hyperoxia and ROS also increase activity and expression of phosphodiesterase 5, inactivating the critical second messenger, cGMP (10). The role of ROS in pulmonary vasoconstriction is further established by the effectiveness of antioxidants in counteracting the effects of ROS on pulmonary vasculature (26,30,33).

The optimal FiO_2 and Pa_{O_2} during ventilation of neonates with PPHN are not known. Traditional management of PPHN included hyperoxic ventilation during the acute phase of PPHN to promote pulmonary vasodilation. However, we found that ventilation with 100% O_2 did not decrease PVR compared with 50% in control and PPHN lambs despite a significant increase in Pa_{O_2} (Fig. 6A). Rudolph and Yuan (34) first reported the relationship between Pa_{O_2} and PVR in young, healthy calves. They found that PVR rose steeply when the Pa_{O_2} fell below 45 to 50 mm Hg, but decreased minimally when the Pa_{O_2} was >50 mm Hg (34). We found a similar relationship between Pa_{O_2} and PVR in control lambs in this study, observing an acute increase in PVR with Pa_{O_2} $<52.5 \pm 1.7$ mm Hg (Fig. 6B). In PPHN lambs, severe hypoxemia with Pa_{O_2} below 13.9 ± 1.1 mm Hg resulted in a steep increase in PVR (Fig. 6B), and PVR decreased only minimally when the Pa_{O_2} rose above 59.6 ± 15.3 mm Hg. This demonstrates that, except in the presence of extreme hypoxemia, the remodeled pulmonary vasculature in PPHN lambs is not responsive to increased FiO_2 or Pa_{O_2}. This may account for the similar decrease in PVR with 21%, 50%, and 100% O_2 ventilation at birth in PPHN lambs (Fig. 3) in contrast with our previous study in control lambs (5). Pa_{O_2} exceeding 60 mm Hg (corresponding to an oxygen saturation of 90%) resulted in very little further decrease in PVR in control and PPHN lambs (Fig. 6). These findings are similar to previous clinical observations in children with bronchopulmonary dysplasia and pulmonary hypertension (35).

We acknowledge several limitations for this study. First, the ductal ligation model represents idiopathic PPHN associated with remodeled pulmonary vasculature. The findings in this study may not be applicable to all forms of pulmonary hypertension. For instance, while right to left shunting is common in human infants with PPHN, ductal occlusion prevents right to left shunt at this level and may contribute to increase right ventricular afterload. Factors known to increase pulmonary vascular sensitivity to oxygen such as hypercarbia and acidosis (34) are often associated with PPHN with acute asphyxia (36) but are not present in our model. However, other studies in asphyxiated piglets have shown similar reductions in PVR with 21% and 100% O_2 resuscitation (37,38). The same set of PPHN lambs were used to study two inter-related questions about the effect of FiO_2 and PVR. We did not see any significant impact of oxygen concentration during resuscitation at birth (Fig. 3) on subsequent PVR response to oxygen after stabilization (Fig. 6). However, the brief period of exposure to iNO might have influenced these results. Moreover, our study design did not allow us to perform mechanistic studies to explain our findings. Our main aim was to determine the physiologic and hemodynamic changes associated with early transition in the pulmonary circulation. These findings now provide the basis for future investigations into the vascular mechanisms underlying these responses.

We conclude that, even in a model of PPHN associated with remodeled pulmonary vasculature, initial resuscitation with 21% or 50% O_2 reduces PVR as effectively as 100% O_2. Exposure of PPHN lambs to initial resuscitation in 21% O_2 allowed a greater response later to iNO compared with 100% O_2. We speculate that infants with *in utero* pulmonary hypertension who are adequately ventilated and briefly resuscitated with 21% O_2 in the delivery room will likely respond with a

significant decrease in PVR without any impairment of subsequent pulmonary vasodilation to iNO. Finally, maintaining Pao_2 significantly above 60 mm Hg may not confer additional pulmonary vasodilation in the management of PPHN. Further clinical studies focused on the effect of varying FiO_2 on PVR in PPHN are warranted.

Acknowledgment. We thank Ikaria for providing iNO and Aeronox delivery systems.

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