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ORIGINAL RESEARCH

Optimal Oxygen Targets in Term Lambs with Meconium Aspiration Syndrome and Pulmonary Hypertension

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Abstract

Optimal oxygen saturation as measured by pulse oximetry (Sp_{O_2}) in neonatal lung injury, such as meconium aspiration syndrome (MAS) and persistent pulmonary hypertension of newborn (PPHN), is not known. Our goal was to determine the Sp_{O_2} range in lambs with MAS and PPHN that results in the highest brain oxygen delivery (bDO_2) and pulmonary blood flow (Qp) and the lowest pulmonary vascular resistance and oxidative stress. Meconium was instilled into endotracheal tubes in 25 near-term gestation lambs, and the umbilical cord was occluded to induce asphyxia and gasping, causing MAS and PPHN. Lambs were randomized into four groups and ventilated for 6 hours with fixed fraction of inspired oxygen (FI_{O_2}) = 1.0 irrespective of Sp_{O_2}, and three groups had FI_{O_2} titrated to keep preductal Sp_{O_2} between 85% and 89%, 90% and 94%, and 95% and 99%, respectively. Tissues were collected to measure nitric oxide synthase activity, 3-nitrotyrosine, and 8-isoprostanes. Throughout the 6-hour exposure period, lambs in the 95–99% Sp_{O_2} target group had the highest Qp, lowest pulmonary vascular resistance, and highest bDO_2 but were exposed to higher FI_{O_2} (0.5 ± 0.21 vs. 0.29 ± 0.17) with higher lung 3-nitrotyrosine (0.67 [interquartile range (IQR), 0.43–0.73] ng/mcg protein vs. 0.1 [IQR, 0.09–0.2] ng/mcg protein) and lower lung nitric oxide synthase activity (196 [IQR, 192–201] mMol nitrite/mg protein vs. 270 [IQR, 227–280] mMol nitrite/mg protein) compared with the 90–94% target group. Brain 3-nitrotyrosine was lower in the 85–89% target group, and brain/lung 8-isoprostane levels were not significantly different. In term lambs with MAS and PPHN, Qp and bDO_2 through the first 6 hours are higher with target Sp_{O_2} in the 95–99% range. However, the 90–94% target range is associated with significantly lower FI_{O_2} and lung oxidative stress. Clinical trials comparing the 90–94% versus the 95–99% Sp_{O_2} target range in term infants with PPHN are warranted.

Keywords: PPHN; lung injury; oxygen saturation

Clinical Relevance

Based on clinical trials, the optimal oxygen saturation target in preterm infants is considered to be 91–95%. However, the optimal target oxygen saturation (Sp_{O_2}) range for term neonates with lung disease and pulmonary hypertension is not known. In term newborn lambs with meconium aspiration and pulmonary hypertension, 95–99% target Sp_{O_2} results in higher pulmonary blood flow and brain oxygen delivery with lower lactate levels but is associated with higher FI_{O_2} and lung 3-nitrotyrosine compared with 90–94% target Sp_{O_2}.

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This article has a data supplement, which is accessible from this issue's table of contents at www.atsjournals.org.

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Table 1. Baseline Characteristics of the Lambs

Groups		85–89% Target Sp_{O_2}	90–94% Target Sp_{O_2}	95–99% Target Sp_{O_2}	Fixed $F_{I_{O_2}}$ 1.0	*P* Value
Sex, F/M		0/6	3/4	4/2	3/3	—
Multiplicity, single/twin/triplet		1/4/1	2/2/3*	4/2/0	1/5/0	—
Birth weight, kg		3.32 ± 0.26	3.63 ± 0.41	3.50 ± 0.41	3.29 ± 0.28	0.17
Gestational age, d		140 ± 1	140 ± 1	139 ± 1	140 ± 1	0.74
Time spent in the target Sp_{O_2} range, %		48 ± 9	55 ± 5	51 ± 10	n/a	0.69
Arterial pH	Baseline fetal	7.31 ± 0.02	7.27 ± 0.02	7.22 ± 0.02	7.26 ± 0.03	0.08
	Before resuscitation	7.00 ± 0.02	6.96 ± 0.04	6.96 ± 0.02	6.98 ± 0.02	0.23
	After 6 h ventilation	7.32 ± 0.03	7.27 ± 0.03	7.33 ± 0.03	7.38 ± 0.04	0.27
Base excess, mmol/L	Baseline fetal	1.4 ± 0.4	−2.4 ± 0.9†‡	−0.7 ± 0.9	1 ± 0.9	0.04
	Before resuscitation	−8.2 ± 1.2	−8.6 ± 1.3	−9.25 ± 0.5	−7.8 ± 0.7	0.89
	After 6 h ventilation	−5.6 ± 1.4	−4.9 ± 1	−2.8 ± 2.5	−1.5 ± 1	0.19
Hemoglobin, g/dl	Baseline fetal	13.3 ± 0.4	15.0 ± 0.6	15.3 ± 0.9	13.6 ± 0.6	0.11
	At 6 h	11.1 ± 0.7	12.6 ± 0.8	12.6 ± 0.9	11.4 ± 0.4	0.14
PRBC transfused	Lambs receiving PRBC, *n*	1/6	1/7	0/6	1/6	—
	Volume of PRBC, ml/kg	12.5	7	0	7.5	0.19
Fluid bolus given	Lambs receiving bolus, *n*	1/6	3/7	1/6	0/6	—
	Volume of bolus, ml/kg	25	10,20,20	10	0	0.21
Plasma lactate, mmol/L	Asphyxia	10.4 ± 1.7	10.7 ± 1.3	11.1 ± 0.7	10.4 ± 1.2	0.73
	At 2 h	7.8 ± 1	8.9 ± 1.8	8.6 ± 1.9	6.2 ± 1.4	0.1

Definition of abbreviations: $F_{I_{O_2}}$ = fraction of inspired oxygen; n/a = not applicable; PRBC = packed red blood cell; Sp_{O_2} = oxygen saturation.
*There were seven lambs in this group because the last set of enrolled lambs was a result of triplet gestation.
†Significantly different from the 85–89% target group.
‡Significantly different from the fixed $F_{I_{O_2}}$ = 1.0 group.

Supplemental oxygen plays an important role in neonatal ICUs (NICUs) in correcting hypoxemia associated with lung disease. The current Neonatal Resuscitation Program guidelines recommend initiating resuscitation with 21% oxygen and titrating fraction of inspired oxygen ($F_{I_{O_2}}$) to achieve 85–95% preductal oxygen saturation as measured by pulse oximetry (Sp_{O_2}) by 10 minutes after birth (1). We have previously shown that this approach is associated with optimal gas exchange and hemodynamics

Table 2. Gas Exchange (Oxygen) and Hemodynamic Parameters Based on Targeted Saturation Range between 15 Minutes and 6 Hours

Parameters	85–89% Target Sp_{O_2}*	90–94% Target Sp_{O_2}*	95–99% Target Sp_{O_2}*	Fixed $F_{I_{O_2}}$ 1.0*	*P* Value
Mean $F_{I_{O_2}}$	0.44 ± 0.24	0.29 ± 0.17	0.5 ± 0.21	1	0.003
$F_{I_{O_2}}$ at 6 h	0.53 ± 0.33	0.32 ± 0.2	0.68 ± 0.18	1	0.009
Preductal Pa_{O_2}, mm Hg	42 ± 13	56 ± 11	58 ± 19	167 ± 66	<0.0001
$Pa_{O_2}/F_{I_{O_2}}$ ratio, mm Hg	141 ± 93	199 ± 95†‡§	125 ± 50	167 ± 66	<0.0001
Pa_{CO_2}, mm Hg	39 ± 4	41 ± 3	40 ± 2	40 ± 4	0.11
Jugular venous PO_2, mm Hg	28.7 ± 6.7	40.3 ± 9.3†	34.9 ± 9.8	52.3 ± 20.7†‡	0.014
Left carotid artery flow/kg, ml/kg/min	19 ± 2	18 ± 3	21 ± 2	18 ± 3	0.27
Left atrial pressure, mm Hg	4.1 ± 1.9	5 ± 2.4	3.5 ± 1.2	5 ± 2.6	0.19
Mean systemic blood pressure, mm Hg	45 ± 4	55 ± 2†	52 ± 3†	48 ± 2	0.035
Pulmonary artery pressure, mm Hg	53 ± 16.3	66.1 ± 12.9	45.4 ± 11.3	54.7 ± 11.2	0.122
Mean pulmonary artery pressure/Mean systemic blood pressure	1.2 ± 0.3	1.2 ± 0.2	0.9 ± 0.2†	1.1 ± 0.1	0.04
Preductal–postductal Sp_{O_2} difference, median (IQR)	11 (2–25)‡§	4 (1–13)§	4 (1–8)§	14 (5–26)	0.001
Pulmonary vascular resistance (left lung), mm Hg/ml/kg/min	0.98 ± 1	0.95 ± 0.7	0.55 ± 0.15†‖	0.78 ± 0.4	0.006
Left pulmonary artery flow, ml/kg/min	54 ± 26	78 ± 51†§	86 ± 24†§	65 ± 15	0.0013
O_2 delivery brain, ml/kg/min	2.4 ± 0.7	2.8 ± 1	3.7 ± 2†	3 ± 1†	0.01
Brain O_2 extraction, ml/kg/min	0.26 ± 0.19	0.21 ± 0.13	0.36 ± 0.07†‖	0.32 ± 0.3†‖	0.0006
Lung O_2 uptake, ml/kg/min	0.7 ± 0.5	0.8 ± 1	2 ± 1.6†‖	1.1 ± 0.7†	<0.0001
Plasma lactate at 6 h, mmol/L	9.3 ± 1.6	9.4 ± 2.4	7 ± 2.3†‖	6.5 ± 3†‖	0.023

Definition of abbreviations: IQR = interquartile range; Pa_{CO_2} = arterial carbon dioxide pressure; Pa_{O_2} = arterial oxygen pressure.
*Average of values obtained every 15 min over the 5 h 45 min were included in the analysis.
†Significantly different from the 85–89% target group.
‡Significantly different from the 95–99% target group.
§Significantly different from the fixed $F_{I_{O_2}}$ = 1.0 group.
‖Significantly different from the 90–94% target group.

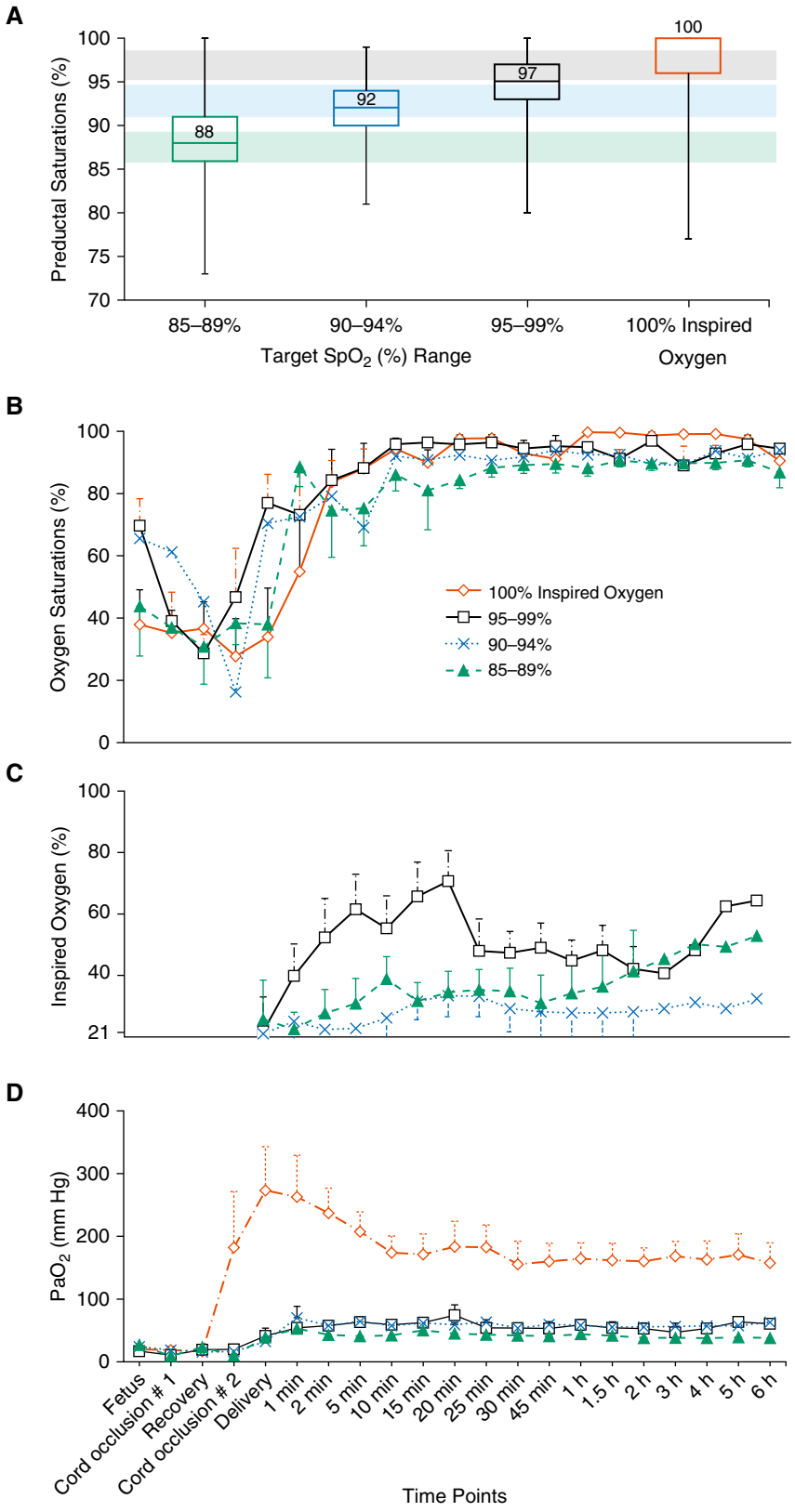

Figure 1. Oxygenation. (*A*) Box plot showing oxygen saturations achieved in the three target saturations groups and fixed 100% inspired oxygen from 15 minutes to 6 hours of postnatal age. Horizontal colored bars reflect the desired target oxygen saturation (Sp_{O_2}) range. There was overlap in

in asphyxiated lambs with meconium aspiration syndrome (MAS) (2). However, there are wide variations in preferred Sp_{O_2} targets for optimal oxygenation during the postresuscitation phase in hypoxic term neonates with persistent pulmonary hypertension of the newborn (PPHN) in the NICU (3, 4). A U.S. survey of practicing neonatologists showed that 70% preferred Sp_{O_2} in the ⩾95% range while managing PPHN, and 6% of this cohort used an FI_{O_2} of 1.0 irrespective of Sp_{O_2} until they were confident that pulmonary vascular reactivity had stabilized (4). Another international survey noted the preferred target Sp_{O_2} to be 91–95% (55.8% of respondents), ⩾96% (37.7%), and 86–90% (6.5% of respondents) (3). The 2019 Consensus Statement of the European Pulmonary Vascular Disease Network (EPVDN) recommends a preductal Sp_{O_2} of 91–95% for term and preterm infants (5). The American Heart Association (AHA)/American Thoracic Society (ATS) guidelines recommend 92–95% Sp_{O_2} for expreterm infants with bronchopulmonary dysplasia and pulmonary hypertension (6).

The goals of supplemental oxygen in PPHN are to reduce pulmonary vascular resistance (PVR) and deliver adequate oxygen to the tissues (especially the brain) without causing oxygen toxicity. Newborn infants are vulnerable to oxidative stress because of immature antioxidant defense mechanisms (7). After resuscitation from perinatal asphyxia, term infants with parenchymal lung disease are at a high risk of hypoxic-ischemic encephalopathy (HIE) and PPHN (8). Hyperoxia in the immediate postnatal period is associated with a higher incidence of HIE in term infants (9). These data suggest that there is a narrow therapeutic window for supplemental oxygen during the management of PPHN. The 2019 European Consensus Guidelines for the management of respiratory distress syndrome recommend 90–94% Sp_{O_2} targets for preterm infants in need of supplemental oxygen (10). However, there are no clinical or translational studies evaluating optimal Sp_{O_2} targets in infants or animal models with PPHN (11). We hypothesized that targeting a preductal Sp_{O_2} of 90–94%, which is similar to the recommendations for preterm infants and term infants with PPHN, is associated with high pulmonary blood flow (Qp), low PVR, high brain oxygen delivery (bDO_2), and low lactate

and markers of oxidative stress in the plasma, lung, and the brain.

The objective of our study was to compare 85–89%, 90–94%, and 95–99% Sp_{O_2} target ranges together with fixed 100% inspired oxygen in a term ovine model of perinatal asphyxia (induced by umbilical cord occlusion) and lung disease (MAS and PPHN) and to determine the target Sp_{O_2} that optimizes gas exchange and hemodynamics with minimal oxidative stress.

Methods

This study was approved by the institutional animal care and use committee at State University of New York at Buffalo. A detailed description of methods is provided in the data supplement (Figure E1 in the data supplement). Briefly, time-dated pregnant ewes (139–142 d gestation; term 145 d) (Newlife Pastures) were sedated, intubated, and anesthetized with 2% isoflurane. Delivery and instrumentation of the lambs were conducted as previously described (12, 13). Fetal lambs were asphyxiated by umbilical cord occlusion, and meconium (5 ml/kg of 20% meconium suspended in ewe amniotic fluid) was instilled into their endotracheal tube as previously described (14). Two 5-minute episodes of umbilical cord occlusion (separated by a 10-minute recovery period) resulted in asphyxia and meconium aspiration during gasping (2, 14).

Twenty-five lambs were randomized before delivery into the following four groups using sealed envelopes: fixed FI_{O_2} of 1.0 irrespective of Sp_{O_2} (to mimic the clinical practice of not weaning FI_{O_2} until pulmonary vascular reactivity is "stable") (4) or variable FI_{O_2} titrated to achieve target preductal Sp_{O_2} (right forelimb) in the ranges of 85–89%, 90–94%, and 95–99%, respectively. Blood gases were obtained from the right carotid artery (preductal), right jugular venous bulb, pulmonary artery, and left atrium. Left carotid and left pulmonary blood flow were continuously measured using ultrasonic flow probes (Transonics). During the study, if the preductal Sp_{O_2} was outside the target range, inspired oxygen was adjusted by 5% each minute. Hypotension (defined as a mean blood pressure of less than 35 mm Hg) was treated with 5–10 ml/kg of fluid boluses. Lambs with anemia (defined as a hematocrit of less than 30%) were transfused with 2.5–5 ml/kg of fetal packed red blood cells collected from the placental end of the umbilical vein. Lambs were ventilated for 6 hours and then killed.

Oxygen uptake from the lungs was calculated as Qp × (systemic arterial oxygen content − pulmonary arterial oxygen content). bDO_2 was estimated by carotid blood flow (Qca) × systemic arterial oxygen content. Cerebral oxygen extraction was calculated by Qca × (systemic arterial oxygen content − jugular venous oxygen content). Values were collected every 15 minutes and averaged for the period from 15 minutes to 6 hours of postnatal life (23 measurements) for each lamb. Plasma lactate was measured at the end of asphyxia and at 2 hours and 6 hours.

Oxidative Stress Markers

3-nitrotyrosine (3-NT) was measured in the lung and brain tissue using an ELISA kit (ab116691; Abcam). Similarly, an OxiSelect 8-isoprostane F2α ELISA kit (Cell Biolabs, Inc) was used to measure 8-isoprostanes in the plasma, lung tissue, and brain tissue. Nitric oxide synthase (NOS) activity (Ultra-Sensitive Assay for Nitric Oxide Synthase; Oxford Biomedical Research) was measured in lung tissue.

Data Analysis and Statistics

See data supplement.

Results

Twenty-five lambs were asphyxiated and ventilated per protocol. Baseline characteristics, including weight, sex, gestational age, and hemoglobin, were similar between the groups (Table 1). All lambs had mixed respiratory and metabolic acidosis with elevated lactate after umbilical cord compression (Tables 1 and 2). None of the lambs received chest compressions or epinephrine, and they recovered from bradycardia with positive pressure ventilation. In the three groups in which FI_{O_2} was titrated to target Sp_{O_2} (85–89%, 90–94%, and 95–99%), the intended range was maintained during approximately half of the ventilated period (Table 1 and Figure 1A). The interquartile range (IQR) of the Sp_{O_2} values was similar to the target range in the 85–89% and 90–94% groups. However, the IQR of the 95–99% target group was lower than expected at 93–97%. Lambs ventilated with fixed FI_{O_2} of 1.0 spent 52 ± 30% time with an Sp_{O_2} of 100%. Data on packed red blood cell transfusion and fluid boluses are shown in Table 1. Baseline fetal arterial blood gases before thoracotomy were similar except for a higher base deficit (−2.4 ± 0.9 mM/L) in lambs targeted to the 90–94% saturation range. There was no difference in base deficit or plasma lactate after asphyxiation and before resuscitation. After 6 hours of ventilation, plasma lactate was significantly lower in the 95–99% target group and the fixed 100% oxygen group (Table 2).

Oxygenation

The desired separation in Sp_{O_2} was achieved ($P < 0.05$ between the groups), although there was significant overlap between the groups (Figure 1A). The median Sp_{O_2} values between 15 minutes and 6 hours among the three saturation targeted groups were as follows: 87%, 92%, 95%, and 100% in the 85–89%, 90–94%, and 95–99% target

Figure 1. (*Continued*). oxygen saturations between the three target Sp_{O_2} groups. However, the saturations achieved by the three target groups were different ($P < 0.0001$). In addition, the 100% fixed inspired oxygen group achieved significantly higher Sp_{O_2} compared with the 85–89% target ($P < 0.001$) and 90–94% target groups ($P = 0.0022$). There was no significant difference between the 95–99% target group and the fixed $FI_{O_2} = 1.0$ group. (*B*) Changes in preductal oxygen saturations during different time points in the lambs ventilated with inspired oxygen to target saturations in the 85–89% range (solid green triangle), the 90–94% range (blue cross), and the 95–99% range (open black square). The lambs ventilated with fixed 100% inspired oxygen are shown in open red diamonds. The three target saturation ranges were different by repeated-measures ANOVA ($P = 0.005$). (*C*) Inspired oxygen to maintain target saturations in the three Sp_{O_2} target groups. The inspired oxygen required to maintain saturations in the 95–99% target range was higher than that in the 90–94% ($P < 0.0001$) and 85–89% ranges ($P = 0.0017$) by repeated-measures ANOVA. (*D*) Partial pressure of oxygen in preductal (right carotid arterial) blood (Pa_{O_2}) in the four groups. Lambs ventilated with fixed 100% inspired oxygen had higher Pa_{O_2} than the three target saturation groups. Pa_{O_2} in the 85–89% group was lower than in the 90–94% ($P = 0.02$) and 95–99% groups ($P = 0.01$). There was no difference between the 90–94% and 95–99% target Sp_{O_2} groups.

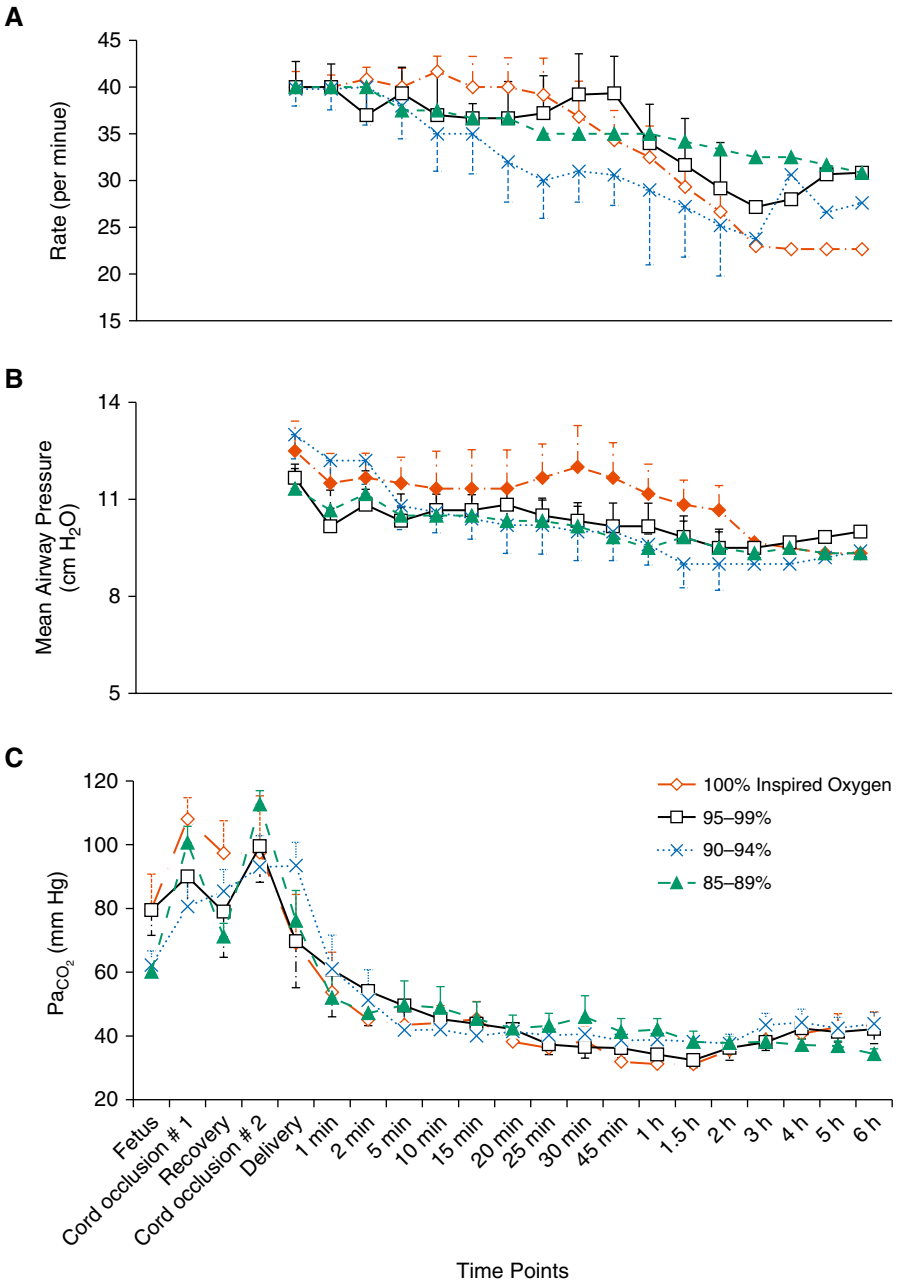

Figure 2. Ventilation parameters. (*A* and *B*) The changes in ventilator rate (*A*) and mean airway pressure (*B*) during 6 hours of postresuscitation phase are shown. (*C*) Changes in the Pa_{CO_2} during asphyxiation and 6 hours of postresuscitation phase. There were no significant differences between the four groups (100% fixed inspired oxygen [open red diamonds], 85–89% target Sp_{O_2} [solid green triangles], 90–94% target Sp_{O_2} [blue crosses], and 95–99% target Sp_{O_2} [open black squares]). Pa_{CO_2} = arterial carbon dioxide pressure.

groups and the $FI_{O_2} = 1$ group, respectively ($P < 0.0001$) (Figure 1A). Among the three groups of variable FI_{O_2}, the target Sp_{O_2} 95–99% group was associated with the highest average FI_{O_2} requirements (Figure 1C and Table 2). Interestingly, the lambs randomized to 90–94% received lower mean FI_{O_2} compared with lambs in the 85–89% target group. As expected, lambs ventilated with $FI_{O_2} = 1.0$ were associated with the highest arterial oxygen pressure (Pa_{O_2}) (Figure 1D and Table 2). Lambs randomized to the 85–89% target Sp_{O_2} range had the lower Pa_{O_2} than those randomized to the 90–94% and 95–99% target groups. Pa_{O_2}/FI_{O_2} ratios were significantly higher in the 90–94% Sp_{O_2} target group (Table 2).

Ventilation

Ventilator parameters were adjusted to target normocarbia (Pa_{CO_2} ~40 mm Hg). The ventilator rate (Figure 2A), mean airway pressures (Figure 2B), and Pa_{CO_2} (Figure 2C) were similar across the groups.

Systemic Hemodynamics

The lambs randomized to the Sp_{O_2} target range of 85–89% had a lower heart rate (151 ± 6 beats/min) (Figure 3A) and a lower mean blood pressure (45 ± 4 mm Hg) (Figure 3B) than the other groups. No significant differences were seen in Qca between the groups (Figure 3C).

Pulmonary Hemodynamics

Lambs randomized to the 85–89% target Sp_{O_2} group had lower Qp (54 ± 27 ml/kg) (Figure 4A) and higher PVR (Figure 4C) than lambs in the other groups. Pulmonary arterial pressures were similar between the groups (Figure 4B). However, smaller variations in pulmonary arterial pressures and systemic blood pressures resulted in significant differences in pulmonary to systemic arterial pressure ratios and preductal to postductal Sp_{O_2} gradients (Table 2). The lambs randomized to the 95–99% target Sp_{O_2} group and the fixed $FI_{O_2} = 1.0$ group had lower PVR compared with the 85–89% target and 90–94% target groups (Figure 4C). Surprisingly, the lambs randomized to the FI_{O_2}=1.0 group had lower Qp than the 95–99% target group, despite exposure to twofold higher FI_{O_2} and the achievement of a threefold higher Pa_{O_2}. The mean pulmonary arterial pressure to systemic blood pressure ratio was greater than 1 in all groups except for the 95–99% target group (Table 2).

Gas Exchange and Oxygen Delivery

Lambs in the 95–99% target Sp_{O_2} group had the highest O_2 uptake in the lung, brain O_2 delivery, and O_2 extraction by the brain (Table 2). Plasma lactate levels were similar in all groups at the end of asphyxia and at 2 hours (Table 1) butwere significantly lower in the 95–99% target Sp_{O_2} group and fixed $FI_{O_2} = 1$ group at 6 hours (Table 2).

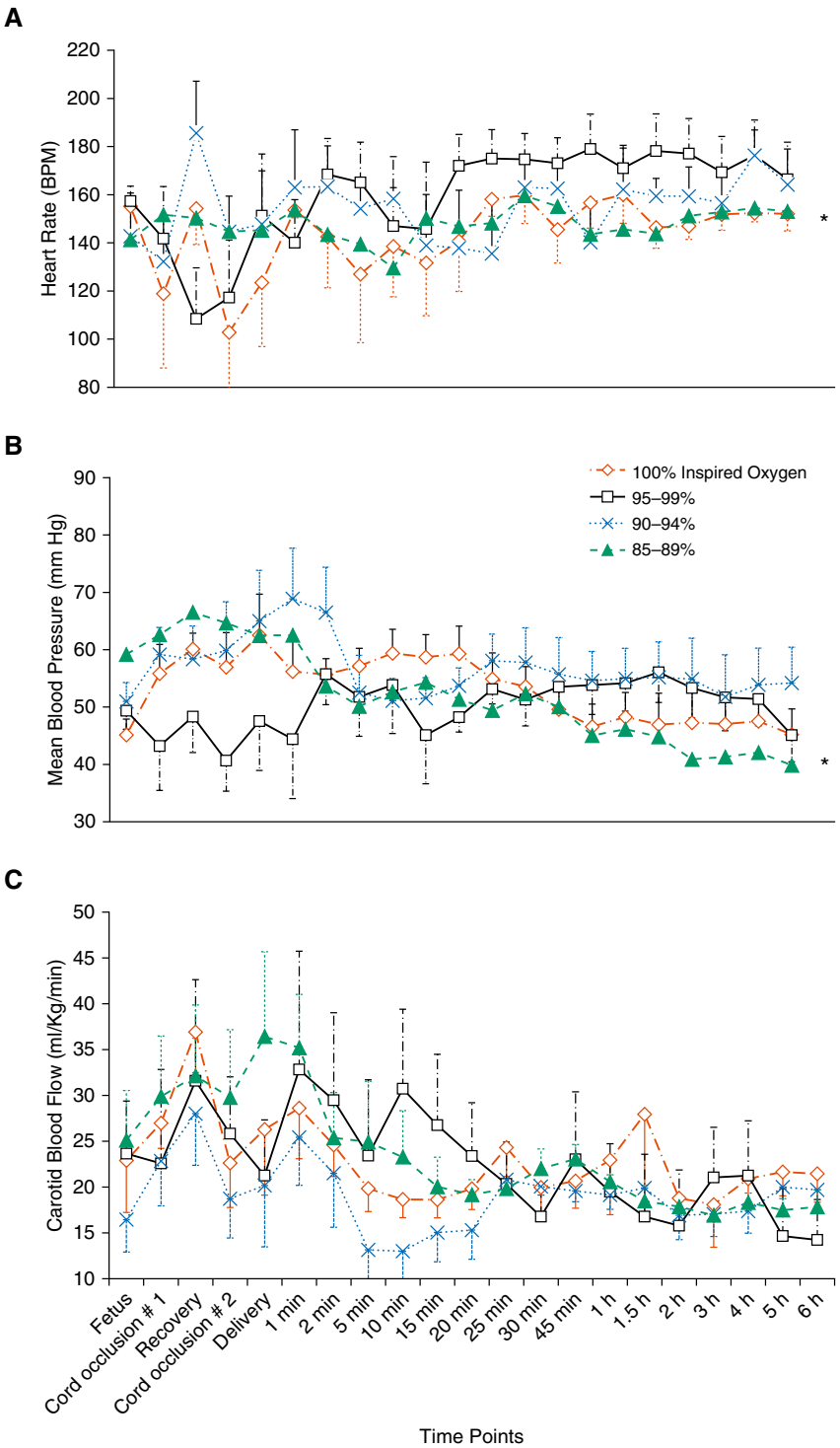

Figure 3. Systemic hemodynamics. (*A–C*) Changes in heart rate (*A*), mean systemic blood pressure (*B*), and left carotid blood flow (*C*) during asphyxiation and 6 hours of postresuscitation phase are shown. The lambs ventilated to maintain target oxygen saturation in the 85–89% range (solid green triangles) had lower heart rates than those in the 90–94% ($P = 0.03$) (blue crosses) and 95–99% ($P = 0.01$) (open black squares) groups. Similarly, lambs ventilated with fixed 100% inspired oxygen (open red diamonds) also had lower heart rates than 90–94% ($P = 0.03$) and 95–99% ($P = 0.008$) oxygen saturation groups. The mean blood pressure was lower in the 85–89% target group compared with the 90–94% ($P = 0.014$) and 95–99% ($P = 0.05$) target groups. There carotid artery blood flows (*C*) were similar between the groups. *Significantly different from the 90–94% and 95–99% target groups. BPM = beats per minute.

Oxidative Stress Markers

Table 3 shows oxidative stress markers in the lung, brain, and plasma. The lowest 3-NT levels were observed in lung tissue obtained from the lambs in the 90–94% target Sp_{O_2} group and in brain tissue in the 85–89% Sp_{O_2} target group. No statistical difference was seen in 8-isoprostanes in the lung, brain, and plasma.

Lung NOS Activity

NOS activity was significantly higher in lungs isolated from lambs in the 85–89% and 90–94% target Sp_{O_2} groups than those in the 95–99% Sp_{O_2} target group and the $FI_{O_2} = 1.0$ group (Table 3).

A summary of the results is shown in Figure E2.

Discussion

Hypoxemic respiratory failure with PPHN is observed in approximately 2 of 1,000 live births (15). However, 22–25% of term infants with birth asphyxia and moderate to severe HIE will develop PPHN (16). Optimal uptake of oxygen from the lungs and oxygenation to the brain without causing oxidative stress is crucial in the postresuscitation management of these infants. In this translational, randomized trial during the postresuscitation phase in a model of parenchymal lung disease and PPHN, we report that targeting 95–99% Sp_{O_2} (with an actual IQR Sp_{O_2} in the 93–97% range) resulted in low PVR and high bDO_2 and oxygen consumption but had higher 3-NT and lower NOS activity in the lung. In contrast, targeting 90–94% Sp_{O_2} (with an actual IQR Sp_{O_2} in the 90–94% range) was associated with a high Pa_{O_2}/FI_{O_2} ratio, low 3-NT, and high NOS activity in the lung but lower bDO_2 and higher PVR. Targeting 85–89% Sp_{O_2} led to low Qp and bDO_2, and a fixed 100% inspired oxygen was associated with supraphysiological Pa_{O_2} with low Qp and no additional improvement in bDO_2.

We speculate the following mechanistic explanation for our findings (Figure E2): The target Sp_{O_2} range of 85–89% is associated with low Pa_{O_2} below the threshold to induce pulmonary vasodilation (greater than 45 ± 0.1 mm Hg in this model) (17, 18). The low Qp and high PVR lead to a gradual increase in FI_{O_2} requirement compared with the 90–94% target group. In addition, the 85–89% target

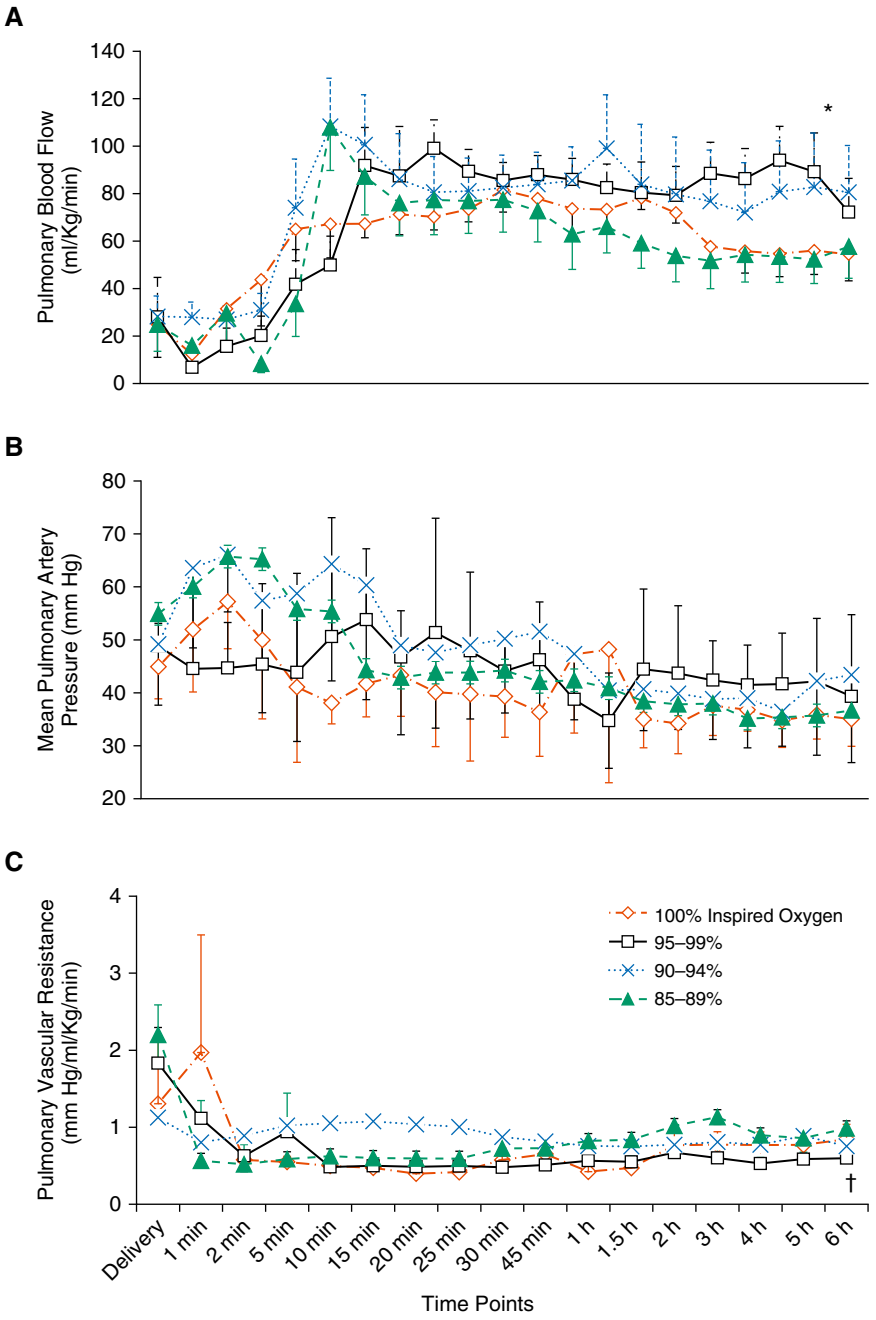

Figure 4. Pulmonary hemodynamics. (*A–C*) Changes in left pulmonary arterial blood flow (*A*), mean pulmonary arterial blood pressure (*B*), and left pulmonary vascular resistance (*C*) during asphyxiation and 6 hours of postresuscitation phase are shown. (*A*) Pulmonary blood flow was lower in the lambs randomized to the 85–89% (solid green triangles) target oxygen saturation range compared with those randomized to the 90–94% ($P = 0.0007$) (blue crosses) and 95–99% ($P = 0.0023$) (open black squares) ranges. Lambs ventilated with fixed 100% inspired oxygen (red diamonds) also had lower pulmonary blood flow than the 90–94% ($P = 0.012$) and 95–99% target groups ($P = 0.04$). (*B*) Mean pulmonary artery pressure was not significantly different between the groups. (*C*) Pulmonary vascular resistance was lower in the 95–99% target group compared with the 85–89% ($P = 0.05$) and 90–94% ($P = 0.05$) target groups. The pulmonary vascular resistance of the fixed 100% inspired oxygen group was lower than that of the 85–89% ($P = 0.05$) and 90–94% ($P = 0.04$) target groups. *Significantly different from the 90–94% and 95–99% target groups. †Significantly different from the 85–89% and 90–94% target groups.

group did not demonstrate reduced markers of oxidative stress in the brain, lung, or plasma compared with the 90–94% target group. These results suggest that a preductal Sp_{O_2} of less than 90% does not offer any distinct advantages at term gestation and is associated with high PVR and lower oxygen delivery to the brain. This finding supports the ATS/AHA and the European EPVDN guidelines (5) recommending Sp_{O_2} of greater than 90% during the management of pulmonary hypertension (6).

Some neonatologists prefer to use high FI_{O_2} (~1.0) during acute phase management of PPHN (4). In the current study, ventilation with an FI_{O_2} of 1.0 resulted in high Pa_{O_2} but did not result in increased oxygen delivery to the brain or induce additional pulmonary vasodilation compared with lambs with a target Sp_{O_2} of 95–99%. Rudolph and Yuan have previously shown that although Pa_{O_2} of less than 45 mm Hg results in hypoxic pulmonary vasoconstriction with elevated PVR in newborn calves, hyperoxia with Pa_{O_2} of more than 100 mm Hg does not cause additional pulmonary vasodilation (17). We have shown similar results in normal lambs, in lambs with PPHN induced by antenatal ductal ligation (12), and in lambs with meconium aspiration (18). Ventilation with 100% oxygen causes cerebral vasoconstriction (19–21) and is associated with increased incidence of HIE (22). In lambs, ventilation with 100% oxygen can induce increased pulmonary arterial contractility (13, 23). In the current study, the fixed 100% inspired oxygen group had lower Qp compared with the 90–94% and 95–99% target groups. In fact, the hemodynamic benefits observed in the 95–99% target group (subsystemic pulmonary arterial pressure, low PVR, and high bDO_2) were lost with hyperoxic ventilation with 100% oxygen. The use of 100% inspired oxygen did not minimize fluctuations in Sp_{O_2} (Figure 1A) and increased the preductal–postductal Sp_{O_2} difference. Our findings do not support the practice of continuous use of 100% inspired oxygen without titrating based on Sp_{O_2} during PPHN management and the support the recommendations of the ATS/AHA workgroup suggesting the limitation of FI_{O_2} and avoiding hyperoxic ventilation

Table 3. Oxidative Stress Markers Based on Targeted Oxygen Supplementation and Saturation Ranges

Markers	85–89% Target Sp_{O_2} [*Median (IQR)*]	90–94% Target Sp_{O_2} [*Median (IQR)*]	95–99% Target Sp_{O_2} [*Median (IQR)*]	Fixed $F_{I_{O_2}}$ 1 [*Median (IQR)*]	*P* Value
Lung 3-NT, ng/mcg protein	0.16 (0.1–0.17)*	0.10 (0.09–0.20)*	0.67 (0.43–0.73)	0.25 (0.21–0.33)	0.005
Brain 3-NT, ng/mcg protein	0.37 (0.3–0.5)*†	0.63 (0.36–0.88)	0.80 (0.6–1)	0.69 (0.60–0.80)	0.017
Lung 8-isoprostane, pg/ml	5,336 (1,360–5,962)	3,194 (547–5,848)	2,385 (1,631–2,403)	1,004 (821–1,186)	0.5
Brain 8-isoprostane, pg/ml	3,883 (3,198–10,266)	4,281 (3,646–4,915)	8,433 (3,084–8,851)	14,765 (8,829–21,135)	0.1
Plasma 8-isoprostane, pg/ml	463 (434–721)	1,249 (1,110–1,512)	1,277 (836–2,106)	725 (434–1,083)	0.4
Lung NOS, mmol nitrite/mg protein	263 (234–291)*†	270 (227–280)*†	196 (187–206)	207 (188–214)	0.001

Definition of abbreviations: 3-NT = 3-nitrotyrosine; NOS = nitric oxide synthase.
*Significantly different from the 95–99% target group.
†Significantly different from the fixed $F_{I_{O_2}} = 1.0$ group.

($F_{I_{O_2}} > 0.6$) if possible in term infants with PPHN (6).

What is the optimal choice between the 90–94% and 95–99% Sp_{O_2} target ranges during postresuscitation management of infants with PPHN? Based on our results and contrary to our hypothesis, at least in the short term, there are distinct, statistically significant benefits in pulmonary hemodynamics, gas exchange, and oxygen delivery by targeting 95–99% Sp_{O_2} compared with 90–94% Sp_{O_2}. Interestingly these differences were observed with similar preductal Pa_{O_2} (58 ± 19 mm Hg vs. 56 ± 11 mm Hg) and Sp_{O_2} (median, 92% vs. 95%) but with significantly higher $F_{I_{O_2}}$ exposure in the 95–99% target group compared with that in the 90–94% group throughout the study (0.5 ± 0.21 vs. 0.29 ± 0.1) and at 6 hours (0.68 ± 0.18 vs. 0.32 ± 0.2). We speculate that higher $F_{I_{O_2}}$ and alveolar Pa_{O_2} in the 95–99% target group might play a role in reducing pulmonary arterial pressure below systemic pressure and in limiting extrapulmonary right-to-left shunting, leading to optimal pulmonary and systemic hemodynamics associated with this target group. Because we failed to maintain lambs within the target 95–99% range, these hemodynamic benefits were associated with actual preductal Sp_{O_2} in the 93–97% range. However, a higher $F_{I_{O_2}}$ in this target group was associated with a significant rise in lung 3-NT (a pulmonary vasoconstrictor) and lower lung NOS activity (vasodilator). We acknowledge that a longer study (more than 6 h) might have demonstrated the negative consequences of this imbalance in vasoactive mediators on pulmonary circulation.

Targeting 90–94% Sp_{O_2} results in significantly low $F_{I_{O_2}}$ requirement (and highest $Pa_{O_2}/F_{I_{O_2}}$ ratios), with low 3-NT levels in the lung and preservation of lung NOS activity. We speculate that low oxidative stress and preservation of NOS in the lung may potentially be a lung-protective strategy and explain the improved survival of PPHN infants managed with gentle ventilation (24).

There are several limitations to this study. We believe our findings reflect a short-term impact (~6 h) of targeting these Sp_{O_2} ranges in a model of asphyxia and lung disease with PPHN. We were only able to maintain Sp_{O_2} in the assigned target range only about half of the duration of the study. Although a statistically different Sp_{O_2} was achieved in different groups, there was considerable overlap between the groups. Specifically, the adherence to the desired range in the 95–99% target group was low, with the IQR being 93–97%. The gas exchange and hemodynamic advantages observed with the 95–99% target range probably reflects preductal Sp_{O_2} in the mid-90s. We acknowledge that longer-term clinical studies with strict adherence to Sp_{O_2} targets (possibly by using automated $F_{I_{O_2}}$ control) may show different findings. In the clinical setting, targeting a narrow range of preductal Sp_{O_2} is difficult, similar to our experience. A wider range of Sp_{O_2} and the use of automated $F_{I_{O_2}}$ control may assist in tighter control of $F_{I_{O_2}}$ to limit fluctuations in Sp_{O_2}. Contrary to our expectations, oxidative stress markers in the lung were not significantly higher in the fixed $F_{I_{O_2}} = 1.0$ group compared with 95–99% target group. We attempted normocarbia by maintaining a Pa_{CO_2} of approximately 40 mm Hg. We did not evaluate the effect of permissive hypercapnia on gas exchange and hemodynamics. We did not perform histological analysis of lung tissue. Finally, many infants with PPHN are treated with inhaled nitric oxide. Evaluating the effects of pulmonary vasodilators such as nitric oxide at different Sp_{O_2} target ranges would be clinically important. Because the changes in pulmonary hemodynamics induced by inhaled nitric oxide makes the interpretation of the effects of oxygen difficult, we did not use pulmonary vasodilators in our study. PPHN from causes other than parenchymal lung injury and MAS may have different results.

This study has several strengths. The model closely mimics perinatal asphyxia and MAS, a leading cause of PPHN (25), especially in Africa and Asia (26). Unlike postnatal models of MAS conducted in 1–3 day old piglets created by meconium "pushed" by positive pressure (27, 28), this model uses negative pressure generated during gasping to induce aspiration to a fluid-filled fetal lung before a decrease in PVR at birth. In this model, ventilation–perfusion mismatch may play a predominant role in causing hypoxemia in addition to pulmonary hypertension. Frequent blood gases from the right carotid artery, pulmonary artery, left atrium, and jugular venous bulb enabled us to calculate oxygen uptake from the lung and extraction by the brain.

In conclusion, targeting saturations in the range of 95–99% was associated with low PVR, high Qp, oxygen delivery to

lung and brain, and the highest oxygen extraction by the brain. The target range of 85–89% is associated with high PVR, low Qp, and low oxygen delivery to the brain and cannot be recommended. Continuous ventilation with 100% oxygen did not offer any hemodynamic benefits compared with the 95–99% target group despite high Pa_{O_2} and $F_{I_{O_2}}$ exposure, and weaning $F_{I_{O_2}}$ is important during PPHN management. Although the preductal saturation target of low 90s, as recommended by the European Consensus Statement, results in low oxygen toxicity and increased pulmonary blood flow in our current study, it markedly limits oxygen extraction by the brain compared with that in the 95–99% target group (which reflects achieved preductal Sp_{O_2} of 93–97%). This study and several clinical trials on Sp_{O_2} targeting in preterm infants (29) reflect the difficulties in maintaining Sp_{O_2} in a narrow target range (30). Multicenter clinical trials evaluating the optimal preductal Sp_{O_2} target in term infants with PPHN are direly needed. Pending further clinical studies, it may be prudent to consider a wider range of preductal Sp_{O_2} in the low- to mid-90s (91–97%) in the management of suspected or established PPHN. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

References

1. Weiner GM, Zaichkin J, editors. Textbook of Neonatal Resuscitation (NRP), 7th ed. Elk Grove Village, IL: American Academy of Pediatrics; 2016.
2. Rawat M, Chandrasekharan PK, Swartz DD, Mathew B, Nair J, Gugino SF, *et al*. Neonatal resuscitation adhering to oxygen saturation guidelines in asphyxiated lambs with meconium aspiration. *Pediatr Res* 2016;79:583–588.
3. Nakwan N, Chaiwiriyawong P. An international survey on persistent pulmonary hypertension of the newborn: a need for an evidence-based management. *J Neonatal Perinatal Med* 2016;9:243–250.
4. Alapati D, Jassar R, Shaffer TH. Management of supplemental oxygen for infants with persistent pulmonary hypertension of newborn: a survey. *Am J Perinatol* 2017;34:276–282.
5. Hansmann G, Koestenberger M, Alastalo TP, Apitz C, Austin ED, Bonnet D, *et al*. 2019 updated consensus statement on the diagnosis and treatment of pediatric pulmonary hypertension: the European Pediatric Pulmonary Vascular Disease Network (EPPVDN), endorsed by AEPC, ESPR and ISHLT. *J Heart Lung Transplant* 2019;38:879–901.
6. Abman SH, Hansmann G, Archer SL, Ivy DD, Adatia I, Chung WK, *et al*.; American Heart Association Council on Cardiopulmonary, Critical Care, Perioperative and Resuscitation; Council on Clinical Cardiology; Council on Cardiovascular Disease in the Young; Council on Cardiovascular Radiology and Intervention; Council on Cardiovascular Surgery and Anesthesia; and the American Thoracic Society. Pediatric pulmonary hypertension: guidelines from the American Heart Association and American Thoracic Society. *Circulation* 2015;132:2037–2099.
7. Auten RL, Davis JM. Oxygen toxicity and reactive oxygen species: the devil is in the details. *Pediatr Res* 2009;66:121–127.
8. Singh BS, Clark RH, Powers RJ, Spitzer AR. Meconium aspiration syndrome remains a significant problem in the NICU: outcomes and treatment patterns in term neonates admitted for intensive care during a ten-year period. *J Perinatol* 2009;29:497–503.
9. Kapadia V, Wyckoff MH. Oxygen therapy in the delivery room: what is the right dose? *Clin Perinatol* 2018;45:293–306.
10. Sweet DG, Carnielli V, Greisen G, Hallman M, Ozek E, Te Pas A, *et al*. European consensus guidelines on the management of respiratory distress syndrome - 2019 update. *Neonatology* 2019;115:432–450.
11. Neff TA. Routine oximetry: a fifth vital sign? *Chest* 1988;94:227.
12. Lakshminrusimha S, Swartz DD, Gugino SF, Ma CX, Wynn KA, Ryan RM, *et al*. Oxygen concentration and pulmonary hemodynamics in newborn lambs with pulmonary hypertension. *Pediatr Res* 2009;66: 539–544.
13. Lakshminrusimha S, Russell JA, Steinhorn RH, Swartz DD, Ryan RM, Gugino SF, *et al*. Pulmonary hemodynamics in neonatal lambs resuscitated with 21%, 50%, and 100% oxygen. *Pediatr Res* 2007; 62:313–318.
14. Lakshminrusimha S, Mathew B, Nair J, Gugino SF, Koenigsknecht C, Rawat M, *et al*. Tracheal suctioning improves gas exchange but not hemodynamics in asphyxiated lambs with meconium aspiration. *Pediatr Res* 2015;77:347–355.
15. Walsh-Sukys MC, Tyson JE, Wright LL, Bauer CR, Korones SB, Stevenson DK, *et al*. Persistent pulmonary hypertension of the newborn in the era before nitric oxide: practice variation and outcomes. *Pediatrics* 2000;105:14–20.
16. Lakshminrusimha S, Shankaran S, Laptook A, McDonald S, Keszler M, Van Meurs K, *et al*. Pulmonary hypertension associated with hypoxic-ischemic encephalopathy-antecedent characteristics and comorbidities. *J Pediatr* 2018;196:45–51.e3.
17. Rudolph AM, Yuan S. Response of the pulmonary vasculature to hypoxia and H+ ion concentration changes. *J Clin Invest* 1966;45:399–411.
18. Lakshminrusimha S, Konduri GG, Steinhorn RH. Considerations in the management of hypoxemic respiratory failure and persistent pulmonary hypertension in term and late preterm neonates. *J Perinatol* 2016;36:S12–S19.
19. Rosenberg AA, Murdaugh E, White CW. The role of oxygen free radicals in postasphyxia cerebral hypoperfusion in newborn lambs. *Pediatr Res* 1989;26:215–219.
20. Watson NA, Beards SC, Altaf N, Kassner A, Jackson A. The effect of hyperoxia on cerebral blood flow: a study in healthy volunteers using magnetic resonance phase-contrast angiography. *Eur J Anaesthesiol* 2000;17:152–159.
21. Floyd TF, Clark JM, Gelfand R, Detre JA, Ratcliffe S, Guvakov D, *et al*. Independent cerebral vasoconstrictive effects of hyperoxia and accompanying arterial hypocapnia at 1 ATA. *J Appl Physiol (1985)* 2003;95:2453–2461.
22. Kapadia VS, Chalak LF, DuPont TL, Rollins NK, Brion LP, Wyckoff MH. Perinatal asphyxia with hyperoxemia within the first hour of life is associated with moderate to severe hypoxic-ischemic encephalopathy. *J Pediatr* 2013;163:949–954.
23. Lakshminrusimha S, Steinhorn RH, Wedgwood S, Savorgnan F, Nair J, Mathew B, *et al*. Pulmonary hemodynamics and vascular reactivity in asphyxiated term lambs resuscitated with 21 and 100% oxygen. *J Appl Physiol (1985)* 2011;111:1441–1447.
24. Gupta A, Rastogi S, Sahni R, Bhutada A, Bateman D, Rastogi D, *et al*. Inhaled nitric oxide and gentle ventilation in the treatment of pulmonary hypertension of the newborn: a single-center, 5-year experience. *J Perinatol* 2002;22:435–441.
25. Steurer MA, Baer RJ, Oltman S, Ryckman KK, Feuer SK, Rogers E, *et al*. Morbidity of persistent pulmonary hypertension of the newborn in the first year of life. *J Pediatr* 2019;213:58–65, e4.
26. Nakwan N, Jain S, Kumar K, Hosono S, Hammoud M, Elsayed YY, *et al*. An Asian multicenter retrospective study on persistent pulmonary hypertension of the newborn: incidence, etiology, diagnosis, treatment and outcome. *J Matern Fetal Neonatal Med* 2020;33:2032–2037.
27. Wiswell TE, Peabody SS, Davis JM, Slayter MV, Bent RC, Merritt TA. Surfactant therapy and high-frequency jet ventilation in the management of a piglet model of the meconium aspiration syndrome. *Pediatr Res* 1994;36:494–500.
28. Wiswell TE, Foster NH, Slayter MV, Hachey WE. Management of a piglet model of the meconium aspiration syndrome with high-frequency or conventional ventilation. *Am J Dis Child* 1992;146:1287–1293.
29. Askie LM, Darlow BA, Finer N, Schmidt B, Stenson B, Tarnow-Mordi W, *et al*.; Neonatal Oxygenation Prospective Meta-analysis (NeOProM) Collaboration. Association between oxygen saturation targeting and death or disability in extremely preterm infants in the neonatal oxygenation prospective meta-analysis collaboration. *JAMA* 2018;319:2190–2201.
30. Lakshminrusimha S, Manja V, Mathew B, Suresh GK. Oxygen targeting in preterm infants: a physiological interpretation. *J Perinatol* 2015;35: 8–15.