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Inhaled Nitric Oxide Improves Gas Exchange and Lowers Pulmonary Vascular Resistance in Severe Experimental Hyaline Membrane Disease

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ABSTRACT

To determine the effects of inhaled nitric oxide (NO) on pulmonary hemodynamics and gas exchange in experimental hyaline membrane disease (HMD), we studied 16 premature lambs (0.78 term) in two separate protocols. All animals were treated with exogenous surfactant before mechanical ventilation. In protocol 1, we measured the acute response to brief treatment with inhaled NO (20 ppm, 20 min) after 2 h of mechanical ventilation with fraction of inspired oxygen of 1.00 (n = 5). After 2 h, brief NO treatment lowered pulmonary vascular resistance from 0.26 ± 0.05 to 0.16 ± 0.03 mm Hg · (mL/min)$^{-1}$ ($p < 0.01$) and improved gas exchange (arterial Po_2, 44 ± 9 mm Hg baseline to 168 ± 45 mm Hg NO, $p < 0.01$; arterial Pco_2 45 ± 5 mm Hg baseline to 35 ± 4 mm Hg NO, $p < 0.05$). In protocol 2, to determine whether early and continuous treatment with inhaled NO could sustain improvement in gas exchange and pulmonary hemodynamics in severe HMD, we compared the physiologic effects of ventilation with high inspired oxygen concentrations for 3 h with NO (20 ppm, n = 6) and without NO (controls, n = 5). After 3 h, the NO treatment group had sustained reduction in pulmonary vascular resistance (0.10 ± 0.01 mm Hg · (mL/min)$^{-1}$ NO *versus* 0.25 ± 0.04 mm Hg · (mL/min)$^{-1}$ control, $p < 0.05$), increased left pulmonary artery blood flow (204 ± 24 mL/min NO *versus* 109 ± 15 mL/min control, $p < 0.05$), and increased arterial Po_2 (114 ± 27 mm Hg NO *versus* 36 ± 11 mm Hg control, $p < 0.05$). We conclude that inhaled NO causes marked and sustained improvement in pulmonary hemodynamics and gas exchange in severe experimental HMD. We speculate that pulmonary vasoconstriction and ventilation/perfusion abnormalities contribute to the pathophysiology of severe respiratory failure in preterm newborns, and early treatment with inhaled NO may be effective in clinical management of selected premature patients with HMD. (***Pediatr Res* 36: 402–408, 1994**)

Abbreviations

NO, nitric oxide
HMD, hyaline membrane disease
PVR, pulmonary vascular resistance
Q_{LPA}, left pulmonary artery blood flow
PIP, peak inspiratory pressure
PEEP, positive end expiratory pressure
FiO_2, fraction of inspired oxygen
Pao_2, arterial partial pressure of oxygen
$Paco_2$, arterial partial pressure of carbon dioxide

Previous studies have demonstrated the vital role of endogenous NO production in decreasing PVR during the transition of the pulmonary circulation at birth in the near-term ovine fetus (1), the potent pulmonary vasodilator effects of inhaled NO in the late-gestation and newborn lamb (2–4), and the potential role of inhaled NO in the treatment of term human neonates with persistent pulmonary hypertension of the newborn (5–8). However, most of these studies examined near-term and term subjects. Mechanisms of pulmonary vasoregulation in the premature fetus and postnatal pulmonary adaptation in the premature newborn have received little attention.

After premature birth, severe respiratory failure is often the result of surfactant deficiency, and treatment with exogenous surfactant can cause dramatic improvements in oxygenation. However, exogenous surfactant therapy results in suboptimal responses in up to 50% of human newborns thought to have HMD (9), suggesting that other problems of prematurity (*e.g.* structural lung immaturity or altered vascular responses to dilator stimuli) or complications of standard interventions contribute to respiratory failure.

We have recently reported that endogenous NO production modulates basal pulmonary vascular tone as early as 78% of term gestation and contributes to the increase in pulmonary blood flow after delivery in extreme

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prematurity (10). In addition, inhaled NO caused marked increases in pulmonary blood flow after NO synthase inhibition, demonstrating marked responsiveness of the vascular smooth muscle cell to NO very early in gestation. Whether pulmonary vasoregulation in the premature subject is altered during prolonged mechanical ventilation with high inspired oxygen concentrations is unknown.

The premature lamb has been extensively studied as a model of severe HMD (11). Survival with exogenous surfactant treatment and mechanical ventilation at delivery varies depending on the gestational age of the lamb and the type of surfactant given (12, 13). We have observed worsening gas exchange and increased PVR in premature lambs delivered at 115 d gestation (0.78 term) when mechanical ventilation was continued beyond 60–90 min after birth, despite treatment with exogenous surfactant at delivery. We hypothesized that exogenous (inhaled) NO could reverse the disturbances in gas exchange and pulmonary hemodynamics associated with prolonged mechanical ventilation of the extremely immature lung. To test these hypotheses, we delivered ovine fetuses at 78% of term gestation in two separate protocols to measure responses to acute and sustained NO inhalation during mechanical ventilation.

METHODS

Mixed-breed (Columbia-Rambouillet) pregnant ewes were used in this study. All procedures and protocols were reviewed and approved by the Animal Care and Use Committee at the University of Colorado Health Sciences Center.

This study includes two separate protocols that examine the effects of inhaled NO on pulmonary hemodynamics and gas exchange in this premature (115 d gestation, 0.78 term; term = 147 d) ovine model of severe HMD. In protocol 1, we studied the effects of brief treatment with inhaled NO after 2 h of mechanical ventilation (n = 5 animals). In protocol 2, we measured the effects of inhaled NO therapy initiated after delivery on pulmonary hemodynamics and gas exchange over a 3-h period of mechanical ventilation (n = 6 animals), compared with controls (n = 5 animals).

Surgical Preparation

The following methods have been previously described (2, 14). Ewes were sedated with i.v. pentobarbital sodium (2–4 g total dose) and anesthetized with intrathecal tetracaine hydrochloride (1% solution, 3 mg). Under sterile conditions, a uterine incision was made and the left forelimb of the fetal lamb was delivered. A skin incision was made in the axilla of the left fetal forelimb after local infiltration with lidocaine (1% solution, 2–3 mL). Polyvinyl catheters (20 gauge; Martech Medical Products, Lansdale, PA) were advanced into the ascending aorta through the axillary artery and into the superior vena cava through the axillary vein. A left thoracotomy was performed, exposing the heart and great vessels. A catheter was inserted into the main pulmonary artery by direct puncture through purse-string sutures. This catheter was guided into position with a 14-gauge i.v. placement unit (Angiocath; Travenol, Deerfield, IL), and secured by tightening the purse-string suture as the introducer was withdrawn. The main pulmonary artery catheter was inserted between the ductus arteriosus and the pulmonic valve. A left atrial catheter was inserted in the medial portion of the left atrial appendage. An ultrasonic flow transducer (6 mm, Transonic Systems Inc., Ithaca, NY) was placed around the left pulmonary artery to measure blood flow to the left lung.

Study Design—General

After stabilization of physiologic parameters, pancuronium was administered to the fetus (0.1 mg/kg), the fetal head was exteriorized, and a tracheotomy was performed with placement of an endotracheal tube (3.0 mm inner diameter). All animals were treated with exogenous surfactant (Infasurf, kindly provided by E. A. Egan, M.D.) at an estimated dose of 3 mL/kg (105 mg phospholipid/kg) before the first breath. Mechanical ventilation was initiated with a continuous-flow, time-cycled, pressure-limited, neonatal ventilator at the following settings: PIP, 35 cm H_2O (3.4 kPa); PEEP, 6 cm H_2O (0.6 kPa); rate, 30 breaths per minute; inspiratory time, 1.0 s; and FiO_2, 1.00. After 30 min of mechanical ventilation, the umbilical cord was ligated and a 5% dextrose solution was infused to provide 10 mL/h crystalloid and 1 mg/kg/h pentobarbital.

Mechanical ventilator settings were modified during the course of studies based on results of postductal arterial blood gas samples. Changes in PIP were determined by measurements of $Paco_2$. If $Paco_2$ was < 35 mm Hg (4.7 kPa), then the PIP was reduced to 30 cm H_2O (2.9 kPa). If subsequent measurements of $Paco_2$ were < 35 mm Hg (4.7 kPa), then the PIP was reduced to 25 cm H_2O (2.5 kPa). The maximum PIP delivered was 35 cm H_2O (3.4 kPa). If $Paco_2$ was greater than 45 mm Hg (6 kPa), then the ventilator rate was increased to a maximum of 60 breaths/min. The inspiratory time was then decreased to maintain an inspiratory to expiratory ratio of ≤1.0. PEEP was changed according to Pao_2. If Pao_2 was <100 mm Hg (13.3 kPa), PEEP was maintained at 6 cm H_2O (0.6 kPa). With Pao_2 > 100 mm Hg (13.3 kPa), PEEP was decreased to 5 cm H_2O (0.5 kPa). If Pao_2 was > 200 mm Hg (26.7 kPa), PEEP was decreased to 4 cm H_2O (0.4 kPa). The management strategy was guided by target $Paco_2$ and Pao_2 values, with guidelines for ventilator adjustment as described above. We did not attempt to correct metabolic acidemia with infusions of base in these experiments. Main pulmonary artery, aortic, and left atrial catheters were connected to a Gould-Statham P23 ID pressure transducer (Gould, Inc., Oxnard, CA). Calibrations of pressure transducers were performed with a mercury column manometer. Q_{LPA} and heart rate were measured using the ultrasonic flow transducer. PVR was calculated

as (pulmonary artery pressure − left atrial pressure)/ Q_{LPA} in mm Hg · (mL/min)$^{-1}$. Blood samples for pH, Po_2, Pco_2, percent oxygen saturation, and percent methemoglobin were withdrawn anaerobically into Natelson glass pipettes and analyzed at 39.5°C using a Radiometer OSM3 blood gas analyzer (Copenhagen, Denmark). Blood Hb concentration and oxyhemoglobin saturation were measured colorimetrically in duplicate using a hemoximeter (Radiometer).

The NO gas (Airco, Riverton, NJ) used in these experiments was certified at a concentration of 450 ppm NO (chemiluminescence method) with less than 1% contamination by other oxides of nitrogen. The NO tank was specially equipped with a stainless steel regulator and low flow meter for delivering finely gauged flow rates. NO was introduced into the afferent limb of the ventilator circuit through a ¼ × ¼-inch luer adaptor fitted within 1 m of the endotracheal tube, thus mixing with the fixed flow rate of circuit gas (10 L/min). The rate of NO flow was changed to yield an inspired NO concentration of 20 ppm. The resulting concentrations of inhaled NO were verified using chemiluminescence (Thermo Environmental Instruments, model 14A, Franklin, MA) or an electrochemical sensor calibrated against a reference NO tank (Sensorstik, Exidyne Instruments, Philadelphia, PA).

Experimental Design

Protocol 1. *Effects of brief treatment with inhaled NO on pulmonary hemodynamics and gas exchange after 2 h of ventilation in the preterm lamb (n = 5, weight = 1.73 ± 0.11 kg).* After at least 1 h of recovery and stabilization of physiologic parameters, baseline hemodynamic and arterial blood gas measurements were recorded. An endotracheal tube was placed by tracheostomy and surfactant was instilled before the first mechanical ventilator breath. Mechanical ventilation was initiated (as described above); after 2 h, inhaled NO (20 ppm) was delivered for 20 min. Hemodynamic and arterial blood gas measurements were repeated and the NO was then discontinued. Mechanical ventilator settings were kept constant immediately before, during, and after NO treatment.

Protocol 2. *Effects of early inhaled NO treatment on pulmonary hemodynamics and gas exchange during 3 h of mechanical ventilation (NO treatment group, n = 6; controls, n = 5).* After recovery from surgery, baseline hemodynamic and arterial blood gas measurements were recorded. An endotracheal tube was placed, surfactant instilled, and mechanical ventilation initiated (as described above). In the NO treatment group, inhaled NO (20 ppm) was administered throughout the 3-h study period. All animals were treated with FiO_2 = 1.00 (due to blending of the NO mixture with inspired gases, measured FiO_2 for the NO treatment group was 0.94).

After the study, animals were killed with T-61 euthanasia solution (American Hoechst, Somerville, NJ). Fetal weight was recorded and appropriate catheter placement was verified.

Statistical Analysis

Statistical comparisons of continuous variables in protocol 1 were performed using one-way repeated measures analysis of variance. Where significant differences were identified, post-hoc analysis was performed using Fisher's least significant difference test. The statistical analysis of differences between the NO treatment group and control group in protocol 2 was performed using two-way analysis of variance for repeated measures (with time and treatment group identified as independent variables and the interaction between them analyzed). Comparisons of responses to each intervention (between treatment groups) at the 3-h time point in protocol 2 were performed using the *t* test. The level of statistical significance was set at $p < 0.05$; results are reported as mean ± SEM.

RESULTS

Protocol 1

Effects of brief treatment with inhaled NO on pulmonary hemodynamics and gas exchange after 2 h of ventilation in the preterm lamb (n = 5). Mechanical ventilation after surfactant treatment with FiO_2 = 1.00 increased Pao_2 9.6-fold from baseline. However, despite continued ventilation, Pao_2 progressively decreased over the subsequent 2-h study period from 241 ± 65 to 44 ± 9 mm Hg (32.1 ± 8.7 to 5.9 ± 1.2 kPa; $p < 0.01$). Brief treatment with inhaled NO (20 ppm) abruptly decreased $Paco_2$ by 22% (Table 1), increased Pao_2 (Fig. 1*A*), and decreased PVR by 39% (Fig. 1*B*). After discontinuing NO inhalation, gas exchange and hemodynamic parameters returned to baseline over 10 min (Fig. 1). Heart rate at baseline was 204 ± 6 bpm and did not significantly change over the study period. Systemic arterial pressure was 47 ± 3 mm Hg (6.3 ± 0.4 kPa) at baseline; measurements were lower than baseline after 30 min of ventilation (37 ± 4 mm Hg, 4.9 ± 0.5 kPa; $p < 0.05$) and after the 20-min NO treatment period (37 ± 4 mm Hg, 4.9 ± 0.5 kPa; $p < 0.05$). Left atrial pressure was higher than baseline measurements (2.2 ± 0.2 mm Hg, 0.3 ± 0.03 kPa) at 30 min of ventilation (5.6 ± 1.4 mm Hg, 0.8 ± 0.19 kPa; $p < 0.05$) and at 60 min of ventilation (3.6 ± 0.5 mm Hg, 0.5 ± 0.07 kPa; $p < 0.05$); however, after 60 min of ventilation, left

Table 1. *Protocol 1: arterial blood gas data for 2-h study**

Study period	pH	$PaCO_2$ (kPa)
Baseline	7.25 ± 0.03	6.1 ± 0.3
30 min	7.36 ± 0.03†	3.7 ± 0.5
60 min	7.28 ± 0.04	4.4 ± 0.8
90 min	7.15 ± 0.02†	6.0 ± 0.7
120 min	7.13 ± 0.02	6.0 ± 0.7
140 min (NO 20 ppm × 20 min)	7.17 ± 0.03	4.7 ± 0.5‡
150 min (off NO)	7.11 ± 0.03	5.6 ± 0.7

* Acute response to inhaled NO after 2 h of ventilation and blood gas data after withdrawing NO are also shown. mm Hg = kPa · 7.5.
† $p < 0.01$ *vs* previous study period.
‡ $p < 0.05$ *vs* previous study period.

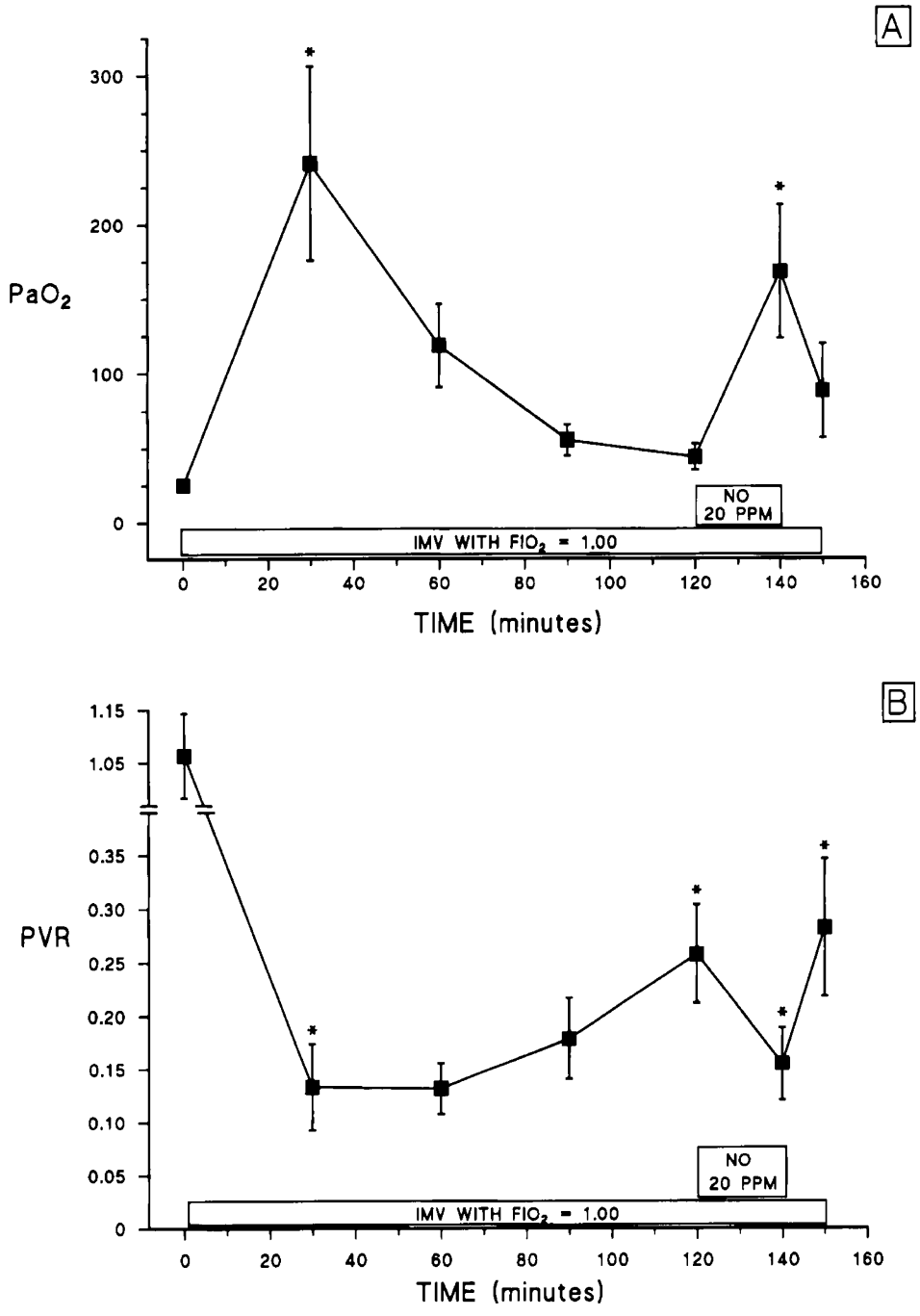

Figure 1. *A*, Pao_2 (mm Hg) for the 2-h ventilation group in protocol 1, showing progressive decline in oxygenation over the 2-h study period, with a marked increase in Pao_2 in response to low-dose inhaled NO at the 2-h time point. Pao_2 returns to baseline after NO is discontinued. *, $p < 0.01$ *vs* 120-min time point; kPa = mm Hg/7.5. *B*, PVR [mm Hg · $(mL/min)^{-1}$] for the 2-h ventilation group in protocol 1, showing progressive increase in PVR over the 2-h intermittent mandatory ventilation period, with a marked decrease in PVR in response to low-dose inhaled NO at the 2-h time point. PVR returns to baseline after NO is discontinued. *, $p < 0.01$ *vs* 120-min time point.

atrial pressure was not different from baseline. PIP was decreased from 35 cm H_2O (3.4 kPa) to 30 ± 2 cm H_2O (2.9 ± 0.2 kPa) by 60 min of ventilation in response to decreased $Paco_2$, and PEEP was decreased from 6 cm H_2O (0.6 kPa) to 4.8 ± 0.5 cm H_2O (0.5 ± 0.05 kPa) by 60 min of ventilation in response to increased Pao_2. There were no significant differences in ventilator rate or mean airway pressure during the study period.

Protocol 2

Effects of early inhaled NO treatment on pulmonary hemodynamics and gas exchange during 3 h of mechanical ventilation (NO treatment group, n = 6; controls, n = 5). Baseline arterial blood gas and hemodynamic measurements in treatment and Control animals were similar (Table 2). However, after 3 h of ventilation, Pao_2 was

Table 2. *Protocol 2: arterial blood gas data for 3-h continuous NO treatment in control group (n = 5) vs NO treatment group (n = 6)**

Study period	Study group	pH	$PaCO_2$ (kPa)	PaO_2 (kPa)
Baseline	Control	7.26 ± 0.02	6.0 ± 0.3	3.1 ± 0.3
	NO treatment	7.24 ± 0.02	6.3 ± 0.4	3.1 ± 0.3
60 min	Control	7.24 ± 0.04	5.7 ± 0.5	12.3 ± 3.7
	NO treatment	7.28 ± 0.05	3.7 ± 0.4†	20.8 ± 4.0
120 min	Control	7.15 ± 0.04	6.1 ± 0.9	10 ± 5.3
	NO treatment	7.12 ± 0.05	5.7 ± 0.5	11.7 ± 3.1
180 min	Control	7.06 ± 0.08	8.5 ± 1.5	4.8 ± 1.5
	NO treatment	7.06 ± 0.06	6.4 ± 0.3	15.2 ± 3.6†

* mm Hg = kPa · 7.5.
† $p < 0.05$ *vs* control group.

3.2-fold higher with NO treatment than in the control group, despite similar ventilator settings (Table 3). NO treatment attenuated the decline in Q_{LPA} and the increase in PVR associated with prolonged mechanical ventilation (Fig. 2). At the end of the study, animals were killed, catheter positions were verified, and body weights were recorded. Body weight for the two groups of animals was not different (1.95 ± 0.09 kg with NO treatment; 1.85 ± 0.12 kg in control).

DISCUSSION

We hypothesized that during tidal-volume mechanical ventilation with high inspired oxygen concentrations after delivery of extremely premature lambs, altered pulmonary vasoregulation leads to increased PVR, decreased pulmonary blood flow, and worsening gas exchange despite a good initial response to exogenous surfactant. We found that the pulmonary hypertension associated with severe HMD in the extremely premature lamb is responsive to exogenous NO, and that early and sustained treatment with low-dose NO in this model attenuates the decline in gas exchange and pulmonary perfusion during prolonged mechanical ventilation.

The premature fetus is characterized by both structural and functional pulmonary immaturity, including surfactant deficiency. Pulmonary immaturity and HMD lead to respiratory failure after premature delivery, and exogenous surfactant therapy can decrease the severity of the respiratory insufficiency (15). In some experimental models of HMD, PVR falls in response to mechanical ventilation alone, and surfactant therapy does not change the direction or magnitude of systemic-to-pulmonary shunting across the patent ductus arteriosus (16, 17). However, progressive deterioration after surfactant therapy occurs in a subset of premature human newborns with HMD, leading to severe pulmonary hypertension with right-to-left shunting across the ductus arteriosus (18–21). Recent studies have shown that the association of pulmonary hypertension with severe HMD leads to increased mortality despite surfactant therapy (22).

Table 3. *Protocol 2: ventilator settings 3-h IMV study; control group (n = 5) vs NO treatment group (n = 6)*

Study period	Study group	PIP (kPa)	PEEP (kPa)	RATE (breaths/min)	$\bar{P}aw$ (kPa)
Baseline	Control	3.4 ± 0	0.6 ± 0	30 ± 0	1.96 ± 0
	NO treatment	3.4 ± 0	0.6 ± 0	30 ± 0	1.96 ± 0
60 min	Control	3.1 ± 0.1	0.5 ± 0.1	30 ± 0	1.76 ± 0.1
	NO treatment	3.2 ± 0.1	0.6 ± 0	30 ± 0	1.86 ± 0.1
120 min	Control	3.1 ± 0.1	0.6 ± 0.04	34 ± 4	1.86 ± 0.1
	NO treatment	3.0 ± 0.1	0.5 ± 0.02	30 ± 0	1.76 ± 0.1
180 min	Control	3.2 ± 0.1	0.6 ± 0.04	38 ± 5	1.86 ± 0.1
	NO treatment	3.2 ± 0.1	5.6 ± 0.04	35 ± 5	1.86 ± 0.1

* cm H_2O = kPa · 10.2. $\bar{P}aw$, mean airway pressure.

The etiology of this apparent surfactant-failure has been elusive. Multiple mechanisms may be involved, including surfactant inactivation caused by alveolar-capillary leak and pulmonary edema, and the production of pulmonary vasoconstrictor agents or diminished endogenous vasodilator formation. Mechanical factors are known to contribute to the increase in pulmonary blood flow at birth. Changes in alveolar surface tension and perivascular tissue pressure (23, 24) and changes in pulmonary endothelial morphology (endothelial cell "flattening") also occur with the onset of ventilation (25, 26). It is likely that these structural changes in pulmonary vascular endothelial cells at birth are associated with functional changes such as augmentation of endogenous NO production, contributing further to pulmonary vasodilation (7). It is also possible that, after birth, the mechanical effects of rhythmic distention of the lung could alter endogenous NO formation by the immature pulmonary vascular endothelial cell, accounting for suboptimal responses to otherwise effective therapies for severe respiratory failure in prematurity, such as surfactant replacement. However, the effects of ventilation with hyperoxia on the endogenous production of vasoconstrictor substances (*e.g.* endothelin) and vasodilators (*e.g.* NO and prostaglandin I_2) and the adverse effects of ventilation and hyperoxia on the structural integrity of the immature lung require further study.

These findings have potential implications for the clinical management of severe respiratory failure in premature infants. The newborn lamb at 115 d gestation develops profound respiratory failure despite treatment with exogenous surfactant and may not exemplify the potentially viable human born prematurely. However, despite the extreme pulmonary immaturity in this ovine model, the pulmonary circulation is markedly responsive to inhaled NO, suggesting a potential therapeutic role in the management of the preterm newborn with pulmonary hypertension. We speculate that the early use of low-dose inhaled NO could play a role in the management of the premature subject with severe respiratory failure unresponsive to exogenous surfactant therapy (27) by improving ventilation/perfusion matching and reducing PVR. However, we caution that little is known of the potential pulmonary and systemic toxicities that could occur in the premature subject exposed to inhalational NO, and studies designed to carefully assess such toxicities are essential before routine clinical application.

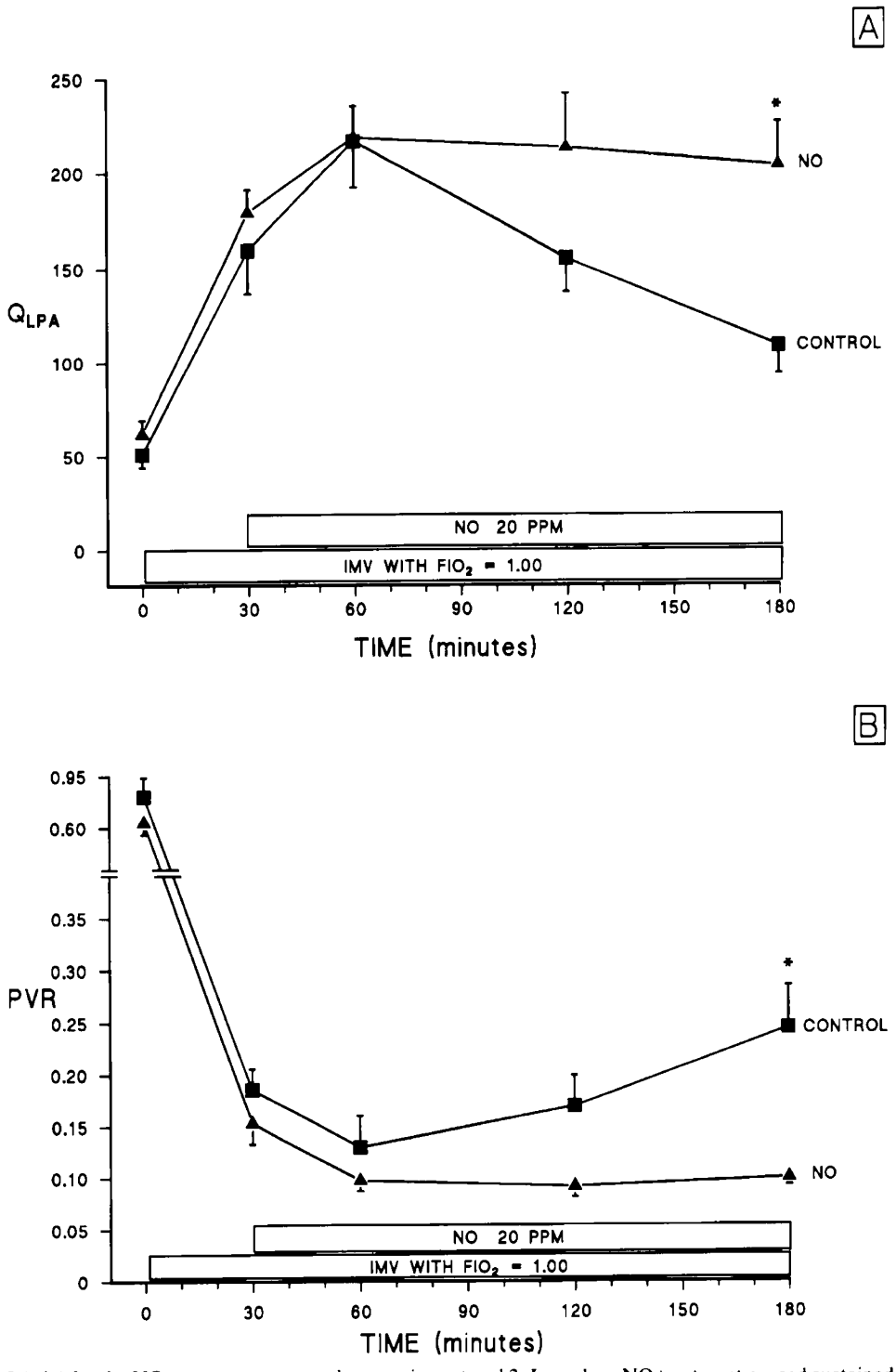

Figure 2. *A*, Q_{LPA} (mL/min) for the NO treatment *vs* control groups in protocol 2. Low-dose NO treatment caused sustained increase in Q_{LPA} over the entire 3-h study period compared with controls. *, $p < 0.05$, NO treatment group *vs* control group at 3-h time point. *B*, PVR [mm Hg · $(mL/min)^{-1}$] for the NO treatment *vs* control groups in protocol 2. Low-dose NO treatment caused sustained decrease in PVR over the entire 3-h study period compared with controls. *, $p < 0.05$, NO treatment group *vs* control group at 3-h time point.

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