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Changes in the Pulmonary Circulation during Birth-Related Events

DAVID F. TEITEL, HARRIET S. IWAMOTO, AND ABRAHAM M. RUDOLPH

Cardiovascular Research Institute and the Department of Pediatrics, Physiology, and Obstetrics and Reproductive Sciences, University of California, San Francisco, California 94143

ABSTRACT. At birth, pulmonary vascular resistance decreases dramatically, allowing pulmonary blood flow to increase and oxygen exchange to occur in the lungs. To determine the extent to which ventilation of the fetus's lungs, oxygenation of the lungs, and umbilical cord occlusion can account for this decrease in resistance, we studied 16 chronically instrumented, near-term sheep fetuses *in utero*. We performed the experiment in a sequential fashion: we first studied the effects of ventilation alone (without oxygenation) on pulmonary vascular resistance and blood flow, and then determined the additive effects of oxygenation and cord occlusion. We calculated pulmonary vascular resistance from measurements of vascular pressures and measurements of pulmonary blood flow obtained by injecting radionuclide-labeled microspheres. We found that ventilation alone caused a large but variable increase in pulmonary blood flow, to 401% of control, no change in pulmonary arterial pressure, and a doubling of left atrial pressure. Thus, pulmonary vascular resistance fell dramatically, to 34% of control. Oxygenation caused a modest further increase in pulmonary blood flow and a decrease in mean pulmonary arterial pressure, so resistance fell to 10% of control. Umbilical cord occlusion caused no further changes in pressure, flow, or resistance. Unexpectedly, the fetuses' pulmonary blood flow responses to ventilation fell into two groups: the mean increase was maximal in eight of the 16 fetuses but was only 20% of the cumulative increase in the other eight. We found no differences between the two groups of fetuses to explain their different responses. We conclude that ventilation and oxygenation together can account for the decrease in pulmonary vascular resistance to levels that occur at birth. Moreover, ventilation alone can account for most of this decrease. (*Pediatr Res* 27: 372–378, 1990)

Abbreviations

IVC, inferior vena cava
LA, left atrium

In the circulation of both fetuses and newborns, the main role of the right ventricle is to deliver blood to the gas exchange circulation for uptake of oxygen and removal of carbon dioxide. In the fetus this delivery is achieved by virtue of the pulmonary vascular resistance being very high. Right ventricular output is thus diverted away from the lungs and toward the placenta, through the ductus arteriosus (1–4). Immediately at birth, as the lungs become the organ of gas exchange, pulmonary vascular resistance must fall dramatically, allowing pulmonary blood flow to increase and oxygen exchange to occur in the lungs. If pulmonary vascular resistance does not fall, the syndrome of persistent pulmonary hypertension of the newborn occurs, often leading to death.

Which of the many events that occur at birth are responsible for the normal decrease in pulmonary vascular resistance is not fully understood. Three major events of the birth process that could be responsible are ventilation, or rhythmic gaseous distension, of fetal lungs, oxygenation of the lungs, and occlusion of the umbilical cord. Two of these events—ventilation and oxygenation—have been studied in acutely exteriorized fetal sheep. Rhythmic ventilation of the fetal lung with a high nitrogen gas, but not with a liquid, produced a decrease in pulmonary vascular resistance (5, 6), although this decrease was significantly less than that seen during oxygen ventilation (5, 7, 8). Exposure of the fetus to high ambient oxygen tension in a hyperbaric chamber supported the hypothesis that oxygen exposure rather than gaseous ventilation of the fetal lung is the major component responsible for the fall in pulmonary vascular resistance (9, 10).

However, the metabolic effects of acute anesthesia and surgery may have altered the pulmonary vascular response in these studies, because this response is considered to be at least partly mediated by vasoactive metabolites (11). Studies of chronically instrumented fetal sheep *in utero* have recently been performed, investigating the effects of ventilation and oxygenation on various cardiovascular functions (12–15), but none has directly addressed the effects of these events on pulmonary vascular resistance. A recent study by Blanco *et al.* (15) did find that nitrogen ventilation was associated with a large increase in the fraction of microspheres injected in inferior vena cava becoming trapped in the lungs. However, pulmonary blood flow could not be calculated and thus the relative effects of nitrogen ventilation and oxygenation could not be assessed.

The purpose of our study was to determine to what extent sequential ventilation of fetal lungs, oxygenation of fetal lungs, umbilical cord occlusion, or a combination of these events can account for the decrease in pulmonary vascular resistance, and thus the large increase in pulmonary blood flow, that normally occur at birth. To remove the superimposed effects of acute anesthetic and surgical stresses, and other components of the birth process, such as prenatal hormonal surges, labor, delivery, and cold exposure, we studied near-term fetal sheep *in utero* 2–3 d after surgery.

MATERIALS AND METHODS

Sixteen fetal sheep were studied at 134.9 ± 1.2 d of gestation (term is about 145 d). The fetuses were of normal weight (3.6 ± 0.6 kg) and had normal blood gases (see Results) and Hb concentrations (109 ± 16 g/L) at the onset of the study. The study was approved by the Committee of Animal Research at the University of California, San Francisco.

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Correspondence and reprint requests: David F. Teitel, Box 0130, University of California at San Francisco, San Francisco, CA 94143.
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Surgical preparation. The surgical protocol has been described previously (4, 16). Briefly, the ewe underwent a midline laparotomy under spinal (1% tetracaine hydrochloride) and supplemental intravenous (ketamine hydrochloride) anesthesia. The fetus also received local anesthesia (0.25% lidocaine hydrochloride) for each skin incision. Through a small uterine incision, the fetal hind limbs were exposed individually and polyvinyl catheters were advanced to the descending aorta and IVC via each pedal artery and vein. Two catheters were also advanced into the main umbilical vein via a peripheral tributary localized from the same uterine incision. This incision was closed after placement of a large polyvinyl catheter in the amniotic cavity for subsequent pressure measurements. A second uterine incision was then made over the left chest. A left lateral thoracotomy was performed and catheters were placed in the ascending aorta via the internal thoracic artery and directly in the pulmonary artery and LA. An 8F multiple side-hole polyvinyl catheter was left in the pleural cavity for drainage. The thoracotomy was closed and a midline incision was made in the neck. The trachea was exposed and ligated proximally, and an endotracheal tube (4.5 mm ID) was inserted directly and advanced to the region of the carina. The proximal end of the tube, which was just outside the skin, was attached to two pieces of 12F polyvinyl tubing via a Y connector and filled with 0.9% NaCl solution. One piece of tubing was sealed and the other was connected to another 12F tubing that was placed in the amniotic cavity, to allow free drainage of tracheal fluid postoperatively. The neck incision was closed. The umbilical cord was then located and a silicone rubber balloon occluder was placed around it, just distal to the abdomen. Antibiotics (400 mg of kanamycin sulfate and 1 million U of penicillin G potassium) were instilled in the amniotic cavity and 0.9% warmed saline was added to replace loss of amniotic fluid. The uterine incision was closed. All vascular catheters were filled with heparin sodium (1000 U/mL), sealed and exteriorized along with the other tubing to the left flank of the ewe. The abdominal incision was closed in layers and the ewe was returned to the cage for recovery. Antibiotics (400 mg of kanamycin sulfate and 1 million U of penicillin G potassium) were administered i.v. to the ewe and into the amniotic cavity daily.

Experimental protocol. We performed four sequential experiments 48–72 h after surgery, control, ventilation alone (*i.e.* ventilation with the same gas concentrations as in the fetus), oxygenation (ventilation with 100% oxygen), and umbilical cord occlusion. During each experiment, for the calculation of pulmonary vascular resistance, we measured pulmonary blood flow by injection of radionuclide-labeled microspheres and measured mean pressures in the pulmonary artery and the LA. We also assessed indicators of oxygenation and acid-base status. Before beginning experimental measurements, we waited for at least 15 min after the intervention for pressures and blood gases to stabilize. In order, we measured pressures, sampled blood gases, and injected the radionuclide-labeled microspheres. This acquisition of data was achieved within 5 min and during hemodynamic stability.

Control. The ewe was placed in a study cage and allowed free access to alfalfa pellets and water. For control experiments, after vascular catheters were connected to Statham P23Db strain-gauge transducers (Statham Instruments, Oxnard, CA), pressures were recorded continuously throughout the study on a direct-writing polygraph (Beckman Instruments, San Jose, CA). Fetal blood samples were obtained from the ascending aorta for determination of pH, P_{CO_2}, and P_{O_2} (Corning 158 pH/blood gas analyzer, Medfield, MA), and of Hb concentration and hemoglobin oxygen saturation (Radiometer OSM2 hemoximeter, Copenhagen, Denmark). Radionuclide-labeled microspheres (selected from ^{57}Co, ^{51}Cr, ^{153}Gd, ^{114}In, ^{54}Mn, ^{95}Nb, ^{113}Sn, ^{85}Sr, and ^{65}Zn), 15 μm in diameter, were then injected into the IVC while reference blood samples were withdrawn from the ascending aorta, descending aorta, and pulmonary artery at a rate of 4 mL/min. Fetal or maternal blood was then given to replace the blood loss.

Ventilation. To assess the effects of ventilation on pulmonary vascular resistance, the two polyvinyl tubes connected to the tracheal tube were opened and the tracheal fluid was allowed to drain by gravity. A mixture of nitrogen, oxygen, and carbon dioxide was balanced to match the fetal blood gases obtained during the control experiment. The gas mixture was approximately 92% nitrogen, 3% oxygen, and 5% carbon dioxide. Before ventilation was begun, this gas mixture was briefly allowed to flow through the polyvinyl tubing at a rate of about 10 L/min so that the fetus would not be exposed to high concentrations of oxygen at the onset of ventilation. The tubing was then connected to a specially designed pump that had an adjustable inspiratory popoff valve proximal to the tracheal tube, a solenoid immediately distal to the tracheal tube, and an adjustable expiratory valve distal to the solenoid. The solenoid was intermittently closed to allow gas to enter the tracheal tube and ventilate the fetal lung, and the inspiratory:expiratory time ratio was about 0.8:1. Expired gases of the fetus were washed out of the system during expiration by the continuous flow of gases so that rebreathing would not occur. Tracheal pressures (fluid filled tube in the distal tracheal tube connected to Statham P23Db pressure transducers) were measured in 15 fetuses and tidal volume (Fleisch type 00 pneumotacograph, Medical Inc., Richmond VA, Validyne model DP45-16 pressure transducer and CD15 carrier demodulator, Validyne Engineering Corporation, Northridge, CA) was measured in nine (Table 1). The ventilation settings were initially adjusted to deliver a tidal volume of about 20–30 mL, similar to that used in newborn lambs (17) and humans (18). Positive end-expiratory pressure was applied to counterbalance amniotic pressure. As the lungs become more compliant throughout the study, minor adjustments were made in the ventilation settings, to maintain inspiratory pressure constant. Ventilatory settings are presented in Table 1. After variables stabilized, blood samples were obtained as above and two sets of radionuclide-labeled microspheres were injected, one into the IVC and the other into the LA, during withdrawal of reference samples as described in the Control section. The microspheres were injected slowly, over several phases of inspiration and expiration (about 20–30 s). Replacement blood was then infused into the fetus.

Oxygenation. To assess the effects of oxygenation on pulmonary vascular resistance, the gas mixture was then changed to 100% oxygen and ventilation was continued. Carbon dioxide was not added to the oxygen because its addition in the first few studies increased fetal P_{CO_2}. This increase probably occurred because placental blood flow fell during oxygenation (4), impairing carbon dioxide removal. After variables stabilized, blood samples were obtained, microspheres were injected into the IVC and the LA, and replacement blood was infused.

Umbilical cord occlusion. To assess the effects of umbilical cord occlusion on pulmonary vascular resistance, the balloon around the umbilical cord was fully inflated to occlude the umbilical blood vessels and thus abolish placental blood flow (4). After variables stabilized, the experimental protocol was repeated. In four of the 16 fetuses, cord occlusion could not be studied, because of a faulty balloon in two and the development of pneumothoraces, which led to cardiovascular decompensation, in two.

Upon completion of the last experiment, the ewe was killed by injection of large doses of sodium pentobarbital and the fetus was removed from the uterus and weighed. The lungs were removed, weighed, and placed in formalin. They were then carbonized in an oven, ground into a coarse powder, and placed in plastic vials to a uniform height of 3 cm. Radioactivity of all organs, tissues, and reference blood samples was counted in a 1000-channel multichannel pulse-ht analyzer (Norland, Fort Atkinson, WI). Sp act of each isotope within a sample was calculated by the least-squares method (19). In all fetuses, adequacy

Table 1. *Ventilatory settings in sheep fetuses during ventilation, oxygenation, and umbilical cord occlusion**

Variable	Ventilation	Oxygenation	Cord occlusion
Respiratory rate (breaths/min)	50 ± 8 (15)†	57 ± 12 (13)	57 ± 13 (11)
Peak inspiratory pressure (kPa)	3.6 ± 1.3 (15)	3.5 ± 1.2 (14)	3.3 ± 1.2 (12)
End expiratory pressure (kPa)	0.4 ± 0.8 (15)	0.5 ± 0.8 (14)	0.5 ± 0.8 (12)
Tidal volume (mL)	23.3 ± 14.6 (9)	35.4 ± 15.9 (9)	30.7 ± 10.4 (9)

* During ventilation, fetuses received a mixture of nitrogen, oxygen, and carbon dioxide balanced to match their blood gases during the control experiment. Pressures are referenced to amniotic cavity pressure. There were no statistically significant differences between experiments for any of the variables.

† Data are presented as the mean ± SD. The number of fetuses is given in parentheses.

of microsphere mixing was assessed by comparing the activity of each isotope in symmetrical organs (cerebral hemispheres and kidneys), and adequacy of microsphere numbers was determined in each organ, tissue and reference sample to ensure accuracy of the technique to within 10% (20).

Calculations. Pulmonary vascular resistance was calculated as the difference between mean pulmonary arterial pressure and mean left atrial pressure divided by pulmonary blood flow. For the six fetuses in which were were unable to measure left atrial pressure for technical reasons, we used the mean values obtained from the other fetuses during the same experiment.

During the control experiment, because there is no left-to-right shunt through the ductus arteriosus (21), pulmonary blood flow was measured by injecting microspheres into the IVC and withdrawing blood samples from the pulmonary artery. This injection and withdrawal technique excludes bronchial flow. To measure bronchial flow, in six fetuses we also injected microspheres into the LA during the control experiment. We found that bronchial flow was relatively constant and quite small, always less than 3% of combined ventricular output. We then subtracted this value from the pulmonary flow measurements in the remaining experiments.

During ventilation, oxygenation, and umbilical cord occlusion, a different technique was used. The reason is that upon ventilation, pulmonary vascular resistance falls and blood flow increases dramatically. Thus, a left-to-right shunt through the ductus arteriosus cannot be excluded. To measure pulmonary blood flow in the presence of a left-to-right shunt requires a technique that determines the contribution of left ventricular output to pulmonary blood flow. Therefore, during ventilation, oxygenation, and umbilical cord occlusion, we injected microspheres labeled with different radionuclides simultaneously into both the IVC and the LA and calculated pulmonary blood flow as the difference between combined ventricular output and the sum of blood flows to the fetal body and placenta (4). Combined ventricular output was calculated as the sum of left and right ventricular outputs. Blood flows to fetal body and placenta were calculated from the LA injections and reference blood withdrawals from the ascending and descending aorta (4).

Left ventricular output was calculated as the sum of blood flow to the fetal body and the placenta, plus the left-to-right ductus arteriosus shunt, less the right-to-left ductus arteriosus shunt. The right-to-left ductus arteriosus shunt was calculated from the IVC and LA injections and the ascending and descending aortic blood withdrawals. We assumed that the IVC microspheres that cross the foramen ovale and are ejected by the left ventricle are distributed similarly to the LA microspheres ejected by the left ventricle, and that the concentration of IVC microspheres in the descending aorta that are ejected by the right ventricle is similar to the concentration of IVC microspheres in the right ventricle. The formula thus derived (4) is:

$$Q_{DRL} = (S^{I}_{LB} - (S^{I}_{UB} \times S^{L}_{LB}/S^{L}_{UB})) \times (Q_{UB} + Q_{LB} - Q_{FO})/(S^{I}_{TOT} - S^{I}_{FO}),$$

where Q represents flow; S represents the number of counts of an isotope; I and L represent the IVC and LA isotopes, respectively, and DRL, LB, UB, FO, and TOT represent the ductus arteriosus right-to-left shunt, the lower body and placenta, the upper body, the foramen ovale, and the total body and placenta, respectively.

The left-to-right ductus arteriosus shunt was calculated by assuming that the ratio of the number of LA microspheres in the lower body and placenta to the number of LA microspheres in the lungs is equal to the ratio of the left ventricular contribution to the lower body and placental flow to the ductus arteriosus left-to-right shunt. The left ventricular contribution to the lower body and placental flow is the total lower body and placental flow less the right-to-left ductus arteriosus shunt. The formula thus derived (4) is:

$$Q_{DLR} = (Q_{LB} - Q_{DRL})/(S^{L}_{LB}/S^{L}_{LG}),$$

where DLR represents the ductus arteriosus left-to-right shunt, and LG represents the lungs.

Right ventricular output was calculated as systemic venous return (equal to the blood flow to the fetal body and placenta) less the right-to-foramen ovale shunt. This foramen ovale shunt was calculated from the IVC and LA injections, assuming that IVC microspheres that cross the foramen ovale to the left ventricles are distributed similarly to the LA microspheres, and that blood flow to the lower body and placenta approximates IVC return. The shunt is thus calculated as the product of the number of IVC microspheres that cross the foramen ovale and the inverse of the concentration of IVC microspheres in the IVC (4):

$$Q_{FO} = S^{I}_{FO} \times (Q_{LB}/S^{I}_{TOT}).$$

Analysis of data. In this study, we assessed the sequential effects of ventilation, oxygenation, and umbilical cord occlusion. Determination of their independent effects was not possible because the order of the experiments could not be randomized. One reason is that we were concerned that oxygenation of the fetal lungs might induce multiple and perhaps irreversible metabolic and hemodynamic consequences, so that subsequent ventilation without oxygenation could not be studied. Another reason is that the umbilical cord cannot be occluded before oxygenation. Thus, the protocol is composed of four sequential experiments, each serving as the control for the next. Data from each of these experiments were analyzed by the Mann-Whitney U test, comparing only the data obtained during one experiment with data obtained during the experiment immediately preceding it. Statistical significance was considered present when $p \leq 0.01$. All data are presented as mean ± 1 SD.

RESULTS

Effects of ventilation, oxygenation, and umbilical cord occlusion. In the 16 sheep fetuses studied, pulmonary vascular resistance decreased dramatically to 34% of control values, during ventilation alone (from 0.26 ± 0.18 to 0.088 ± 0.12 kPa·min·kg/mL) (Fig. 1), decreased further during oxygenation, to 10% of control (0.027 ± 0.103 kPa·min·kg/mL), and did not change further after cord occlusion (0.029 ± 0.015 kPa·min·kg/mL).

These changes in pulmonary vascular resistance were calculated from and reflect changes in pulmonary blood flow and

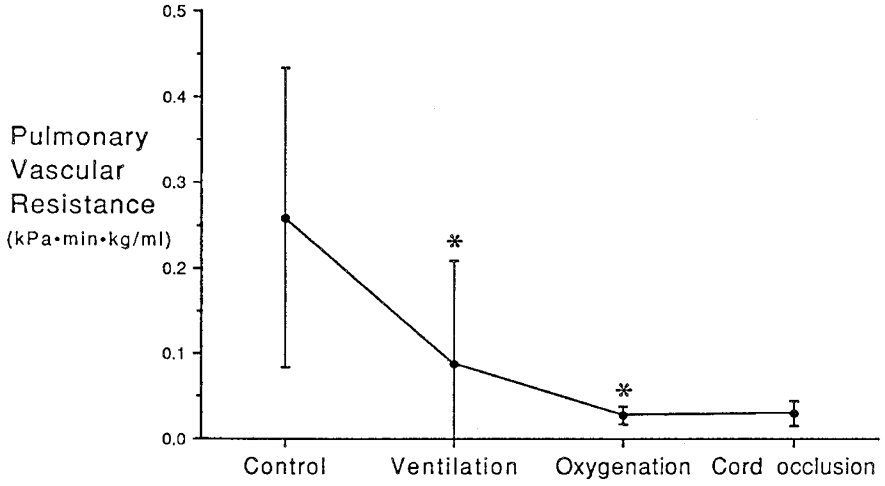

Fig. 1. Pulmonary vascular resistance in the 16 fetal sheep during the experiments. Data are presented as the mean ± 1 SD. *Significantly different from the experiment immediately preceding it, $p \leq 0.001$.

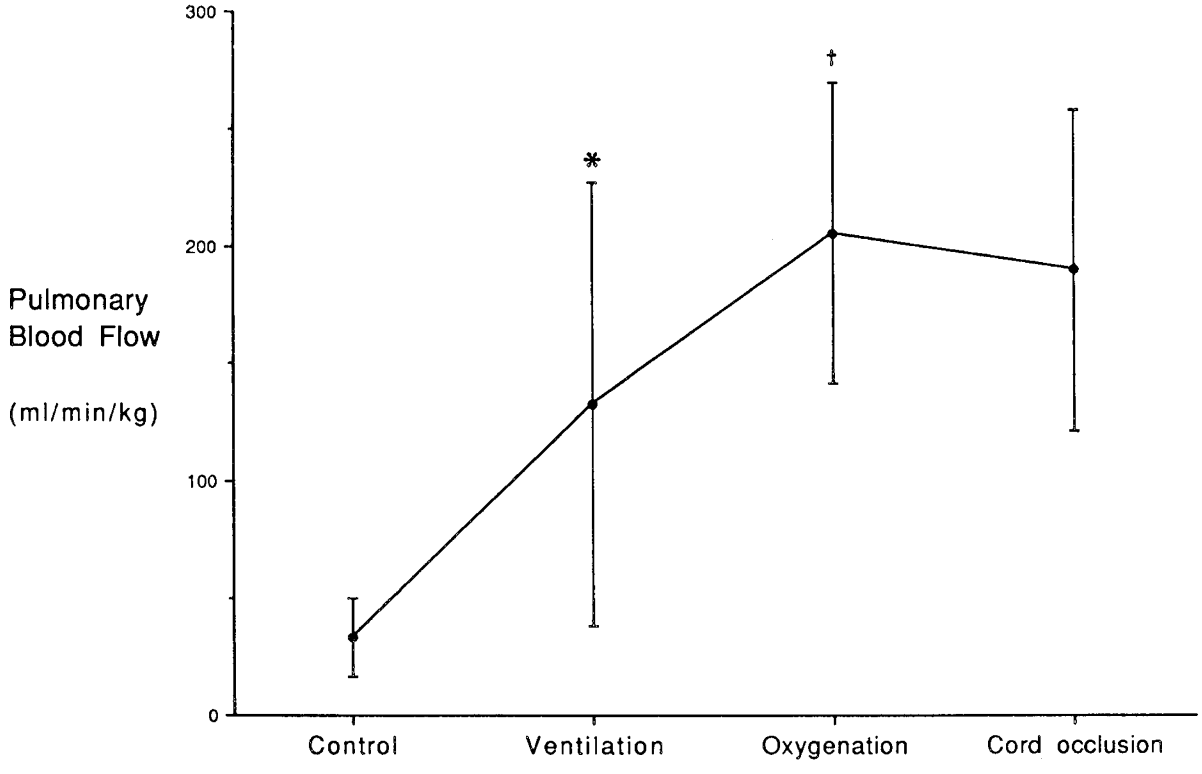

Fig. 2. Pulmonary blood flow during the experiments. Data are presented as the mean ± 1 SD for the 16 fetal sheep. *Significantly different from the experiment immediately preceding it, $p \leq 0.001$. †Significantly different from the experiment immediately preceding it, $p \leq 0.005$.

vascular pressures. Pulmonary blood flow (33 ± 17 mL·min^{-1}·kg^{-1} fetus) was similar to that previously measured in chronically instrumented fetuses of similar gestational ages (2, 3), constituting 9% of combined ventricular output. Ventilation alone increased pulmonary blood flow dramatically, to 401% of control values (133 ± 94 mL·min^{-1}·kg^{-1} fetus) (Fig. 2). The variability of this increase in pulmonary blood flow was marked, however, which led us to separate the fetuses into two groups, as described below. Oxygenation increased pulmonary blood flow further to a mean of 623% of control (206 ± 64 mL·min^{-1}·kg^{-1} fetus). Cord occlusion did not cause any further change in pulmonary blood flow (190 ± 69 mL·min^{-1}·kg^{-1} fetus).

Mean pulmonary arterial pressure was normal in the control experiment and did not change during ventilation (Table 2). During oxygenation there was a small but significant decrease in pressure. Because this decrease was similar to that seen in mean systemic arterial pressure, it cannot be explained by partial closure of the ductus arteriosus. After umbilical cord occlusion, there was no further change in mean pulmonary or mean systemic arterial pressure.

Left atrial pressure could be measured in only 10 fetuses for technical reasons. In association with the large increase in pulmonary blood flow during ventilation alone, mean left atrial pressure doubled (Table 2). It did not change further, during oxygenation or cord occlusion.

Systemic arterial blood gases and Hb oxygen saturation were normal in the control experiment, and did not change during ventilation alone (Table 3). Oxygenation caused a large increase in P_{O_2} and Hb oxygen saturation, but did not change pH or P_{CO_2}. Cord occlusion did not change these variables significantly, but there was much greater variability in P_{CO_2} and pH, probably because of the inability of some fetuses to maintain adequate CO_2 exchange in the lungs, because of pulmonary immaturity.

Table 2. *Mean vascular pressures during experiments**

	Control	Ventilation	Oxygenation	Cord occlusion
Pulmonary arterial pressure (kPa)	7.1 ± 1.1 (15)†	7.3 ± 1.2 (15)	6.3 ± 0.8 (15)‡	6.4 ± 2.1 (12)
Systemic arterial pressure (kPa)	6.9 ± 0.8 (15)	7.1 ± 0.8 (15)	6.4 ± 0.8 (15)‡	7.7 ± 2.1 (12)
Left atrial pressure (kPa)	0.5 ± 0.7 (12)	1.2 ± 0.5 (10)‡	1.3 ± 0.7 (10)	1.2 ± 0.7 (7)

* Pressures are referenced to amniotic cavity pressure.

† Data are presented as the mean ± 1 SD. The number of fetuses is given in parentheses.

‡ Significantly different from the value during the immediately preceding experiment, $p \leq 0.01$.

Table 3. *Ascending aortic blood gases and Hb oxygen saturations during experiments**

Variable	Control	Ventilation	Oxygenation	Cord occlusion
pH	7.37 ± 0.06 (15)	7.35 ± 0.07 (16)	7.34 ± 0.09 (16)	7.29 ± 0.15 (13)
P_{O_2} (kPa)	2.4 ± 0.4 (15)	2.5 ± 0.5 (16)	28.7 ± 20.5 (16)†	35.1 ± 22.4 (13)
P_{CO_2} (kPa)	7.3 ± 0.8 (15)	7.2 ± 0.8 (16)	6.8 ± 1.3 (16)	7.7 ± 2.8 (12)
Hb O_2 saturation	0.47 ± 0.13 (16)	0.46 ± 0.12 (16)	0.97 ± 0.06 (16)†	0.95 ± 0.10 (16)

* Data are presented as the mean ± 1 SD. The number of fetuses is given in parentheses.

† Significantly different from the value during the immediately preceding experiment, $p \leq 0.01$.

Table 4. *Comparisons of variables in major and minor responders during control experiment and oxygenation**

Variable	Major responders	Minor responders	All animals
Gestational Age at study (days)	135 ± 1 (7)†	135 ± 1 (8)	135 ± 1 (15)
Weight (kg)	3.5 ± 0.6 (8)	3.8 ± 0.6 (8)	3.6 ± 0.6 (16)
Days following surgery	2.3 ± 0.7 (8)	2.0 ± 0.0 (8)	2.1 ± 0.5 (16)
Control			
pH	7.37 ± 0.07 (8)	7.39 ± 0.03 (7)	7.37 ± 0.06 (15)
P_{O_2} (kPa)	2.4 ± 0.3 (8)	2.4 ± 0.4 (7)	2.4 ± 0.4 (15)
P_{CO_2} (kPa)	7.3 ± 0.8 (8)	7.2 ± 0.8 (7)	7.3 ± 0.8 (15)
Pulmonary blood flow (mL/min/kg)	33 ± 19 (8)	33 ± 15 (7)	33 ± 17 (15)
Pulmonary arterial mean pressure (kPa)	7.1 ± 1.6 (7)	7.1 ± 0.5 (8)	7.1 ± 1.1 (15)
Combined ventricular output (mL/min/kg)	401 ± 84 (8)	378 ± 69 (8)	390 ± 75 (16)
Oxygenation			
P_{O_2} (kPa)	28.7 ± 20.0 (8)	28.7 ± 22.4 (8)	28.7 ± 20.5 (16)
P_{CO_2} (kPa)	6.9 ± 1.5 (8)	6.5 ± 1.3 (8)	6.8 ± 1.3 (16)
Pulmonary blood flow (mL/min/kg)	195 ± 76 (8)	217 ± 52 (8)	206 ± 64 (16)

* Major responders had increases in pulmonary blood flow ≥50% of the cumulative increase during the study. Minor responders had increases ≤50%. No difference between groups was statistically significant).

† Data are presented as the mean ± 1 SD. The number of fetuses is given in parentheses.

Major versus minor responders during ventilation alone. The individual changes in pulmonary blood flow were extremely variable (Fig. 2). In some fetuses the majority of the increase occurred during ventilation alone, whereas in others there was almost no increase until oxygenation. This finding led us to separate the fetuses according to their response to ventilation and examine the reasons for this variability. We arbitrarily divided the fetuses into two groups: major responders, which showed an increase in pulmonary blood flow during ventilation alone that was at least 50% of the cumulative increase (the difference between pulmonary blood flow during control measurements and after cord occlusion), and minor responders, which showed an increase of less than 50%. Interestingly, eight fetuses were major responders and eight were minor responders. The major responders had an increase in flow during ventilation that was equal to the cumulative increase (103 ± 52%), whereas the minor responders had a much smaller increase (20 ± 17%).

When we examined the measured variables that could have caused this disparity between the major and minor responders, we found that none of those variables showed statistically significant differences between the two groups (Table 4). Indices of maturity and postoperative stability (gestational age, wt, and days after surgery), of initial pulmonary vascular tone (initial blood gases and pulmonary blood flows and pressures), of ventricular function (combined ventricular output), and of adequacy of alveolar ventilation during oxygenation (blood gases and pulmonary blood flow during oxygenation) were remarkably similar. Adequacy of alveolar ventilation during ventilation alone (without oxygenation) could not be assessed, although there was no change in the method of ventilation in either group when oxygenation was established. Of those fetuses in which sex was recorded, the majority in both groups were female (six of seven of the major responders, four of six of the minor responders).

DISCUSSION

Three major events of the birth process are ventilation, or rhythmic gaseous distension of the lungs, oxygenation, and loss of the umbilical-placental circulation. In this study in fetal sheep, we found that two of these events, ventilation and oxygenation, together can account for the decrease in pulmonary vascular resistance, and thus for the large increase in pulmonary blood flow, that normally occur at birth. Moreover, most of the decrease in pulmonary vascular resistance, on average, nearly two-thirds, is accounted for by ventilation alone. It is important to keep in mind that this was a sequential experiment: we determined the independent effects of ventilation on pulmonary vascular resistance, and then studied the additive effects of oxygenation and cord occlusion. In addition, the large mean effect of ventilation alone occurred because one-half of the fetuses

showed a maximal effect of ventilation on pulmonary vascular resistance, without a further increase during oxygenation or cord occlusion. The remaining fetuses showed only a very small decrease in resistance during ventilation.

Our finding that, on average, about two-thirds of the decrease in pulmonary vascular resistance occurs during ventilation alone is much larger than the previously accepted value of about one-third (7). The studies upon which this value is based presented conflicting results: whereas initial studies by Dawes *et al.* (6) suggested that the gas mixture is not important in determining the fall in pulmonary vascular resistance during ventilation, later studies from the same laboratory found that ventilation alone achieved only about 40% of the fall in resistance that ventilation with oxygenation achieved (8). Lauer *et al.* (5) found that ventilation alone decreased pulmonary vascular resistance greatly, thereby increasing pulmonary blood flow to 342% of control, which is very similar to our findings. However, they did not compare this effect to ventilation with oxygenation. The ability for oxygenation alone (without ventilation) to greatly decrease pulmonary vascular resistance has been confirmed by studies performed under hyperbaric conditions (9), during isolated right lung oxygen ventilation while recording left lung blood flow (8), and during liquid oxygen ventilation (5). Dawes (7) summarized the findings of these and other studies by stating that "vasodilation caused by gaseous expansion of the lungs, irrespective of gas consumption, is a substantial part (one third, at least) of the acute pulmonary vasodilatation caused by rhythmic positive pressure ventilation with room air."

The reason we found a larger decrease than previously accepted may be that these earlier studies were performed on acutely exteriorized fetuses (5, 6, 8–10). An acute stress such as that caused by the anesthesia and surgery used to exteriorize a fetus can greatly alter production and inhibition of various metabolic agents. Altered metabolite production and inhibition could have attenuated the slow second phase of the decrease in pulmonary vascular resistance in those studies, as characterized by Leffler *et al.* (22). Evidence for this possibility is that this slow second phase, which lasts for 10–20 min after the rapid first phase, has been shown to be attenuated by the prostaglandin synthesis inhibitor, indomethacin (22). [In contrast, the first phase, a rapid decrease that lasts for only 30 s, is not altered by indomethacin but may be altered by direct mechanical effects of ventilation: the establishment of a gas-liquid interface in the alveoli may decrease perivascular pressures and thus distend the small arterioles and decrease resistance (22).] Further evidence that prostaglandin metabolites are important in the decrease of pulmonary vascular resistance is that prostaglandin I_2, a potent pulmonary vasodilator, is produced in response to either mechanical ventilation (23–25) or breathing in recently delivered fetal lambs. In addition, the production of prostaglandin E_1, prostaglandin D_2, and bradykinin and the inhibition of leukotrienes C_4 and D_4 may affect pulmonary vascular resistance (11). Thus, the variable but generally lesser effects of ventilation alone in the previous studies may be ascribed to the variable effects of the study protocols on the metabolic milieu of the pulmonary vascular bed.

An unexpected finding of this study is the great variability in the response of fetal pulmonary blood flow to the effects of ventilation alone. In one-half of the fetuses, the mean increase in pulmonary blood flow during ventilation alone was maximal, whereas in the other half it was only about 20% of the cumulative response. Interestingly, Cook *et al.* (26) found a similar variability in their study of nitrogen and air ventilation: of the six fetuses studied, two showed no effect of nitrogen ventilation but a large effect upon changing to air, two showed a small effect of nitrogen and a larger response to air, and two showed a large increase in pulmonary blood flow during nitrogen ventilation with no further change upon exposure to air. To explain these findings, Cook *et al.* (26) noted that nitrogen had the greatest effect on the smallest fetuses. However, we were unable to identify the reasons for the variability we found. It was not on a purely arithmetic basis. That is, the major responders did not begin with lower control flows or have lower maximal flows. In fact, the two groups had remarkably similar pulmonary blood flows both during control measurements and during ventilation with 100% oxygen. The groups were also not different in their overall maturity, either with respect to gestational age or to wt. In addition, differences in P_{O_2} were not responsible for the differences between major and minor responders, because both during control measurements and during ventilation alone, the minor responders were neither more hypoxic nor more hypercapnic than the major responders. Finally, adequacy of alveolar ventilation was probably not responsible for the difference between the groups. Although we were not able to determine the adequacy of alveolar ventilation during ventilation alone, during oxygenation, P_{O_2} and P_{CO_2} values were similar in the two groups, without the method of ventilation having been changed in either group.

Although our data cannot explain the reason for the marked difference between the pulmonary vasodilatory responses of the two groups of fetuses, this difference may have important implications for future studies. First, it may be important in uncovering the metabolic processes responsible for an incomplete decrease in pulmonary vascular resistance at birth. Second, evaluation of the concentrations and fluxes of the putative metabolic agents involved may demonstrate different fates of these agents in major and minor responders. However, careful evaluation of lung mechanics is critical in future studies to ensure that the differences between the responses of the pulmonary vascular bed are not caused solely by differences in pulmonary function. In particular, lung volumes and compliance may have been significantly different in the two groups. In addition, it would be of interest to determine whether static gaseous distension of the lungs (*i.e.* distension without ventilation) can induce a similar decrease in pulmonary vascular resistance. Static distension does increase lung compliance in fetal sheep (27), and has been shown to decrease pulmonary vascular resistance to some degree in acutely exteriorized fetal sheep (28).

In summary, this study in fetal sheep shows that two major events during the birth process, ventilation and oxygenation of the fetus's lungs, together can account for the decrease in pulmonary vascular resistance, and thus for the large increase in pulmonary blood flow, that normally occur at birth. Moreover, ventilation alone accounts for most of this change; it was nearly two-thirds. The increase in pulmonary blood flow during ventilation is variable: one-half of the fetuses showed a large increase during ventilation, and one-half showed only a minimal increase. This variability is probably mediated in part by alterations in a variety of vasoactive metabolites. By using an *in utero* preparation to investigate the metabolic differences between fetuses that do and do not respond to ventilation alone, the processes responsible for an incomplete decrease in pulmonary vascular resistance and thus for the syndrome of persistent pulmonary hypertension of the newborn may be better elucidated.

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