The Effects of Lung Distension, Oxygenation, and Gestational Age on Fetal Behavior and Breathing Movements in Sheep¹

SHABIH U. HASAN AND ANITA RIGAUX

*Departments of Paediatrics and Reproductive Medicine Research Group, The University of Calgary,
Calgary, Alberta, Canada T2N 4N1*

ABSTRACT. Lung distension with 100% O₂ at a continuous positive airway pressure of 30 cm H₂O may induce continuous fetal breathing movements (FBM) in sheep. The objectives of this study were 1) to investigate the relative roles of lung distension and oxygenation and 2) to test the hypothesis that FBM can be induced during labor, when normally they are greatly reduced or absent. We studied 13 chronically instrumented, unanesthetized fetal sheep between 128 and 144 d of gestation (term = 147 ± 2 d). Each fetus was instrumented to record sleep states, diaphragm electromyogram, blood pressure, arterial pH, and blood gas tensions. The fetal lungs were distended via an *in situ* endotracheal tube with four different concentrations of O₂ (0, 21, 50 and 100%) at a continuous positive airway pressure of 10, 20 and 30 cm H₂O in a randomized order. No change in any recorded physiologic variable was observed at 129 ± 1 or 132 ± 1 d of gestation. At 135 ± 1 and 138 ± 1 d, in response to a continuous positive airway pressure of 30 cm H₂O and 100% O₂, pH decreased ($p = 0.0004$ and 0.005 , respectively) and arterial O₂ tension increased ($p = 0.004$ and 0.02 , respectively). However, increases in 1) breathing time, 2) breathing time/low-voltage electrocortical activity ratio, 3) duration of arousal, and 4) length of single breathing epochs were observed only at 138 ± 1 d. Lung distension with N₂ resulted in a decrease in FBM. Six fetuses were studied during labor. Significant increase in total breathing time, stimulation of breathing during quiet sleep, and increased duration of arousal were observed with an increase in fetal arterial O₂ tension. The onset of breathing always coincided with the onset of arousal. We conclude that fetal maturity, an increase in fetal arterial O₂ tension, and a change in state from quiet sleep toward arousal are necessary conditions for the induction of FBM. (*Pediatr Res* 30: 193–201, 1991)

ID, inner diameter
OD, outer diameter
PaO₂, arterial O₂ tension
PaCO₂, arterial CO₂ tension

Since the early studies of Dawes *et al.* (1), it has been known that fetal sheep make irregular, rapid, and episodic breathing movements (2). One of the many unique and vital changes occurring at birth is the establishment of continuous breathing. Mechanisms for the control of FBM or the initiation of continuous breathing at birth remain unknown. In sheep, during late gestation (115–120 d; term = 147 ± 2 d), FBM occupy 40% of the total time and occur normally only during LV-ECoG and are absent during HV-ECoG. Although the precise control mechanism for FBM has yet to be explained, FBM are susceptible to modulation. Isocapnic hypoxemia suppresses and normoxic hypercapnia stimulates FBM (3, 4); morphine, meclizolam, indomethacin, pilocarpine, doxapram, aminophylline, caffeine, and 5-hydroxytryptophan stimulate, whereas general anesthetics, barbiturates, diazepam, and prostaglandin E₂ depress FBM (2, 5–8).

We have recently shown that distending fetal lungs with 100% O₂ at a CPAP of approximately 30 cm H₂O may induce continuous FBM (9). However, the relative roles of distending pressure and oxygenation remain unknown. Nor is it known if these FBM can be induced during spontaneous onset of labor when normally these are absent or greatly reduced. Therefore, we designed a study 1) to investigate the relative roles of oxygenation and distending pressure by using various concentrations of O₂ and different levels of distending pressures and 2) to test the hypothesis that FBM can be induced during spontaneous onset of labor.

Abbreviations

BP, blood pressure
CPAP, continuous positive airway pressure
EMG_{NK}, neck electromyogram
EOG, electro-oculogram
FBM, fetal breathing movements
ECOG, electrocorticogram
HV-ECOG, high-voltage ECOG or electrocortical activity
LV-ECOG, low-voltage ECOG or electrocortical activity

MATERIALS AND METHODS

Animal preparation. We studied 13 fetal sheep between 128 and 144 d of gestation. Surgery was performed on time-dated, pregnant ewes of mixed breed between 124 and 132 (127 ± 2) d of gestation using 4% halothane in O₂ for induction of anesthesia and 1.5 to 2% for maintenance. After the skin was cleansed with iodine and 70% alcohol, the abdominal wall was opened by a midline incision using sterile technique. Avoiding major vessels, the uterine cavity was opened and the fetal head and neck were exteriorized. The uterine wall and fetal skin were brought together with several Babcock forceps to minimize loss of amniotic fluid. An incision was made at the upper dorsal part of the neck to tunnel four color-coded electrode wires to record ECOG, EOG, EMG_{NK}, and diaphragm electromyogram. The electrodes were constructed from Teflon-coated stainless steel wire of which a 5.0-mm segment at the end of each was scraped bare (Cooner

Received December 3, 1990; accepted April 8, 1991.

Correspondence and reprint requests: Dr. Shabih U. Hasan, Department of Paediatrics, Faculty of Medicine, University of Calgary, 3330 Hospital Drive N.W., Calgary, Alberta, Canada T2N 4N1.

Supported by the Foothills Hospital Research and Development Committee and the Alberta Children's Hospital Research Foundation.

¹ Presented in part at the Annual Meeting of the Society for Pediatric Research, May 7–11, 1990, Anaheim, CA.

Wire Co., Chatsworth, CA; AS 632). Each electrode was enveloped in polyvinyl tubing (1 mm ID and 2 mm OD; Portex, UK) and sealed with silicone of one of four different colors. To record the ECoG, a pair of electrodes was implanted bilaterally over the parietal area through two holes drilled in the fetal skull 2.5 cm apart and 4 mm behind the coronal sutures. These electrodes were fixed to the fetal skull using rubber pads and cyanoacrylate glue. To record the EOG, a silver-coated, oval-shaped electrode was embedded around the lateral rectus muscle of one eye through an incision made along the lateral bony orbital ridge; the reference electrode was implanted 3 to 4 cm below the orbit. EMG_{NK} electrodes were sutured in the lateral neck muscle 5 cm apart and 5 cm below the occiput. ECoG, EOG, and EMG_{NK} were used to define the fetal behavioral state. To place polyvinyl catheters (1 mm ID and 2 mm OD; Portex, Hythe, Kent, UK) in the fetal carotid artery and jugular vein, a 5- to 7-cm ventral incision was made lateral to the trachea, starting just below the thyroid cartilage. The arterial catheter was used to monitor BP and draw blood samples for the analysis of pH and blood gas tensions; antibiotics and fluid were administered through the venous catheter. To apply CPAP to the fetal lungs, the trachea was exposed through the ventral neck incision and a 7.5-cm endotracheal tube (4.8 mm ID and 7.9 mm OD; Tygon, Norton Plastics, Akron, OH) was inserted in the trachea and advanced for 5 to 6 cm through an incision made just below the thyroid cartilage. The endotracheal tube was transfixed (0-silk) to prevent dislodgement from the trachea and two (size 4 silk) threads were tied around the trachea to avoid air leaks that might occur during the lung distension experiments. The free end of the endotracheal tube was connected to a serrated Y connector (polypropylene, 6.4 mm OD; Nalgene 6152, Sybron Corp., Rochester, NY), which in turn was connected to two polyvinyl tubes (4.8 mm ID and 7.9 mm OD, Tygon) that served as inspiratory and expiratory extensions of the endotracheal tube. This step was undertaken to minimize the dead space.

After removing the Babcock forceps, the fetus was further exteriorized to expose the lower chest area. A 5-cm incision was made 3 cm above the lower subcostal margin to expose the right hemidiaphragm. Three s.c. tunneled electrode wires were implanted in the diaphragm and sutured securely along the 10th intercostal space. Although we used only two diaphragm electrode wires at any given time, the third wire was implanted to obtain the best possible combination of diaphragmatic signals. For common grounding purposes, a single reference electrode was also anchored firmly at the site of the initial tunneling. Two polyvinyl catheters were placed in the amniotic cavity by securing those at the back of the fetal head. One of the two amniotic catheters (2.4 mm ID and 4 mm OD; Tygon) was used for monitoring amniotic pressure and also for the electronic subtraction of fetal BP. The second amniotic catheter (4.8 mm ID and 7.9 mm OD; Tygon) was connected (perfectum adapters; Popper & Sons, Inc., New Hyde Park, NY) to one of the endotracheal tube extensions to facilitate the tracheoamniotic fluid flow, whereas the second extension of the endotracheal tube remained plugged when no experiments were being conducted. Every effort was made to keep the exposed fetal parts covered to minimize heat loss. After the instrumentation was completed, the fetus was returned to the uterine cavity and all incisions were sewn in layers.

A polyvinyl catheter (2.4 mm ID, 4 mm OD; Tygon) was placed in the jugular vein of the pregnant ewe for infusion of antibiotics and fluids. All fetal electrode wires and catheters were exteriorized through the maternal left flank and connected to the recording apparatus.

Postoperatively, the ewes had free access to water, hay, and alfalfa pellets. They received 10⁶ IU of benzylpenicillin and 80 mg of gentamicin i.v., and 20 mg of gentamicin were injected into the amniotic cavity; the fetus received 75 000 IU of benzylpenicillin and 10 mg of gentamicin i.v. for 4 d. The fetal and maternal vascular catheters were flushed with heparinized saline

(heparin 10 IU/mL) twice daily. To maintain patency, fetal arterial catheters were infused continuously with heparinized saline (heparin 1 IU/mL) at a rate of 1.0 mL/h (Harvard infusion pump, model no. 901; Harvard Apparatus Co., Inc., South Natick, MA).

Data acquisition and analysis. All the exteriorized fetal electrode wires were connected to the NeuroLog System (Medical Systems Corporation, Greenvale, NY). The amniotic pressure and fetal BP were recorded with Statham P23 ID pressure transducers (Gould Inc., Instrument Division, Cleveland, OH). Subsequently, the appropriately amplified and filtered ECoG, EOG, EMG_{NK}, and diaphragm electromyogram signals were filtered such that the following respective frequency ranges were recorded: 0.5 to 40 Hz, 5 to 40 Hz, 50 Hz to 1 kHz, and 50 Hz to 1 kHz. Amniotic pressure, BP, and electrophysiologic signals were recorded on an eight-channel recorder (Gould Brush 2800S) and on a video-cassette recorder using an eight-channel Neuro-corder (DR-886) (Neurodata Instruments Corporation, New York, NY).

The analyses were done manually for each recorded minute and included measurements of 1) total time of a given experiment; 2) total breathing time (BT); 3) percentage of breathing time during total time (% BT); 4) percentage of breathing time during LV-ECoG (% BR LV); 5) percentage of breathing time during HV-ECoG (% BR HV); 6) percentage of breathing time/low voltage (% BT/LV) (a value of more than 100% indicates presence of breathing during HV-ECoG); 7) percentage of eye movements during total time (% EYE); 8) percentage of nuchal muscle activity during total time (% NECK); 9) percentages of LV- and HV-ECoG (% LV and % HV, respectively); 10) duration of arousal during the entire experiment in minutes; 11) longest single apneic episode in minutes; 12) longest single breathing epoch in minutes; 13) time lag to the onset of continuous breathing movements if present; 14) arterial pH and blood gas tensions; 15) hematocrit; 16) systolic, diastolic, and mean BP; and 17) heart rate (bpm). Fetal heart rate was calculated from the peaks of systolic BP. The percentage of eye, nuchal and low and high voltage ECoG were measured to delineate the sleep states and were not the end points of this study.

Electrographic criteria were used to define active sleep, quiet sleep, and awake states (10, 11). Active sleep was defined by the simultaneous presence of LV-ECoG and eye movements, and absence of neck muscle tone; quiet sleep was defined by the simultaneous presence of HV-ECoG and neck muscle tone, and absence of eye movements. Fetal wakefulness was defined by the simultaneous presence of LV-ECoG, eye movements, and neck muscle tone (10, 11).

Experimental design. All experiments were done at least 3 d after surgery and only after fetal breathing had returned to the normal range for at least 24 h. No experiments were done if the ewe appeared clinically unwell. In view of the diurnal variability in fetal breathing patterns, all experiments were started between 1100 and 1200 h and completed by 0400 h, and lasted an average of 16 h. In view of the length of the experiments, at least 3 d were allowed to elapse before another experiment was done on the same fetus. No other experiments were done on these fetal preparations.

Before each experiment, a control period was established: recordings were made for at least 3 h showing both LV- and HV-ECoG, which normally coincided with periods of breathing and nonbreathing, respectively. After tracheal suction was done through one of the two loops of the endotracheal tube extension, the fetal lungs were distended with 0% O₂ (100% N₂) for uniform distension at a CPAP of 30 cm H₂O; this pressure was achieved in increments of 10 cm H₂O to avoid sudden increases in intrathoracic pressure. This particular step in the protocol was added to optimize uniform lung distension and minimize the confounding variable of lower CPAP at the outset of the experiments. Thereafter, the fetal lungs were distended with four concentrations of O₂ (0, 21, 50, and 100%) at three levels of CPAP

(10, 20 and 30 cm H₂O) in a randomized order, yielding 12 subsets for each experiment. A given amount of pressure and/or O₂ concentration was maintained for at least two complete cycles of electrocortical activity (one cycle = one cycle of LV-ECoG plus one cycle of HV-ECoG). Various levels of CPAP were achieved using a Baby Bird Ventilator (Bird Corp, Palm Springs, CA; model no. 5900). All changes in CPAP and/or O₂ concentrations were made only during HV-ECoG to maintain consistency and to avoid the effects of FBM that normally occur during LV-ECoG. O₂ concentration was adjusted with a Baby Bird O₂ blender and monitored by an O₂ analyzer (Hudson Ventronics; model no. 5584 EC). Because a change in fetal temperature may stimulate breathing (12, 13), the inspired gases were humidified and heated to 39.5°C before administration (Fisher & Paykel Ltd., servo-controlled model no. 328 humidifier) (Allied Products, Medical Division, Panmure, Auckland, New Zealand).

Six ewes were studied during labor. No attempt was made either to postpone or to induce labor. Because intrauterine pressure rises during labor, CPAP was gradually increased to 40, 50, and 60 cm H₂O to raise PaO₂. Furthermore, during labor, due to time constraints, it was not always possible to use the lower pressures (10 and 20 cm H₂O). However, at higher pressures, N₂ was always added to the experimental protocol.

Arterial pH and blood gas tensions were determined on 0.3-mL aliquots during the control period, before tracheal suction, after tracheal suction, and during both LV- and HV-ECoG states. If stimulation of breathing occurred, frequent blood gas determinations were done. Hematocrit was checked before and during the experiment, and blood was replaced as required. The blood samples were kept on ice until the time of analysis and the temperature of the blood gas analyzer was modified to 39.5°C (Instrumentation Laboratory System, Lexington, MA).

These studies were approved by the Animal Care Committee of the University of Calgary.

Statistical analysis. The fetuses were divided into five groups: 1) 129 ± 1 d, 2) 132 ± 1 d, 3) 135 ± 1 d, 4) 138 ± 1 d, and 5) during labor. The analyses were done using multivariate analysis of variance based on two-factor experiments with repeated measurements on one factor (14, 15). Comparisons were made between control and 12 experimental conditions for each recorded variable. Similarly, comparisons were also made between the various gestational ages. The data in the text are presented as the mean ± SD, and a *p* value of less than 0.05 was considered to be significant.

RESULTS

Gestational age 129 ± 1 d. Three fetuses were studied in this gestational age group. There were no statistically significant differences in any of the variables. In one fetus, PaO₂ increased from 2.91 to 4.53 kPa (21.8 to 34 torr; 1 torr = 0.1333 kPa) at a CPAP of 30 cm H₂O and 100% O₂ with no changes in any of the cardiorespiratory or behavioral responses (Fig. 1).

Gestational age 132 ± 1 d. Eight fetuses were studied in this group. There were no significant changes at a CPAP of 10 or 20 cm H₂O at any O₂ concentration. However, when the lungs were distended with 100% O₂ at a pressure of 30 cm H₂O, arterial pH decreased to 7.29 ± 0.7 and PaO₂ increased to 8.77 ± 7.34 kPa (65.8 ± 55.1 torr) from the control values of 7.36 ± 0.04 and 3.23 ± 0.45 kPa (24.2 ± 3.4 torr), respectively (*p* = 0.06 and 0.16, respectively). The large SD in pH and PaO₂ resulted from the fact that PaO₂ increased in only three of eight fetuses. The pH in these fetuses decreased from control values of 7.37, 7.33, and 7.39 to 7.28, 7.19, and 7.29; PaO₂ increased from 3.29, 3.87, and 3.07 kPa to 12.45, 21.19, and 12.32 kPa (24.7, 29, and 23 torr to 93.4, 159, and 92.4 torr) during control and experimental conditions, respectively. PaCO₂ remained unchanged. However, there were no significant changes in any of the breathing or behavioral responses. For example, among the three oxygenated fetuses, breathing movements occurred for 52.5, 48.2, and 49.7%

Fig. 1. Effects of lung distension at 130 d of gestation. Representative tracing showing the control period (A) and the effects of lung distension with CPAP of 30 cm H₂O with nitrogen and with 100% O₂. Note that lung distension at this gestational age with 0% O₂ (nitrogen) (B) or with 100% O₂ (C) had no effect on behavior or FBM.

of the total time under control conditions and 48, 25.4, and 43% during experimental conditions, suggesting that there was no association between an increase in PaO₂ and the incidence of breathing movements at this gestational age. All fetal breathing activity took place during LV-ECoG. There were no statistically significant changes in BP or heart rate at any CPAP or O₂ concentration.

Gestational age 135 ± 1 d. Results from the six fetuses in this group are summarized in Table 1. In all six fetuses, statistically significant increases in PaO₂ and PaCO₂ and a decrease in pH were observed at a CPAP of 30 cm H₂O and 100% O₂. The PaO₂ and PaCO₂ increased from 2.73 ± 0.61 and 5.33 ± 0.60 to 15.30 ± 5.76 and 6.68 ± 1.37 kPa (20.5 ± 4.6 and 40 ± 4.5 to 114.8 ± 43.2 and 50.1 ± 10.3 torr), respectively, and pH decreased from 7.34 ± 0.03 to 7.25 ± 0.04 (*p* = 0.004, 0.01, and 0.0004, respectively). However, once again, all measured cardiorespiratory and behavioral responses remained unchanged. Only one of six fetuses manifested 24.5 and 16.9% of its breathing during HV-ECoG at a CPAP of 20 cm in conjunction with 50 and 100% oxygen, respectively. The PaO₂ in this fetus increased from 2.66 to 8.80 kPa (20 to 66 torr), pH decreased from 7.28 to 7.25 and PaCO₂ remained constant at 6.40 and 6.28 kPa (48 and 47.1 torr) during control and experimental conditions, respectively. The lag time from changing the O₂ mixture to the onset of breathing was 5.4 and 15.2 min. The same fetus also breathed during lung distension with 100% O₂ at a CPAP of 30 cm. On this occasion, the PaO₂ was 9.60 kPa (72 torr), pH decreased to 7.23, and PaCO₂ increased to 6.80 kPa (51 torr); 37.6% of the total breathing occurred during HV-ECoG.

At this gestational age, the longest single apneic episode remained unchanged, whereas the longest single breathing epoch was significantly decreased when the lungs were distended with

Table 1. *Cardiorespiratory and behavioral responses to lung distension and oxygenation in six fetuses at 135 ± 1 d gestational age**

Variable	Control	CPAP level (cm H ₂ O)											
		10				20				30			
		100% N ₂	21% O ₂	50% O ₂	100% O ₂	100% N ₂	21% O ₂	50% O ₂	100% O ₂	100% N ₂	21% O ₂	50% O ₂	100% O ₂
% BT	42.8 ± 11.9	19.8 ± 22.5	31.7 ± 22.3	24.6 ± 23.8	29.8 ± 21.1	18.3 ± 22.7	45.7 ± 28.8	44.7 ± 18.5	40.4 ± 20.0	19.2 ± 13.6†	40.0 ± 20.3	41.6 ± 21.0	44.6 ± 29.5
% BT/LV	88.9 ± 7.9	41.8 ± 40.0	66.3 ± 46.6	52.5 ± 50.3	71.8 ± 13.4	40.8 ± 48.8	79.4 ± 6.2	79.2 ± 26.8	78.9 ± 36.0	43.7 ± 29.0†	89.9 ± 43.4	75.0 ± 43.5	81.2 ± 38.2
Dur. arousal (min)	3.4 ± 6.0	0.2 ± 0.4	1.2 ± 1.2	1.3 ± 2.7	0.3 ± 0.7	1.1 ± 1.4	0.6 ± 1.2	1.8 ± 2.1	8.6 ± 15.5	1.0 ± 1.4	2.3 ± 2.9	4.8 ± 4.9	16.1 ± 21.9
Long. ap. prd. (min)	26.2 ± 6.8	19.9 ± 8.3	23.9 ± 15.3	44.6 ± 26.4	17.0 ± 10.6	27.5 ± 13.1	20.6 ± 11.9	13.6 ± 11.4	15.5 ± 7.2	26.4 ± 11.1	17.8 ± 6.1	20.5 ± 9.6	24.0 ± 18.4
Long. br. prd. (min)	27.9 ± 8.0	5.1 ± 5.3‡	10.0 ± 8.5	12.2 ± 8.2	10.1 ± 7.1	6.6 ± 7.7‡	20.2 ± 6.9	15.7 ± 14.2	22.8 ± 15.5	7.8 ± 3.8‡	14.5 ± 6.7	18.2 ± 9.5	19.7 ± 13.1
pH	7.34 ± 0.03	7.33 ± 0.05	7.35 ± 0.03	7.34 ± 0.06	7.32 ± 0.06	7.33 ± 0.07	7.33 ± 0.06	7.33 ± 0.05	7.32 ± 0.07	7.32 ± 0.04	7.31 ± 0.03‡	7.32 ± 0.05	7.25 ± 0.04§
Paco ₂ (kPa)	5.3 ± 0.6	5.1 ± 0.8	4.9 ± 0.9	5.5 ± 0.5	5.0 ± 0.7	5.5 ± 1.4	5.4 ± 0.7	5.4 ± 0.5	5.3 ± 0.9	5.3 ± 0.8	5.3 ± 0.7	5.6 ± 0.4	6.7 ± 1.4†
Pao ₂ (kPa)	2.7 ± 0.6	2.5 ± 0.7	2.9 ± 0.9	2.7 ± 0.6	2.6 ± 0.4	2.5 ± 0.6	2.7 ± 0.5	3.6 ± 2.6	4.1 ± 2.9	2.7 ± 0.6	3.0 ± 0.7	4.1 ± 2.3	15.3 ± 5.8§
HCO ₃ (mmol/L)	20.7 ± 1.1	20.0 ± 6.0	19.9 ± 3.3	21.6 ± 2.5	19.7 ± 3.9	20.7 ± 3.5	21.0 ± 4.2	20.6 ± 2.3	20.6 ± 3.9	20.1 ± 2.2	20.1 ± 3.0	21.4 ± 2.4	21.2 ± 3.4
BD/BE (mmol/L)	-3.4 ± 1.0	-4.1 ± 3.8	-3.5 ± 2.7	-2.7 ± 3.4	-4.8 ± 3.9	-3.5 ± 3.3	-3.3 ± 4.5	-3.5 ± 2.8	-3.5 ± 3.9	-4.2 ± 1.8	-4.4 ± 2.8	-3.0 ± 3.0	-4.9 ± 2.6
Syst BP (mm Hg)	60.0 ± 7.7	63.4 ± 8.6	64.5 ± 10.5	64.6 ± 3.9	62.4 ± 9.2	60.8 ± 11.4	62.0 ± 10.0	61.2 ± 9.6	59.7 ± 8.9	64.8 ± 7.1	63.0 ± 5.4	64.0 ± 4.9	67.2 ± 5.6
Diast BP (mm Hg)	41.7 ± 6.7	43.2 ± 9.1	45.5 ± 9.6	43.2 ± 8.3	43.2 ± 9.5	42.6 ± 10.8	43.8 ± 9.0	43.2 ± 7.0	40.7 ± 10.3	43.8 ± 7.9	43.8 ± 7.4	44.3 ± 5.7	45.8 ± 4.8
Mean BP (mm Hg)	47.8 ± 6.7	49.9 ± 8.5	51.8 ± 9.3	50.3 ± 6.3	49.6 ± 9.1	48.7 ± 10.9	49.9 ± 9.1	49.2 ± 7.6	47.0 ± 9.7	50.8 ± 7.4	50.2 ± 6.4	50.9 ± 5.1	52.9 ± 4.8
Heart rate (bpm)	162.0 ± 21.0	164.0 ± 22.0	170.0 ± 40.0	137.0 ± 21.0	140.0 ± 10.0	155.0 ± 18.0	147.0 ± 31.0	151.0 ± 27.0	156.0 ± 32.0	146.0 ± 20.0	164.0 ± 29.0	157.0 ± 28.0	147.0 ± 20.0

* Values are mean ± SD. % BT, percentage of breathing time; % BT/LV, ratio of breathing time during LV-ECoG; Dur. arousal, duration of arousal; Long. ap. prd., longest apneic period; Long. br. prd., longest breathing period; BD/BE, base deficit/base excess; Syst BP, Systolic blood pressure; and Diast BP, Diastolic blood pressure.

† $p \leq 0.02$.

‡ $p \leq 0.05$.

§ $p \leq 0.01$.

|| torr = 0.1333 kPa.

N₂ at any given CPAP; however, the percentage of breathing time was only decreased at a CPAP of 30 cm and 100% N₂ ($p = 0.01$; Table 1).

Gestational age 138 ± d. Six fetuses were studied in this group. The results are summarized in Table 2. Breathing time and duration of arousal significantly increased in response to lung distension with 100% O₂ at a CPAP of 30 cm H₂O ($p = 0.04$ and 0.03 , respectively). There was a significant increase in PaO₂ and a decrease in pH and base excess. Lung distension with N₂ at a CPAP of 20 and 30 cm H₂O decreased the percentage of total breathing time. The longest apneic episode decreased at a CPAP of 30 cm and 50 and 100% O₂, but did not reach statistical significance ($p = 0.13$). The single longest breathing episode decreased at 10 and 20 cm H₂O with most inspired O₂ concentrations. Cardiovascular responses remained unchanged (Table 2). In four of six fetuses, lung distension with 100% O₂ at a CPAP of 30 cm H₂O resulted in the induction of breathing, which remained continuous until either CPAP or the O₂ concentration was brought to a lower level. In these four fetuses, breathing time, breathing time/LV-ECOG ratio, duration of arousal, longest single breathing epoch, PaO₂, and base deficit significantly increased from control values of $32.5 \pm 10.9\%$, $70.7 \pm 24.1\%$, 0 ± 0 min, 18.4 ± 8.2 min, 2.8 ± 0.7 kPa, and 3.8 ± 1.8 mmol/L to $100 \pm 0\%$, $163.4 \pm 11.43\%$, 27 ± 7.8 min, 44.4 ± 11.8 min, 31.6 ± 9.5 kPa, and 6.3 ± 2.2 mmol/L, respectively, during lung distension with 100% O₂ at 30 cm H₂O ($p = 0.001$, 0.02 , 0.006 , 0.004 , 0.01 , and 0.025 , respectively), whereas pH and longest apneic episode decreased from 7.28 ± 0.04 and 39.3 ± 23.5 min to 7.22 ± 0.03 and 0 ± 0 min, respectively ($p = 0.02$ and 0.04 , respectively). Percentages of breathing during HV-ECOG under control and experimental conditions were 0 ± 0 and 26.1 ± 18 , respectively ($p = 0.04$). The PaCO₂ did not change ($p = 0.56$). Also, cardiovascular responses remained unchanged. The lag time varied from 9 to 30 min (9.9 ± 14.1 min). The onset of arousal always coincided with the onset of breathing and was significantly longer than observed during control periods ($p = 0.03$; Fig. 2). There was an inverse correlation between pH and PaO₂ ($p = 0.005$).

Labor. Six fetuses were studied during spontaneous onset of labor. All were delivered within 12 h. Two fetuses had only modest increases in PaO₂—to 5.4 and 6.6 kPa from 2.0 and 2.1 kPa, respectively (to 40.3 and 49.6 torr from 15.0 and 16.7 torr, respectively). Both were delivered within 4 h. The percentage of breathing time among all six fetuses was 7.8 ± 5.6 and 42.9 ± 33.7 during control and experimental conditions, respectively ($p = 0.03$). No breathing was noted during HV-ECOG under resting conditions, whereas $38.23 \pm 15.4\%$ of the total breathing occurred during HV-ECOG under experimental conditions. The arterial pH decreased from 7.28 ± 0.10 to 7.26 ± 0.07 , PaO₂ increased from 2.4 ± 0.6 to 20.8 ± 14.5 kPa, and PaCO₂ remained unchanged at 5.9 ± 0.4 and 6.4 ± 0.7 under control and experimental conditions, respectively ($p = 0.04$, 0.03 , and 0.09 , respectively). The increase in PaO₂ was achieved with a CPAP of 30 cm H₂O in two fetuses and with 40, 45, 55, and 60 cm H₂O in the remaining four. Once again, fetal arousal coincided with the onset of breathing (Fig. 3). The breathing movements and PaO₂ gradually decreased as the frequency and amplitude of uterine contractions increased. Systolic, diastolic, and mean BP (mm Hg) were 71 ± 13 and 69 ± 19 , 46 ± 13 and 44 ± 12 , and 54 ± 13 and 53 ± 13 under control and experimental conditions, respectively ($p = 0.74$, 0.46 , and 0.56 , respectively). Heart rate was 157 ± 13 and 146 ± 16 ($p = 0.35$) under control and experimental conditions, respectively.

DISCUSSION

This study provides new evidence that fetal arousal and stimulation of FBM during quiet sleep may be induced by lung distension and an increase in fetal PaO₂. These breathing and behavioral responses are critically dependent on fetal maturity,

an association not observed in the previous study (9). Furthermore, FBM can be stimulated during both LV- and HV-ECOG during labor when, normally, FBM are either absent or are greatly reduced (16).

Isocapnic hypoxemia suppresses fetal breathing through a central mechanism, inasmuch as transection at midcollicular level as well as lateral pontine lesions leads to disinhibition of breathing during hypoxia (17–19). Therefore, it is conceivable that an increase in PaO₂ above the normal fetal level disinhibits the central inhibitory mechanism, which is set for the normal fetal PaO₂ range. However, we did not observe disinhibition of breathing during HV-ECOG until 136 d of gestation, even though this central inhibitory mechanism is operative in fetuses younger as well as older than 136 d. Because we did not investigate the threshold range of PaO₂ for stimulation of breathing, we cannot determine if the lack of stimulation in young fetuses was due to neural immaturity or if a very high level of PaO₂ (which was not achieved in the younger fetuses) is required to stimulate breathing. Current knowledge would suggest that the former hypothesis is correct (20).

An increase in fetal PaO₂ has been produced by several investigators either by placing the ewe in a hyperbaric chamber or by intermittent positive pressure ventilation of the fetal lungs (21–25). However, breathing movements were not measured in any of these studies. Blanco *et al.* (26) were able to oxygenate the fetus by *in utero* ventilation but no change in FBM was observed. The discrepancy between our results and those of Blanco *et al.* was probably due to the younger gestational age of their fetuses (120–127 d); also, we did not notice an increase in FBM until after 134 d of gestation. It is not known if there is a graded breathing response to various levels of arterial PaO₂ or if there is a threshold range of PaO₂ at which arousal and/or stimulation of FBM occur. An interesting observation was the wide variability in the lag time from the change of O₂ concentration to the onset of arousal and breathing. This lag time may represent the time taken for the PaO₂ to increase to a level that is critical for the induction of arousal and/or breathing. An increase in O₂ tension may lead to cerebral vasoconstriction and, consequently, a decreased cerebral blood flow (27). Such changes in cerebral circulation could give rise to a widened arteriovenous CO₂ difference, resulting in a rise in sagittal sinus and tissue PCO₂, and an increase in fetal PCO₂ is well known to stimulate FBM (2, 3, 5).

We observed that an increase in fetal PaO₂, with or without an increase in PaCO₂, and in the presence or absence of continuous FBM, was associated with a significant decrease in arterial pH. This change in metabolic milieu might have been due to alterations in fetal cardiovascular dynamics and fetoplacental blood flow induced by CPAP. An increase in intrathoracic pressure induced by CPAP would be more profound in relatively mature fetuses due to an increase in pulmonary compliance. This decrease in pH (metabolic acidemia) might have resulted from decreased cardiac output, leading to poor tissue perfusion and impaired O₂ delivery to meet the fetal metabolic demands (anaerobic metabolism), and from decreased umbilical and renal blood flows, leading to an accumulation of metabolic end products (28, 29). A decrease in fetal tissue PO₂ in response to increased PaO₂ has been reported by Towell *et al.* (30). The decreased umbilical blood flow might result from attenuated venous return due either to increased intrathoracic pressure or to the direct contractile effects of O₂ on the umbilical cord as suggested by Lewis (31). Because the decrease in pH was not observed when fetal lungs were distended with N₂ or gas mixtures containing less than 100% O₂, the decrease in pH cannot be attributed exclusively to an impairment of cardiac output. Although the effects of *in utero* CPAP on fetal cardiovascular dynamics have not been studied, the effects of *in utero* intermittent positive pressure ventilation are well documented (24, 25). Teitel *et al.* (25) did not observe a decrease in pH with an increase in PaO₂ until the umbilical cord was occluded, but the observations of Blanco *et al.* (26) were similar to ours. Metabolic acidemia is known to stimulate FBM,

Table 2. Cardiorespiratory and behavioral responses to lung distension and oxygenation in six fetuses at 138 ± 1 d gestational age*

Variable	CPAP level (cm H ₂ O)												
	Control	10				20				30			
		100% N ₂	21% O ₂	50% O ₂	100% O ₂	100% N ₂	21% O ₂	50% O ₂	100% O ₂	100% N ₂	21% O ₂	50% O ₂	100% O ₂
% BT	34.9 ± 9.5	13.3 ± 19.5	22.7 ± 28.5	12.6 ± 18.4	11.6 ± 14.5	6.8 ± 7.8†	13.2 ± 15.4	13.2 ± 26.4	16.0 ± 21.9	14.7 ± 16.7†	26.3 ± 27.3	49.7 ± 29.3	76.6 ± 36.4†
% BT/LV	75.4 ± 18.4	26.7 ± 46.2	63.5 ± 31.2	39.7 ± 42.0	36.9 ± 39.1	32.8 ± 30.1	60.0 ± 30.0	31.1 ± 54.0	43.7 ± 46.9	39.0 ± 39.4	37.0 ± 34.8	76.3 ± 45.5	126.3 ± 53.0
Dur. arousal (min)	0.0 ± 0.0	1.4 ± 1.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.58 ± 1.2	0.0 ± 0.0	0.0 ± 0.0	22.3 ± 28.6	18.5 ± 14.5‡
Long. ap. prd. (min)	34.6 ± 19.7	30.0 ± 9.6	23.0 ± 14.1	30.5 ± 6.8	33.2 ± 12.9	37.1 ± 6.2	29.5 ± 13.4	27.2 ± 10.7	30.1 ± 14.6	27.5 ± 11.8	25.6 ± 13.3	15.2 ± 13.6	11.3 ± 14.0
Long. br. prd. (min)	21.2 ± 7.7	7.3 ± 11.0	10.5 ± 14.6	4.7 ± 7.0§	5.3 ± 5.6§	3.0 ± 3.4	5.2 ± 6.0	6.8 ± 11.5	7.3 ± 8.4‡	6.2 ± 7.5§	11.4 ± 17.0	37.02 ± 33.0	34.0 ± 18.5
pH	7.30 ± 0.04	7.33 ± 0.08	7.31 ± 0.01	7.33 ± 0.02	7.31 ± 0.01	7.34 ± 0.05	7.34 ± 0.04	7.34 ± 0.02	7.33 ± 0.03	7.31 ± 0.05	7.31 ± 0.06	7.30 ± 0.05	7.25 ± 0.05
Paco ₂ (kPa)	5.9 ± 0.7	5.1 ± 0.7	4.6 ± 0.4§	5.3 ± 0.5	5.2 ± 0.5	5.3 ± 0.8	5.2 ± 0.5	5.4 ± 0.7	5.1 ± 0.4‡	5.1 ± 0.8†	5.2 ± 0.6†	5.4 ± 1.0	6.2 ± 1.0
Pao ₂ (kPa)	2.9 ± 0.7	3.0 ± 1.1	3.3 ± 1.0	3.0 ± 1.0	3.1 ± 1.1	3.2 ± 0.9	3.3 ± 1.2	3.7 ± 1.8	4.7 ± 4.1	3.1 ± 1.0	3.9 ± 0.8	8.6 ± 7.1	23.4 ± 14.8§
HCO ₃ (mmol/L)	21.3 ± 1.7	19.6 ± 1.1	17.6 ± 4.2	20.2 ± 3.2	19.7 ± 4.4	21.0 ± 2.9	20.7 ± 1.4	20.6 ± 2.8	18.3 ± 2.5	18.9 ± 2.7§	19.4 ± 2.7	19.6 ± 2.7†	19.9 ± 2.3
BD/BE (mmol/L)	-3.6 ± 1.6	-4.3 ± 2.6	-6.3 ± 5.7	-4.1 ± 4.7	-4.7 ± 5.5	-3.1 ± 2.8	-3.1 ± 1.5	-2.9 ± 2.3	-5.1 ± 0.9	-5.2 ± 2.5‡	-4.9 ± 3.2†	-5.0 ± 2.5†	-5.8 ± 2.4†
Syst BP (mm Hg)	67.8 ± 11.1	76.3 ± 13.9	78.0 ± 14.7	70.0 ± 15.2	72.3 ± 13.1	74.0 ± 16.5	72.8 ± 17.5	73.0 ± 15.8	73.0 ± 16.9	70.8 ± 18.3	74.2 ± 13.6	66.8 ± 16.7‡	66.4 ± 15.2
Diast BP (mm Hg)	44.5 ± 10.8	45.5 ± 6.6	51.5 ± 9.0	45.8 ± 10.7	43.8 ± 11.0	47.3 ± 11.4	47.8 ± 13.0	45.5 ± 11.7	47.5 ± 12.5	45.6 ± 12.8	50.0 ± 10.2‡	45.8 ± 11.5	41.2 ± 9.0
Mean BP (mm Hg)	52.2 ± 10.0	55.8 ± 10.0	60.3 ± 10.9	53.9 ± 12.1	53.3 ± 11.5	56.2 ± 13.1	56.1 ± 14.4	54.7 ± 13.1	56.0 ± 13.9	58.0 ± 13.3	57.1 ± 12.5	56.0 ± 12.83	52.5 ± 10.3
Heart rate (bpm)	140.0 ± 12.0	146.0 ± 17.0	146.0 ± 16.0	145.0 ± 13.0	143.0 ± 12.0	144.0 ± 14.0	147.0 ± 16.0	132.0 ± 25.0	145.0 ± 18.0	138.0 ± 10.0	134.0 ± 15.0	144.0 ± 28.0	144.0 ± 23.0

* Values are mean ± SD. % BT, percentage of breathing time; % BT/LV, ratio of breathing time during LV-ECOG; Dur. arousal, duration of arousal; Long. ap. prd., longest apneic period; Long. br. prd., longest breathing period; BD/BE, base deficit/base excess; Syst BP, Systolic blood pressure; and Diast BP, Diastolic blood pressure.

† $p \leq 0.05$.

‡ $p \leq 0.03$.

§ $p \leq 0.02$.

|| $p \leq 0.01$.

Fig. 2. Effects of lung distension at 139 d of gestation. Representative tracing showing the control period (A) and the effects of lung distension with 0% O₂ (100% nitrogen) (B) and with 100% oxygen (C). The pattern of FBM was similar during the control period (A) and during lung distension with nitrogen (B). However, stronger and continuous FBM occurred during both LV- and HV-ECoG when oxygen concentration was changed to 100% (C) and (D). The onset of continuous FBM coincided with the onset of arousal (C).

Fig. 3. Effects of lung distension during labor. Representative tracing showing the control and the effects of lung distension during spontaneous onset of labor at 142 d of gestation. During the control period (A), no spontaneous breathing activity was present. Lung distension with 0% O₂ (100% nitrogen) at a CPAP of 30 cm H₂O did not induce breathing movements (B); however, 100% O₂ induced breathing (C), which continued during both LV- and HV-ECoG (D).

but such stimulation of breathing has a latency period of 60 to 400 min, depending on the agent used to produce metabolic acidosis (32, 33). The lag period in our studies was 0 to 30 min, and, although the decrease in pH in our younger fetuses did not stimulate breathing, in the studies by Molteni *et al.* (32), stimulation of breathing by metabolic acidosis was independent of gestational age. Therefore, our results do not suggest that metabolic acidemia played a primary role in the induction of continuous FBM. In the present study, cardiovascular responses during experiments were not different from the control condition. Similar results have been reported previously (9, 26).

The incidence of total breathing time under control conditions

was similar to that reported previously by several investigators (2, 5). However, the length of the single longest breathing epoch as well as the overall incidence of total breathing time was significantly decreased in response to lung distension with N₂ at higher pressure in fetuses of greater than 134 d of gestation. This decrease in breathing might have been due to the stimulation of slowly adapting pulmonary stretch receptor-fiber-afferent systems, inasmuch as their activity during fetal life has been reported (34).

Because the onset of fetal arousal always coincides with the onset of continuous breathing, we cannot determine if there is a causal relationship between arousal and the onset of breathing

or if a common modulator such as an increase in fetal PaO_2 mediated both behavioral and breathing responses. Arousal is known to influence respiratory activity during adult as well as fetal life (10, 35). On the other hand, respiratory stimuli such as hypercapnia, hypoxia, airway occlusion, and bronchopulmonary irritation may induce arousal (11, 36). The threshold level for arousal is dependent on the prevailing sleep state (11). It is conceivable that bronchopulmonary irritation produced by high concentrations of inspired O_2 or increased concentrations of prostaglandins in the pulmonary circulation might have led to arousal via rapidly adapting receptors or nonmyelinated C fibers (37). The increased prostaglandins I_2 (prostacyclin) and prostaglandin E_2 concentrations might have resulted from either direct pulmonary mechanical effects of lung distension or from increased pulmonary blood flow due to the static distension of fetal lungs, respectively (38, 39). This mechanism appears to be unlikely, inasmuch as prostaglandins, especially prostaglandin E_1 and E_2 , are not only metabolized rapidly by the lungs, but are also known to suppress fetal and neonatal breathing (39–41). However, to the best of our knowledge, effects of prostaglandin I_2 (prostacyclin) on FBM have not been studied. In a well-executed study, Adamson *et al.* (42) induced continuous fetal breathing by occluding the umbilical cord and providing the fetus with a mixture of 50% O_2 in air at a CPAP of 3 mm Hg. Umbilical cord release resulted in a significant decrease in breathing activity, suggesting that the response might have been mediated by a hormone or a neurotransmitter produced by the placenta. They also observed the invariable presence of arousal at the onset of continuous breathing movements (42). Similar breathing and behavioral changes have been reported in human as well as sheep fetuses at the time of birth (43, 44). Although arousal was always present at the initiation of continuous breathing, breathing, once initiated, continued during all sleep states.

Several studies, including our present study, have shown that fetal breathing movements decrease 2 to 3 d before labor and almost cease during the active stage of labor (16, 45). The possible mechanisms for this decrease in breathing have been discussed in detail by Berger *et al.* (16). Nathanielsz *et al.* (46) have reported that episodic increases in myometrial tone, known as contractures, occurred at a rate of approximately 1/h and were associated with a shift from fetal breathing activity to quiescence. These episodic contractures may be associated with the decreased cotyledonary blood flow, leading to a decrease in fetal PaO_2 . Considering this mechanism, one would expect a more profound decrease in fetal PaO_2 during active labor that could account for complete fetal apnea; however, in our studies we could oxygenate the fetus in labor, thereby alleviating hypoxemia and inducing breathing movements. However, during the final hours of labor, we were unable to oxygenate the fetus and, consequently, we did not observe any change in breathing or behavioral responses.

In summary, we have shown that fetal maturity, an increase in PaO_2 , and a change in state from quiet sleep toward arousal are necessary conditions for the induction of continuous FBM. Furthermore, FBM can be stimulated during labor during both LV- and HV-ECOG. The underlying mechanisms of these new and fascinating responses remain unknown and warrant further studies.

Acknowledgments. The authors thank Dr. Tak Fung for painstaking statistical analysis, Naomi Anderson for editorial comments, and Michelle Cavanaugh for excellent secretarial support. We are especially indebted to Dr. James E. Fewell for providing thoughtful suggestions during these experiments.

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