Arterial Oxygen Tension Threshold Range for the Onset of Arousal and Breathing in Fetal Sheep¹

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ABSTRACT. Mechanisms for the control of episodic fetal breathing movements or the onset of continuous breathing at birth remain unknown. Lung distension with 100% O₂ at a continuous positive airway pressure of 30 cm H₂O may induce arousal and continuous breathing. To investigate 1) the threshold range of arterial oxygen tension (PaO₂) for the onset of arousal and breathing and 2) the graded response of breathing to various levels of PaO₂, we studied 10 fetal sheep between 135 and 142 d of gestation (term = 147 ± 2 d). Each fetus was instrumented to record sleep states, diaphragmatic electromyogram, arterial pH, and blood gas tensions. PaO₂ threshold was determined through an indwelling O₂ sensor catheter. Fetal lungs were distended at a continuous positive airway pressure of 40 cm H₂O with 100% N₂ or with O₂ ranging from 40 to 100% via an *in situ* endotracheal tube. At the onset of arousal (*n* = 10), PaO₂, arterial carbon dioxide tension, and Hb O₂ saturation increased from control values of 21.7 ± 0.75 torr (2.9 ± 0.09 kPa), 41.8 ± 1.1 torr (5.47 ± 0.15 kPa), and 52.9 ± 2.6% to 65.6 ± 9.6 torr (8.74 ± 1.28 kPa), 46.9 ± 1.3 torr (6.25 ± 0.17 kPa), and 92.9 ± 2.06%, respectively, whereas the pH decreased from 7.31 ± 0.006 to 7.27 ± 0.009 (mean ± SEM; *p* = 0.001, 0.04, 0.002, and 0.001, respectively). Seven of 10 fetuses breathed continuously. In these fetuses, PaO₂ and arterial carbon dioxide tension further increased and pH decreased; however, no further significant increase in Hb O₂ saturation was observed. Breathing stopped at a PaO₂ of 38.5 ± 9.5 torr (5.1 ± 1.3 kPa) but could be restarted by increasing the PaO₂. In response to an increase in fetal PaO₂ (30–60 torr; 4–8 kPa), amplitude of breathing and total respiratory output significantly increased from the control values. Further increases in PaO₂ or Hb O₂ saturation did not significantly affect the respiratory output. Hb O₂ saturation and arterial pH had the most confounding effects on frequency and amplitude of breathing, respectively. Among the various sleep states, arousal had the most profound effects on respiratory output. We conclude that 1) very high levels of PaO₂ are not necessary to initiate arousal and continuous breathing and 2) further increments in Hb O₂ saturation above the threshold level have no significant effect on breathing responses. (*Pediatr Res* 32: 342–349, 1992)

Abbreviations

AP, amniotic pressure

BP, blood pressure
CPAP, continuous positive airway pressure
EMG_{Di}, Diaphragmatic electromyogram
EMG_{NK}, nuchal electromyogram
EOG, electro-oculogram/eye movements
FBM, fetal breathing movements
ECoG, electrocorticogram
HV-ECoG, high-voltage ECoG or electrocortical activity
LV-ECoG, low-voltage ECoG or electrocortical activity
ID, inner diameter
OD, outer diameter
PaO₂, arterial oxygen tension
PaCO₂, arterial carbon dioxide tension

Fetuses of mammalian species make episodic breathing movements (1, 2). To maintain an independent life, the fetus must establish continuous breathing at birth. Mechanisms for the control of episodic FBM or the establishment of continuous breathing at birth remain unknown. In sheep, during late gestation, FBM occupy 40% of the total time and normally occur only during LV-ECoG but are absent during HV-ECoG (3). Recent studies have shown that an increase in fetal PaO₂ achieved by distending the fetal lungs with 100% O₂ at a CPAP of 30 cm H₂O may stimulate FBM during both LV- and HV-ECoG (4, 5). In our previous study (5) initiation of breathing was always associated with the onset of fetal arousal and was critically dependent on fetal maturity. However, in these studies, fetal PaO₂ levels were as high as 250 torr (33.3 kPa). Furthermore, the range of PaO₂ has also been very wide (4, 5), and it is not known whether there is a graded response of FBM to various levels of PaO₂. Therefore, we designed a study to investigate 1) the PaO₂ threshold range for the onset of arousal and breathing and 2) the graded response of breathing to various levels of PaO₂.

MATERIALS AND METHODS

Animal preparation. Ten time-dated pregnant ewes underwent surgery between 129 and 133 d of gestation, and the fetuses were studied between 135 and 142 d of gestation (term = 147 ± 2 d). Surgery was performed under halothane general anesthesia; 4% for induction and 1.5 to 2% for maintenance. Instrumentation of the fetus included implantation of 1) four Teflon-coated and color-coded electrode wires (Conner Wire Company, Chatsworth, CA), 2) a carotid arterial catheter, 3) a jugular venous catheter, 4) an endotracheal tube, and 5) two amniotic catheters. The details of surgical procedures have been given elsewhere (5). Briefly, a pair of electrode wires was implanted over the parietal area through two holes drilled in the fetal skull to record ECoG, whereas the EOG was obtained by implanting a silver-coated, oval-shaped electrode in the lateral rectus muscle of one eye. The EMG_{NK} was recorded by suturing a pair of electrodes in the

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lateral neck muscle. ECoG, EOG, and EMG_{NK} were used to define the fetal sleep states. The EMG_{Di} was recorded from a pair of electrodes implanted in the right hemidiaphragm through an incision in the 10th intercostal space. The fetal carotid artery was catheterized with a 5 Fr neonatal O₂ sensor catheter (Neocath, NA 1005, Biomedical Sensors Ltd., High Wycombe, England) to continuously monitor the fetal PaO₂, record BP and heart rate, and withdraw blood samples to analyze pH and blood gas tensions; a polyvinyl catheter (2.0 mm ID, 3.0 mm OD; Portex, Hythe, Kent, UK) was placed in the jugular vein to administer antibiotics. To apply CPAP to the fetal lungs, a polyvinyl endotracheal tube (4.8 mm ID, 7.9 mm OD; Tygon Norton Plastics, Akron, OH) was inserted through an incision 2.0 cm below the thyroid cartilage, advanced for 5–7 cm, and transfixed (0-silk) to the trachea. Two (4.0 silk) sutures were tied around the trachea to minimize the air leak during experiments. To decrease the dead space, 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). The free ends of the Y connector were, in turn, connected to two polyvinyl extension tubes (4.8 mm ID, 7.9 mm OD, Tygon) serving as inspiratory and expiratory extension tubes. Two polyvinyl catheters (4.9 mm ID, 7.0 mm OD and 2.0 mm ID, 3.0 mm OD; Tygon) were placed in the amniotic cavity and secured to the back of the fetal neck. The smaller diameter amniotic catheter was used to monitor the AP and also to subtract the fetal BP, whereas the larger amniotic catheter was connected (Perfectum Adapters; Popper & Sons, Inc., New Hyde Park, NY) to one of the Y-endotracheal tubes to facilitate the tracheoamniotic fluid flow. The second extension of the endotracheal tube remained plugged when no experiments were being conducted. In addition to the fetal instrumentation, a polyvinyl catheter (2.4 mm ID; 4.0 mm ID) was inserted in the maternal jugular vein to infuse fluids intraoperatively and to administer antibiotics postoperatively. After the instrumentation was completed, the fetus was returned to the amniotic cavity and all fetal and maternal incisions were sewn in layers. Routine postoperative management included food and water *ad libitum*, antibiotic prophylaxis for 4 d, and heparinized saline flush and infusion (Harvard infusion pump, model 901; Harvard Apparatus Co., Inc., South Natick, MA) to maintain the patency of vascular catheters.

Data acquisition and analysis. Immediately after surgery, the ewes were housed in metabolic carts and electrophysiologic signals from ECoG, EOG, EMG_{NK}, and EMG_{Di} along with AP and BP were recorded on an eight-channel chart recorder (Gould 2800S; Gould Inc., Cleveland, OH). ECoG, EOG, EMG_{NK}, and EMG_{Di} were amplified and filtered through NeuroLog System (NL 100 AK, 102G 125/6; Medical Systems Corporation, Greenvale, NY) to record frequency ranges of 0.5 to 40 Hz, 5 to 40 Hz, 50 Hz to 1 kHz, and 50 Hz to 1 kHz, respectively. The AP and fetal BP were calibrated twice daily and recorded with Statham P23 ID pressure transducers. During experiments, all signals were digitized using an eight-channel Neuro-corder (Neurodata Instruments Corporation, New York, NY) and recorded on a videocassette recorder (model FVH C4000; Fisher, Toronto, Ontario, Canada). The O₂ sensor catheter was connected to the monitor (Neocath neonatal oxygen monitor, NM-1001-0) and calibrated at least once a day and more often if a discrepancy existed between the monitor and the blood gas analyzer (IL 1301; Instrumentation Lab Systems, Lexington, MA). The response time of the Neocath O₂ monitor system is 60 ± 30 s (90% step change). Furthermore, this system measures O₂ tensions at the subject's temperature.

Continuous FBM were defined as those that not only remained continuous but also occurred during both LV-ECoG and HV-ECoG. Electrographic criteria were used to define the fetal behavioral states. Active sleep was defined by the simultaneous presence of LV-ECoG and eye movements and absence of nuchal tone; quiet sleep was defined by the simultaneous presence of HV-ECoG and nuchal tone, and absence of eye movements.

Fetal arousal was defined by the simultaneous presence of LV-ECoG, nuchal tone, and eye movements (6, 7).

The analyses of cardiorespiratory and behavioral responses included 1) delineation of all three behavioral states as described above; 2) measurement of lag period in min, defined as the time elapsed between the change of O₂ concentration and the onset of arousal and/or breathing; however, the change in PaO₂ does not include the 60 ± 30-s response time of the monitoring system; 3) integration of EMG_{Di} and the integrated signal measurement of frequency (F; the number of diaphragmatic contractions per min of breathing time), amplitude of breathing (JEMG_{Di}) in arbitrary units, and respiratory output (F × EMG_{Di}) during control and at various PaO₂ levels as well as during active sleep, quiet sleep, and awake states; control period refers to the time when no experimental maneuvers were being made; 4) measurement of arterial pH and blood gas tensions; 5) indirect measurements of Hb O₂ saturation; both blood gas tensions and Hb O₂ saturations were corrected to 39.5°C; 6) systolic, diastolic, and mean BP; and 7) heart rate (beats/min), calculated from the peaks of the systolic BP. To investigate the graded response of breathing to various levels of PaO₂, PaO₂ was divided into 1) control, 2) 30–60 torr (4.0–8.0 kPa), 3) 61–120 torr (8.0–16 kPa), and 4) 121–300 torr (16–40 kPa). The corresponding mean ± SEM and range of Hb O₂ saturations were 1) 52.9 ± 2.6%, range 40.4–64.1%; 2) 86.3 ± 1.94%, range 73.3–94.1%; 3) 97.2 ± 0.47%, range 95.1–99.2%; and 4) 99.7 ± 0.1%, range 99.1–99.9%.

Experimental design. No experiments were done for at least 4 d after surgery and only after fetal breathing, arterial pH, and blood gas tensions were within the physiologic range, which in our laboratory usually occurs within 48 h. The experimental design is shown in Figure 1. Before each experiment, the physiologic variables AP, BP, ECoG, EOG, EMG_{NK}, and EMG_{Di} were recorded overnight on a chart recorder. For detailed future analyses, all physiologic variables were recorded on a videocassette for at least 3 h before and during the entire experiment. Experiments were conducted between 1000 h and 1800 h to avoid the morning trough and evening peak periods of FBM, respectively. As soon as the control period was obtained, fetal trachea was suctioned through one of the endotracheal tube extensions to remove the existing tracheal fluid, the patency of both endotracheal tubes was checked to avoid pneumothorax, and fetal lungs were distended at a CPAP of 40 cm H₂O (relative to atmospheric pressure) using a Baby Bird Ventilator (Bird Corp., Palm Springs, CA) with either N₂ or 40% O₂ in randomized order. In our previous study (5), fetal lungs were distended with CPAP of 30 cm H₂O. However, after completing the protocol in that study, we increased the CPAP to 40 cm H₂O and found that an increased number of fetuses could be oxygenated. Therefore, based on those (unpublished) observations, we elected to choose 40 cm H₂O instead of 30 cm H₂O. Nitrogen was added to the protocol as a control for gaseous distension. Forty percent O₂ concentration was chosen because in our previous studies, 21% O₂ had no significant effect on either breathing or behavior (4, 5). Thereafter, O₂ concentration was increased in increments of 20% until an increase in PaO₂ and/or effects on breathing or behavior were observed. Various concentrations of O₂ were not randomized because, in the past, with a high initial O₂ concentration the fetal PaO₂ rose rapidly, making the determination of PaO₂ threshold impossible. Each concentration of O₂ was maintained for at least 2 h. All changes in gas concentration were made only during HV-ECoG. However, once the breathing was established in both LV-ECoG and HV-ECoG, O₂ concentration was decreased gradually until the breathing ceased. As soon as the cessation of breathing in quiet sleep was observed, O₂ concentration was again increased until a change in breathing and/or behavior was observed.

Statistical analysis. The onset of breathing and arousal and the graded response of breathing to various levels of PaO₂ and Hb O₂ saturations were analyzed using analysis of variance for

Fig. 1. Experimental design. First, a control period was recorded. Each control period consisted of at least two episodes of LV-ECoG and HV-ECoG that normally coincided with periods of breathing and apnea, respectively. Then tracheal suction was followed by lung distension at 40 cm H₂O with 100% nitrogen. Thereafter, the inspired gas mixture was changed to 40% O₂ and increased by 20% until an increase in fetal PaO₂ was noted and/or a change in breathing or behavior was observed. Each concentration of inspired gas was humidified, heated to 39.5°C, and maintained for 1.5 to 2 h.

repeated measures. The significance of differences was assessed by Tukey's procedure (8). The breathing responses were further analyzed while keeping the pH, PaCO₂, PaO₂, and Hb O₂ saturations as a continuous variable. For this purpose, analysis of covariance was carried out with three dependent variables (respiratory output, frequency, and amplitude of breathing), adjustments were made for animal differences, and tests were considered for the impact of pH, PaCO₂, PaO₂, and Hb O₂ saturations. Regression diagnostic techniques were used to assess the adequacy of the fit, and logarithmic transformations were considered for each variable, but the findings remained comparable to the untransformed analysis. Because Hb O₂ saturation is a dependent variable, analysis of covariance was also performed without the Hb O₂ saturation as a covariant. Correlation coefficient analysis was used to determine the association between pH and PaO₂. The changes in pH, PaCO₂, and PaO₂ at the onset of arousal were compared with the control values by paired *t* test. An unpaired *t* test was used to determine whether the arterial pH and blood gas tensions were different in the fetuses that breathed continuously than in those that did not breathe continuously. *n* denotes the number of fetuses. All data are presented as mean $\pm$ SEM, and a *p* less than 0.05 was considered significant.

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

RESULTS

Threshold range of PaO₂ for arousal and breathing. Results from the 10 fetuses manifesting arousal given in Figure 2 show that at the onset of arousal PaO₂ increased from the control value

of 21.7 ± 0.75 torr (2.89 ± 0.1 kPa) to 65.6 ± 9.6 torr (8.78 ± 1.28 kPa, $p = 0.001$), whereas the pH decreased from 7.31 ± 0.006 to 7.27 ± 0.009 ($p = 0.002$). PaCO₂ increased from 41.8 ± 1.1 torr (5.47 ± 0.15 kPa) to 46.9 ± 1.3 torr (6.25 ± 0.17 kPa, $p = 0.041$). Hb O₂ saturation also increased from the control value of $52.9 \pm 2.6\%$ to $92.9 \pm 2.06\%$ at the onset of arousal (analysis of variance; Tukey's procedure confidence interval -37.407 to -51.143).

Seven of 10 fetuses breathed continuously during both LV-ECoG and HV-ECoG. Arterial pH and blood gas tensions from these fetuses are summarized in Figure 3. In these fetuses, PaO₂ increased from a control value of 22.1 ± 0.82 torr (2.95 ± 0.11 kPa) to 69.4 ± 12.7 torr (9.3 ± 1.7 kPa, $p = 0.008$) and 114.8 ± 18.2 torr (15.3 ± 2.43 kPa, $p = 0.002$), whereas pH decreased from 7.30 ± 0.007 to 7.27 ± 0.01 ($p = 0.04$) and 7.24 ± 0.01 ($p = 0.004$) at the onset of arousal and breathing and during breathing in quiet sleep respectively (Fig. 3). The decrease in pH and the increase in PaO₂ during breathing in HV-ECoG were also significant when compared with the arousal values ($p = 0.02$ for both changes). PaCO₂ also significantly increased from control value of 43.2 ± 1.3 torr (5.76 ± 0.17 kPa) to 47.9 ± 1.4 torr (6.39 ± 0.18 kPa) during breathing in quiet sleep, whereas it remained unchanged (46.5 ± 1.9 torr; 6.20 ± 0.25 kPa) at the onset of arousal and breathing ($p = 0.044$ and 0.30 , respectively; Fig. 3). In these fetuses, Hb O₂ saturation increased from a control value of $54.1 \pm 2.4\%$ to $94.53 \pm 4.1\%$ and $96.54 \pm 2.6\%$ at the onset of arousal and stimulation of breathing during quiet sleep, respectively (confidence interval -40.704 to -52.810 and -40.338 to -52.955 , respectively). There was no significant difference in Hb O₂ saturation during arousal and breathing in

Fig. 2. Arterial pH and blood gas tensions during control and at the onset of arousal in all 10 fetuses. pH decreased and both PaCO₂ and PaO₂ increased (mean $\pm$ SEM; $p = 0.002$, 0.041 , and 0.001 , respectively).

Fig. 3. Arterial pH and blood gas tensions in seven fetuses that breathed continuously during both LV-ECOG and HV-ECOG. pH significantly decreased from control values during arousal ($p = 0.04$). Further decrease in pH was observed during breathing in HV-ECOG ($p = 0.004$). The decrease in pH during breathing in HV-ECOG was also significantly different when compared with the arousal values ($p = 0.02$). PaCO₂ changed significantly from the control values only during breathing in HV-ECOG (analysis of variance; $p = 0.044$). PaO₂ significantly increased from the control values during arousal and breathing in HV-ECOG ($p = 0.008$ and 0.002 , respectively). The increase in PaO₂ from arousal values during breathing in HV-ECOG was also significant ($p = 0.02$). *, $p < 0.05$ from the control values; †, $p < 0.05$ from the arousal values.

quiet sleep (confidence interval -6.297 to $+6.519$). Breathing stopped and HV-ECOG prevailed when the PaO₂ was decreased to 38.5 ± 9.5 torr (5.13 ± 1.3 kPa); however, breathing could be restarted by increasing the PaO₂. There were no statistically significant differences in pH, PaCO₂, PaO₂, or gestational ages ($p = 0.369, 0.635, 0.09$, and 0.208 , respectively) of the fetuses that breathed continuously when compared with the fetuses that did not breathe continuously. Figures 4 and 5 are representative tracings from the fetuses that showed arousal and continuous breathing.

The inspired O₂ concentration was 100% for the three fetuses that manifested arousal but did not breathe continuously, whereas the minimum inspired O₂ concentration varied widely among the seven fetuses that manifested both arousal and continuous breathing. Five fetuses manifested arousal and the onset of breathing during administration of 100% O₂, whereas the inspired O₂ concentrations were 40 and 60% O₂ in the remaining two fetuses. Although no lag was observed between the monitored increase in PaO₂ and the stimulation of breathing, a delay of up to 2 min is possible because of the 60 ± 30 -s (90% step change) response time of the indwelling O₂ sensor catheter and monitor system.

Graded response of breathing to various levels of PaO₂ and Hb O₂ saturation. Amplitude and frequency of breathing and total respiratory output under control and various levels of PaO₂ are summarized in Figure 6. In response to an increase in fetal PaO₂ (30–60 torr; 4–8 kPa), both amplitude of breathing and respiratory output increased significantly from control values. The Hb O₂ saturation increased from control values of $52.9 \pm 2.6\%$ (range 40.4–64.1%) to $86.3 \pm 1.9\%$ (range 73.3–94.1%, confidence interval -3.76 to -18.125). Further significant increases in PaO₂ or Hb O₂ saturation did not result in a further increase in these variables (Fig. 6). Frequency of breathing did not significantly increase until the range of PaO₂ was between 60 and 120 torr (8.1–16 kPa) and the Hb O₂ saturation were $97.2 \pm 0.47\%$ (range 96.4–99.2%). Further increase in PaO₂ had no significant effect on the frequency of breathing. Similar to the amplitude of breathing and respiratory output, the increase in frequency was not significantly different among the higher levels of PaO₂ or Hb O₂ saturations (Fig. 6).

Interactions of pH, PaCO₂, PaO₂, and Hb O₂ saturation on breathing responses. When pH, PaCO₂, PaO₂, and Hb O₂ saturation were included as covariants, Hb O₂ saturation had the most profound effect on the frequency of breathing ($p = 0.024$), whereas the decrease in pH had the most significant effect on the amplitude of breathing ($p = 0.05$). Effects of pH on breathing amplitude remained the most significant covariant even when Hb O₂ saturation was excluded from the analysis ($p = 0.021$).

Fig. 4. Representative tracing under control and experimental conditions from a fetus at 140 d of gestation. A, Control recording showing the presence of FBM only during LV-ECOG and the absence of FBM during HV-ECOG. B, Onset of arousal and initiation of breathing, arousal being defined as the simultaneous presence of LV-ECOG, eye movements, and nuchal tone. C, Continuation of breathing during both LV-ECOG and HV-ECOG. D, Continuation of breathing at a PaO₂ of 72.8 torr (9.7 kPa).

However, when the Hb O₂ saturation was excluded as a covariant, the change in PaCO₂ had the most profound effect on the frequency of breathing ($p = 0.015$). Excluding the effects of pH, PaO₂, and Hb O₂ saturation, the rate of change in frequency of breathing was 3.0 per min per unit of PaCO₂ (1 torr or 0.1333

Fig. 5. Representative tracing from a fetus at a gestational age of 137 d showing control, onset, and stimulation of breathing during HV-ECoG. During control (A), an irregular pattern of breathing is present during LV-ECoG and is absent during HV-ECoG. At the onset of breathing, the PaO_2 as recorded from the indwelling O_2 sensor catheter increased from the control value of 23.6 torr (3.15 kPa) to 55 torr (7.33 kPa). Breathing continued during HV-ECoG at a PaO_2 of 64.6 torr (8.61 kPa). During this period, a decrease in pH and an increase in PaCO_2 also was observed.

Fig. 6. Figure showing the JEMG_{Di} (breathing amplitude in arbitrary units), frequency (breaths/min), and respiratory output ($\text{JEMG}_{\text{Di}} \times \text{frequency}$) during control and three different ranges of PaO_2 . Breathing amplitude and respiratory output significantly increased from control values during various PaO_2 levels; however, no graded response was noted among the higher PaO_2 levels. Frequency of breathing significantly increased at PaO_2 levels of greater than 61 torr (8.1 kPa) but again no graded response was observed.

kPa), whereas independent of PaCO_2 and PaO_2 , the amplitude of breathing changed by 0.68 arbitrary units per 0.1 unit change in pH. The effects of pH and PaCO_2 on respiratory output were also significant ($p = 0.009$ and 0.006 , respectively).

Effects of sleep states on breathing. The effects of various sleep states on breathing responses are summarized in Figure 7. Amplitude of breathing increased significantly only during arousal ($p = 0.02$), remaining unaffected during both active and quiet sleep states ($p = 0.62$ and 0.22 , respectively). In contrast, frequency of breathing increased during all sleep states ($p = 0.03$ – 0.02); when compared with the control values, however, this increase was not significantly different among various sleep states ($p = 0.52$ – 0.92). Respiratory output increased during arousal and quiet sleep ($p = 0.003$ and 0.01 , respectively) but did not reach the statistical significance during rapid eye movement sleep ($p = 0.12$; Fig. 7).

Cardiovascular responses. No significant effects on systolic, diastolic, and mean BP or heart rate were noted during lung distension and oxygenation in any sleep state (Table 1).

DISCUSSION

Our present data provide new evidence that high fetal PaO_2 is not required to induce continuous FBM. In fact, the threshold range of PaO_2 falls well within the physiologic range described by other investigators both in sheep and human newborns at or shortly after birth (9, 10). Furthermore, we have shown that an increase in Hb O_2 saturation above the threshold range does not significantly affect the total fetal respiratory output.

Baier *et al.* (4) originally made the observation that a high fetal PaO_2 achieved by distending fetal lungs with 100% O_2 may initiate continuous fetal breathing. We subsequently showed that such stimulation of breathing was critically dependent on fetal maturity and was always associated with the onset of arousal (5). An increase in fetal PaO_2 has been achieved by many investigators (11–15). However, FBM were not observed in any of these studies because of either fetal immaturity or methodologic reasons (11–17). The mechanisms through which an increase in fetal PaO_2

Fig. 7. $JEMG_{Di}$ (breathing amplitude in arbitrary units), frequency (breaths per minute), and respiratory output ($JEMG_{Di} \times$ frequency) during control and various sleep states. Breathing amplitude was significantly higher during arousal ($p = 0.02$) but not during rapid eye movement (REM) or quiet sleep ($p = 0.62$ and 0.22 , respectively). Frequency of breathing significantly increased during all sleep states when compared with the control values. No significant intersleep state changes were observed.

Table 1. Cardiovascular responses during control and various sleep states

	Control	Awake	REM sleep*	Quiet sleep
Systolic BP (torr)†	61.9 ± 3.3	65.5 ± 2.4	65.3 ± 5.6	64.1 ± 3.1
(kPa)	(8.25 ± 0.44)	(8.73 ± 0.32)	(8.70 ± 0.75)	(8.54 ± 0.41)
Diastolic BP (torr)	44.8 ± 2.2	49.2 ± 2.8	48.1 ± 4.7	48.1 ± 2.2
(kPa)	(5.97 ± 0.29)	(6.56 ± 0.37)	(6.41 ± 0.63)	(6.41 ± 0.29)
Mean BP (torr)	50.5 ± 2.5	54.7 ± 2.6	53.9 ± 4.8	53.4 ± 2.5
(kPa)	(6.73 ± 0.33)	(7.29 ± 0.35)	(7.18 ± 0.64)	(7.12 ± 0.33)
Heart rate (beats/min)	156 ± 5.4	159.6 ± 4.8	154.4 ± 5.4	160.5 ± 4.4

* REM, rapid eye movement.

† 1 torr = 0.1333 kPa.

cause stimulation of breathing remain unknown, but several possibilities exist.

In our current study, fetal PaCO₂ increased and pH decreased; both these changes were statistically significant and are well known to stimulate fetal breathing (1, 2, 18, 19). Inasmuch as the increase in PaO₂ and PaCO₂, the decrease in pH, onset of arousal, and stimulation of breathing occur simultaneously, it is difficult to establish a cause and effect relationship. Neither can one differentiate between dependent and independent variables. The reasons for such an increase in PaCO₂ remain unclear. It is conceivable that with an increase in PaO₂ umbilical blood flow decreased, leading to a decrease in the unloading of CO₂, whereas further increase in PaCO₂ during HV-ECOG breathing resulted from increased work of breathing. Also, at that time, further increase in PaO₂ occurred and at least some of the increases in fetal PaCO₂ might have been due to the Haldane effect. We believe that stimulation of breathing, at least during LV-ECOG, might have occurred because of an increase in PaCO₂. However, it is well established that CO₂-stimulated fetal breathing is always restricted to rapid eye movement sleep (1, 2, 18, 19), whereas the stimulation of breathing in our current study occurred during both sleep states. Furthermore, several studies showed that, when fetal sleep states were measured, an increase in fetal PaCO₂ levels as high as 100 torr (13.33 kPa) did not lead to either arousal or breathing during HV-ECOG (18, 19). At the onset of arousal in all 10 fetuses, although there was a statistically significant increase in PaCO₂, we are not aware of any study where an increase of 4–5 torr in PaCO₂ was associated with stimulation of FBM (1, 2, 19–21). However, all hypercapnic studies were done under fetal normoxemic or hypoxemic states, and it is possible that an increase in fetal PaO₂ above the normal fetal level alters the central and/or peripheral sensitivity to CO₂ tension because hypercapnia does not stimulate fetal breathing under hypoxemic conditions (1, 2). Another important and confounding variable in our studies has been the decrease in arterial pH; metabolic acidemia is also well known to stimulate fetal breathing (22, 23). Although there appears to be direct correlation between increased

PaO₂ and decreased pH, the mechanism(s) for this decrease in pH also remain(s) unclear. The implications and possible causes of metabolic acidemia have been discussed in detail in our previous communication (5). In summary, although the increase in PaCO₂ and/or a decrease in pH had significant effects on breathing, they are unlikely to be the sole reason for the stimulation of breathing. This notion is supported in our previous study, where stimulation of breathing was observed while PaCO₂ and pH were kept at constant or near-constant values by high-frequency oscillation (4). However, the important issue of increased intrathoracic pressure and its effects on venous return, organ blood flow, and impairment of cardiac output need to be addressed in future studies.

In our current study, we have endeavored to quantify the relative contributions of pH and PaCO₂ to the amplitude and frequency of breathing and respiratory output. Previous studies by Molteni *et al.* (22) showed that after a minimum lag of 2.5 h a decrease in pH caused an increase in both the depth and the frequency of breathing, whereas Hohimer and Bissonnette (23) reported an increase in the incidence of breathing although they did not provide data regarding the frequency or amplitude of breathing. An increase in fetal PaCO₂ by maternal rebreathing techniques showed an increase in both frequency and amplitude of breathing. However, an increase in PaCO₂, unless compensated, would also cause a decrease in pH. Because Hb O₂ saturation is a dependent variable, analysis of covariance was done with and without it being a covariant. The significant effects of Hb O₂ saturation on breathing frequency might have been caused by Hb O₂ saturation *per se* being dependent on other confounding variables present in our current study. Excluding Hb O₂ saturation as a covariant, analysis of covariance in our current study suggests that the amplitude and frequency of fetal breathing are affected preferentially but not exclusively by fetal arterial pH and PaCO₂, respectively. Furthermore, this analysis also quantifies the per unit change in breathing responses, which has not been reported previously.

At least three studies recently have suggested a role for respi-

ratory inhibitory factor(s) released from the placenta (4, 24, 25). In the study by Adamson *et al.* (24) umbilical cord occlusion led to gasping movements followed by continuous breathing; subsequent cord release resulted in cessation of breathing. However, during cord occlusion, both PaCO_2 and PaO_2 increased significantly to very high levels. In two recent studies, Adamson *et al.* (25) and Baier *et al.* (4) occluded the umbilical cord while maintaining the PaCO_2 near control levels by high-frequency oscillation. However, in the former study (25), baseline PaO_2 was considered to be after the initiation of high frequency oscillation at which time PaO_2 , analyzed at 37°C , ranged from 38 to 47 torr, whereas in the latter study (4) umbilical cord occlusion induced breathing but in association with a further increase in fetal PaO_2 . Therefore, further studies are needed to quantitate the relative roles of oxygenation and umbilical cord occlusion. It is controversial although possible that an increase in fetal PaO_2 in our studies might have led to a reduction (26–28) or cessation of umbilical-placental blood flow (functional cord occlusion), thereby eliminating the presumed blood-borne respiratory inhibitory factor(s) of placental origin. Iwamoto *et al.* (14) showed that the net umbilical blood flow did not decrease in response to fetal oxygenation; however, the relative amount of cardiac output to the placenta decreased. Such a relative decrease could have resulted from a significant increase in the left to right shunt at the ductus arteriosus level, inasmuch as an increase in PaO_2 might lead to a dramatic decrease in pulmonary vascular resistance and an increase in pulmonary blood flow (29). Also, lungs have been shown to be a major site for the metabolism of prostaglandins (30). Therefore, in response to an increase in fetal PaO_2 , increased metabolism of prostanoids might have played a more important role in the stimulation of breathing than did the decrease in umbilical blood flow.

In our current study, the amplitude of breathing and total respiratory output did not show a graded effect to various (higher) levels of PaO_2 . We did not make direct measurements of Hb O_2 saturation; however, indirect measurements of Hb O_2 saturations showed that no further significant increase in Hb O_2 saturation was observed above the threshold level for arousal and breathing. Therefore, it is conceivable that, once the Hb O_2 saturation reached a critical level, further increase in the Hb O_2 saturation regardless of the PaO_2 level had no significant effects on various breathing responses. It was also interesting to note that the frequency of breathing remained unchanged during various sleep states, whereas the amplitude of breathing increased only during the awake state. Such an increase might have been due to wakefulness-related cortical influences that have been shown to play a significant role in maintaining the respiratory drive during the awake state (31). Similarly, the significant increase in respiratory output during the awake state was not surprising; however, the increase in respiratory output during quiet sleep rather than active sleep was unexpected because previous studies in newborn human infants have shown the opposite effects (32, 33). Such a discrepant increase in respiratory output might have been due to the presence of sensory (mechanoreceptor) input resulting from lung distension, inasmuch as such an input is more important during quiet sleep in the very young infant than in the older child or adult (32).

We previously have shown that the initiation of breathing in response to lung distension and oxygenation always coincided with the onset of arousal and it was not clear whether there was a causal relationship between arousal and breathing (5). In our present study, arousal occurred in three fetuses but breathing did not become continuous, suggesting that breathing and arousal could be uncoupled and factors other than arousal may play an important role in the maintenance of breathing. However, in the remaining seven fetuses, arousal did coincide with the onset of continuous breathing, which is consistent with our previous observations (5). Similar behavioral changes have been reported in both human and sheep newborns at birth (9, 10). However, in the study by Baier *et al.* (4), arousal was observed only during

cord occlusion. These discrepancies in fetal behavioral state might have been because of the different criteria used to define fetal arousal as well as different techniques to induce continuous breathing. Most investigators have used the electrographic criteria to define the fetal sleep states (6, 7), whereas Rigatto (34) observed the fetus through a Plexiglas window implanted in the ewe's left flank. However, no studies comparing the electrographic and visual observation have been reported in a chronically prepared fetal sheep. This issue is important because arousal not only has been reported in association with the onset of continuous breathing but also has been regarded as a potent respiratory stimulant (35–37). Mechanisms through which lung distension and oxygenation cause arousal remain unknown. In our previous and current studies, absence of arousal during lung distension with nitrogen suggests that lung distension *per se* does not lead to arousal. This is consistent with the observations made by Sullivan *et al.* (37) that lung inflation failed to arouse dogs from either quiet or active sleep. Our preliminary data suggest that the arousal response may be at least partially mediated via vagal afferents and not afferents from the peripheral chemoreceptors (38, 39).

In summary, this study has demonstrated that the maintenance of fetal breathing and the onset of arousal in response to lung distension and oxygenation does not require very high levels of PaO_2 and that there is no graded response of breathing above a critical level of Hb O_2 saturation. Further studies are warranted to investigate the precise mechanisms through which increased fetal PaO_2 initiate these behavioral and breathing responses and to help us better understand the factors for the onset of continuous extrauterine breathing. Such studies may also have clinical implications inasmuch as disturbances in the control of breathing and behavior are associated with significant neonatal mortality and morbidity (40, 41).

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