Respiratory function in lambs after in utero treatment of lung hypoplasia by tracheal obstruction

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Davey, M. G., S. B. Hooper, M. L. Tester, D. P. Johns, and R. Harding. Respiratory function in lambs after in utero treatment of lung hypoplasia by tracheal obstruction. J. Appl. Physiol. 87(6): 2296–2304, 1999.—Tracheal obstruction (TO) stimulates growth of hypoplastic lungs in the fetus, but there is little knowledge of subsequent postnatal respiratory function. We have determined the effectiveness of TO in fetal sheep with existing lung hypoplasia in restoring postnatal respiratory function. Lung hypoplasia was induced by lung liquid drainage from 112 days of gestation to term (~148 days). We used an untreated group (ULH), a treated group (TLH) in which the trachea was obstructed for 10 days, and a control group. ULH lambs died within 4 h of birth. TLH lambs were hypoxic for the first week and were hypercapnic at 2 days. Pulmonary diffusing capacity, gas volumes, and respiratory compliances were not different between control and TLH lambs. Minute ventilation was not different between the two groups; however, tidal volumes were lower and respiratory frequencies were higher in TLH lambs than in controls for 2 wk after birth. We conclude that 10 days of TO in the presence of initial lung hypoplasia prevents death at birth and returns most aspects of pulmonary function to normal by 1–2 wk after birth.

fetus; newborn; lung function; fetal lung hypoplasia

DESPITE RECENT ADVANCES in perinatal medicine, morbidity, and mortality rates of infants born with congenital diaphragmatic hernia (CDH) remain high because of the associated lung hypoplasia (16). In CDH, pulmonary hypoplasia occurs as a result of the developing fetal lung being unable to expand and hence grow, leading to respiratory insufficiency and pulmonary hypertension after birth (10, 27). However, it is now well established that fetal lung growth can be accelerated by increasing the degree of lung expansion as a result of tracheal obstruction (1, 8, 19), and this knowledge has led to tracheal obstruction being investigated as a prenatal treatment for lung hypoplasia associated with CDH (12).

Recent studies using animal models of lung hypoplasia have analyzed the effects of tracheal obstruction in stimulating lung growth and maturation (7, 20). In fetal sheep, 6 days of tracheal obstruction almost totally reversed an existing lung growth deficit as indicated by pulmonary DNA and protein contents (20). Using CDH to induce pulmonary hypoplasia in fetal sheep, DiFiore et al. (7) demonstrated that 25 days of tracheal obstruction normalized both alveolar number and surface area. Although these studies demonstrate the biochemical and morphometric benefits of using tracheal obstruction to rapidly reverse an existing lung growth deficit, there is little knowledge of the postnatal functional capacity of the lungs after a period of accelerated lung growth in utero. Thus the aim of this study was to assess the effectiveness of tracheal obstruction in reversing the effects of lung hypoplasia on lung function after birth. We used three groups of animals: 1) lambs with untreated lung hypoplasia (ULH), 2) lambs with prenatal treatment of lung hypoplasia (10 days of tracheal obstruction (TLH)), and 3) a control group that had undergone surgery but experienced no tracheal drainage or tracheal obstruction [operated controls (OC)]. We also compared our data with values obtained from a group of control lambs that had experienced no prenatal surgery (unoperated controls (C)) (4).

We chose to induce pulmonary hypoplasia in fetal sheep by prolonged drainage of lung liquid because this method avoids the need for the surgical creation of a diaphragmatic defect and its subsequent repair. To minimize tracheal damage when obstructing the fetal trachea, we developed a technique that permits the removal of the source of tracheal obstruction (a small latex balloon) at the time of birth. To stimulate fetal lung growth in the TLH group, we chose to obstruct the fetal trachea for 10 days because previous studies have shown that the pulmonary growth response is maximal between 6 and 14 days after obstruction of the fetal trachea (6, 20). By comparing postnatal pulmonary function over the first 8 postnatal wk in control lambs, and in lambs with TLH and ULH, we have been able to evaluate the effectiveness of using short-term late-gestational tracheal obstruction as a prenatal treatment for pulmonary hypoplasia.

METHODS

Twenty-five pregnant sheep (Border-Leicester × Merino) underwent aseptic surgery at 112–114 days postmating (term ~148 days). Maternal and hence fetal anesthesia was induced with thiopental sodium (1 g iv) and was maintained with 2% halothane in 98% O2-N2O (50:50 vol/vol). After exposure of the fetal head and neck via a uterine incision, a catheter (2.3 mm OD) was inserted into the fetal trachea ~5 cm below the level of the larynx, such that the tip was 5–7 cm caudal to this point. This catheter allowed the drainage of fetal lung liquid (to induce lung hypoplasia) and the measurement of lung luminal pressure during tracheal obstruction. A saline-filled balloon-tipped catheter (purpose made) was placed such that the balloon lay between the incision site and the larynx. In this position, the inflated
tracheal balloon both prevented the drainage of amniotic fluid (via the upper respiratory tract) during gravimetric drainage of fetal lung liquid and provided a means by which the fetal trachea could be obstructed. The catheter attached to the balloon exited the upper airway via the mouth of the fetus. After surgery, 0.7–0.8 ml of saline was injected into the balloon, creating a luminal pressure of 30–50 mmHg. Balloon pressure (corrected for amniotic pressure) was measured (model P23ID, Statham) every 1–2 days to ensure that the balloon remained inflates. The balloon was not inflated in OC fetuses. Catheters were implanted into a fetal carotid artery and jugular vein to permit blood sampling. A catheter was sutured to the fetal skin for the measurement of amniotic fluid pressure, which was used to adjust other pressure recordings. Fetuses received antibiotics (2 ml im, Depomycin, Intervet) before the fetal head and neck were returned to the uterus to monitor the onset of labor (11). All catheters and electromyographic (EMG) electrodes were sutured to the uterus and the uterine incision was closed. Stainless steel Intervet) before the fetal head and neck were returned to the uterus and the uterine incision was closed. Stainless steel electromyographic (EMG) electrodes were sutured to the uterus to monitor the onset of labor (11). All catheters and electrodes were tracked to the upper right flank of the ewe and exteriorized via a small skin incision. Fetal blood samples were collected every 3–4 days to monitor fetal arterial P02 (PaO2), PCO2 (PaCO2), pH (pH a), and percent O2 saturation of hemoglobin (SaO2) (model ABL510, Radiometer).

Experimental Protocol

We induced lung hypoplasia in 15 fetuses (5 ULH and 10 TLH) by continuously draining lung liquid by gravity into 1-liter sterile bags starting at 112–114 days of gestation. Collection bags were secured to the side of the individual holding pens, below the level of the floor; in this position, the “drainage pressures” were ~15 cm H2O when the ewes were lying down and ~40 cm H2O when the ewes were standing. In ULH fetuses, lung liquid was drained until the onset of labor at term. In TLH fetuses, lung liquid was drained up to 137 days of gestation (after 22.0 ± 0.5 days of drainage), after which the fetal trachea was obstructed from 137 to 147 days of gestation (i.e., the tracheal balloon remained inflated while drainage ceased). Lung luminal pressures (from which amniotic pressure was subtracted) were measured daily (model P23ID, Statham) from 137 to 147 days to confirm that the trachea was obstructed. After the 10 days of tracheal obstruction, the tracheal balloon was then deflated and normal movement of lung liquid was permitted; this allowed a non-treatment period of 2.0 ± 0.7 days (range 0–4 days) before birth.

Of the 10 TLH lambs, 5 were studied until 8 wk of age, and the other 5 died in the perinatal period. Two fetuses died during labor because of umbilical cord entanglement with catheters; one lamb failed to recover from surgery at postnatal day 1; one ewe chewed the vascular catheters of her lamb, resulting in fetal blood loss (day 1); and one lamb died of an unexplained death also at postnatal day 1.

There were two groups of control animals. Five underwent prenatal surgery (OC) after which the tracheal balloon was not inflated, allowing normal flow of lung liquid; after birth pulmonary function measurements were made on these animals for the first 8 postnatal wk. Five C lambs were humanely killed soon after birth (0–1 days); the lungs were removed for determination of lung weights and pulmonary DNA and protein contents.

During periods of tracheal drainage, we recorded the volume of lung liquid collected and the time of collection, enabling lung liquid production rates to be determined. The collected liquids were analyzed for their Na+, K+, and Cl- concentrations (Na+/K+/Cl− analyzer, model 644, Ciba-Corning) to confirm that drained fluid was lung liquid and not amniotic fluid. Uterine EMG activity was recorded from 140 days of gestation in all ewes to identify the onset of labor; at this time exteriorized catheters were cut short and obstructed so as to permit vaginal delivery of the lambs with their catheters intact.

Postnatal Surgery

At 1–2 days after birth, surviving lambs (5 TLH and 5 OC lambs) underwent aseptic surgery [2–3% halothane in balance O2-N2O (50:50 vol/vol)] for the implantation of a saline-filled (0.5–1.5 ml) balloon-tipped catheter into the midthoracic cavity for the measurement of intrapleural pressure (Ppl). The vascular catheters (implanted at fetal surgery) were refitted with three-way stopcocks to permit arterial blood sampling and intravenous drug administration.

Pulmonary Function Studies

At ~2 days after birth and at 1, 2, 4, 6, and 8 wk of age, lambs were placed prone in a sling with openings for their legs. After an arterial blood sample (0.5 ml) was collected, lambs were lightly sedated (pentobarbital sodium, 5–15 mg·kg−1·h−1 iv), and the trachea was intubated (Portex; 5–7 mm OD). Our techniques for measuring pulmonary function in developing lambs have been described previously (4). Briefly, pulmonary diffusing capacity for CO (DLCO; ml·min−1·mmHg−1) was measured by causing the lambs to rebreathe 0.3% CO (10 ml/kg), commencing at functional residual capacity (FRC), for 10–20 s. The rebreathing time was recorded, and the final gas mixture was analyzed for its CO concentration (CO analyzer, P. K. Morgan). The CO rebreathing tests were performed in duplicate, allowing 5 min between tests. FRC was measured by using a closed-circuit He-dilution technique. Lambs breathed 80% He (in 20% O2) for 5 min after which they were connected to a water-sealed spirometer at FRC (Godart Paeditest); spirometer volume was maintained by adding O2 to the circuit. Inspiratory capacity at 30 cmH2O (IC30) and static pulmonary compliances were measured in triplicate by mechanically ventilating lambs until apneic. While Ppl (MacLab, ADInstruments) was recorded, the lungs were inflated to an airway pressure (Paw) of 30 cmH2O, after which lambs passively exhaled (to FRC) into a spirometer (1 liter, Collins). Total lung capacity (TLC; at 30 cmH2O) was calculated by adding IC30 and FRC. Respiratory system compliance (Crs) was obtained by dividing the change in spirometer volume (ΔV) by ΔPaw (30 cmH2O). Chest wall compliance (Cw) was estimated by dividing ΔV by the change in pleural pressure (ΔPpl). Static lung compliance (CL) was derived according the following equation: CL = 1/(ICrs − 1/Cw). Minute ventilation (Vt), tidal volume (Vt), and breathing frequency (f) were measured by using a heated pneumotachograph (size 0, Fleisch) connected to a differential pressure transducer and integrator (model P10CD, Grass). At the end of each study, the spirometers and pneumotachograph were calibrated by using a 100-ml syringe (Hans Rudolph). Pressure transducers were calibrated against a water column, and the volume of the spirometer circuit used for the measurement of FRC was calculated by using the He-dilution technique.

Postmortem

Lambs were painlessly killed either soon after birth (0–1 days), or at 8 wk after birth by an overdose of pentobarbital sodium (iv), and the lungs were removed for estimation of pulmonary dry weight and DNA and protein contents. Body weights and wet lung weights were recorded. To estimate dry lung weight, two samples of wet lung tissue (~1–2 g) were...
Fetal data. Data on tracheal composition (Na+, K+, and Cl−) and drainage rates of lung liquid from ULH and TLH fetuses were grouped into 5-day bins (111–115, 116–120, 121–125, 126–130, 131–135, and 136–140 days of gestational age) and analyzed by two-way repeated-measures ANOVA (treatment and gestational age as factors; version 6.09, SAS). Data on fetal PaO2, PaCO2, pHa, and SaO2, was grouped into 5-day bins as above and also included data from 141–145 and 146–150 days of gestation; these data were also analyzed by using a two-way repeated-measures ANOVA.

Postnatal data. Birth weights and the gestational age at which lambs were born were analyzed by using a one-way ANOVA (treatment as a factor). Postnatal body weights; PaO2, PaCO2, pHa, and SaO2, values (unsedated); and pulmonary function data in OC and TLH lambs were analyzed by using a two-way ANOVA (repeated measures) with treatment and postnatal age as factors. Pulmonary gas volumes (IC30, TLC, Vt, FRC) and Vt were reported at 8TPS. Lung weights and pulmonary DNA and protein contents in control and TLH lambs were analyzed by using a two-between-groups ANOVA to determine the effects of treatment and age. Significant differences between means were identified with a least significant difference test at P < 0.05. All values are expressed as means ± SE. Statistical significance was accepted at P < 0.05 unless otherwise stated.

RESULTS

Fetal Data

Fetal blood gases. All fetuses were considered healthy throughout the studies on the basis of their blood-gas values. There were no significant differences between values of PaO2 (21.2 ± 0.3 Torr), PaCO2 (45.9 ± 0.3 Torr), pHa (7.368 ± 0.002), and SaO2, in TLH and OC fetuses. SaO2, decreased with gestational age in both groups from 69.6 ± 2.7% at 113.2 ± 0.3 days to 57.4 ± 2.1% at 147.3 ± 0.3 days of gestation.

Fetal tracheal fluid. There was no significant difference between tracheal drainage rates of ULH and the two groups of TLH fetuses; in the three groups, drainage rates increased from 6.5 ± 0.5 ml/h at 113.8 ± 0.3 days of gestation to 17.7 ± 1.3 ml/h at 138.0 ± 0.2 days of gestation.

Concentrations of Na+ and Cl− in drained tracheal fluid were not significantly different between ULH and TLH fetuses and did not change with age (146.3 ± 0.2 and 147.9 ± 0.5 mmol/l, respectively). Concentrations of K+ were not different between groups, but they increased from 4.6 ± 0.1 mmol/l at 113.2 ± 0.3 days of gestation to 7.0 ± 0.3 mmol/l at 138.4 ± 0.2 days of gestation. In ULH fetuses, in which lung liquid drainage continued until term, K+ concentration further increased to 9.9 ± 1.3 mmol/l at 147.0 ± 0.3 days of gestation.

Postnatal Data

Body weights. Birth weights of lambs (4.5 ± 0.1 kg) and the gestational ages at which they were born (147.7 ± 0.3 days) were not significantly different among the C, OC, TLH, and ULH lambs. Body weights at each study age were not different between TLH and OC lambs and significantly increased with advancing postnatal age.

Postnatal blood gases in the immediate postnatal period (Fig. 1). The highest PaO2 and SaO2 values, and lowest PaCO2 values, of lambs (3 OC, 4 ULH, and 5 TLH) measured at ~30-min intervals during the first 4 h postnatal are presented in Fig. 1; when blood samples were collected OC and TLH lambs were spontaneously breathing room air, whereas ULH lambs were mechanically ventilated with room air. We are unable to statistically analyze these data because of the low number of observations for OC lambs (n = 3).

ULH LAMBS. Lambs with no prenatal treatment for lung hypoplasia died within 4 h of birth despite the provision of ventilatory support (mechanical ventilation, 60 breaths/min, peak end-expiratory pressure <20 cmH2O, with O2 supplementation). These animals were severely hypoxemic and hypercapnic in the newborn period; in four of the five ULH lambs, PaCO2 levels exceeded 100 Torr, whereas PaO2 and pHa fell below 30 Torr and 6.9, respectively.

TLH LAMBS. In the immediate newborn period (<4 h), TLH lambs were mildly hypoxemic but did not demonstrate the high degree of CO2 retention observed in ULH lambs. One TLH lamb required ventilatory sup-
port (without O$_2$ supplementation) for ~20 min after birth. In contrast to ULH lambs in which postnatal blood-gas status progressively deteriorated, P$_{\text{a}CO_2}$ and P$_{\text{a}O_2}$ in TLH lambs were stable soon after birth and further improved by postnatal day 2; normal values were not seen until 7–14 days after birth.

Arterial blood parameters (2 days to 8 wk, Fig. 2).  

P$_{\text{a}O_2}$. In OC lambs, P$_{\text{a}O_2}$ did not change during the first 8 postnatal wk (105.2 ± 2.5 Torr). P$_{\text{a}O_2}$ of TLH lambs was lower than that of OC lambs at day 2 (57.3 ± 3.5 vs. 97.1 ± 6.7 Torr) and week 1 (82.8 ± 9.2 vs. 103.7 ± 4.9 Torr) but increased to reach control values by 2 wk.

P$_{\text{a}CO_2}$. In OC lambs, P$_{\text{a}CO_2}$ was constant for the first 8 wk after birth (39.1 ± 1.0 Torr). P$_{\text{a}CO_2}$ of TLH lambs was higher than in OC lambs at day 2 (52.6 ± 2.9 vs. 41.8 ± 3.8 Torr;  P < 0.02, unpaired t-test) but was normal thereafter.  

p$_{\text{H}a}$. pH$_a$ was not different between TLH and control lambs, but increased with age from 7.385 ± 0.013 at day 2 to 7.434 ± 0.010 at week 8.

S$_{\text{a}O_2}$. S$_{\text{a}O_2}$ values for OC lambs did not change during the first 8 postnatal wk. S$_{\text{a}O_2}$ of TLH lambs was lower than that of OC lambs at day 2 only (83.1 ± 2.5 vs. 93.6 ± 5.0%;  P = 0.07, unpaired t-test) but was normal thereafter. The rectal temperature (39.7 ± 0.1°C) of lambs was not different between groups and did not change with age.

DLCO (Fig. 3). Absolute values of DLCO in OC and TLH lambs were not different between groups and increased with age from 1.10 ± 0.16 ml·min$^{-1}$·mmHg$^{-1}$ at day 2 to 4.46 ± 0.26 ml·min$^{-1}$·mmHg$^{-1}$ at week 8. When DLCO of OC and TLH lambs was expressed in relation to body weight, values were not different between groups; the mean value increased with age from 0.21 ± 0.03 ml·min$^{-1}$·mmHg$^{-1}$·kg$^{-1}$ at day 2 to 0.30 ± 0.02 ml·min$^{-1}$·mmHg$^{-1}$·kg$^{-1}$ at week 6. When DLCO was expressed in relation to lung volume (FRC measured simultaneously during the DLCO rebreathing procedure), it was not different between TLH and OC lambs, and mean values increased from 6.5 ± 0.8 ml·min$^{-1}$·mmHg$^{-1}$·ml$^{-1}$·10$^{-3}$ at day 2 to 11.9 ± 0.9 ml·min$^{-1}$·mmHg$^{-1}$·ml$^{-1}$·10$^{-3}$ at week 8.

Pulmonary gas volumes (Fig. 4). Absolute values of FRC, I$_{C30}$ and TLC were not different between OC and TLH lambs; values increased, respectively, from 165.6 ± 7.4, 150.7 ± 9.6, and 316.3 ± 14.9 ml at day 2 to 382.7 ± 20.5, 448.5 ± 20.8, and 831.2 ± 24.6 ml at 8 wk. When adjusted for body weight, values of FRC, I$_{C30}$ and TLC were not significantly different in OC and TLH lambs;
values declined, respectively, from 33.2 ± 1.6, 29.9 ± 1.4, and 63.1 ± 2.3 ml/kg at day 2 to 23.6 ± 1.6, 27.5 ± 1.5, and 51.2 ± 2.1 ml/kg at 8 wk.

Ventilatory parameters at rest (Fig. 5). Body weight-adjusted VT in TLH and OC lambs decreased during the first 2 postnatal wk; values in TLH lambs at day 2 (6.4 ± 0.8 ml/kg) and week 1 (5.4 ± 0.3 ml/kg) were lower than in OC lambs (8.9 ± 0.9 ml/kg and 7.8 ± 0.4 ml/kg, respectively) but were not different at other ages. Values of f declined with age in both groups; values of f in TLH lambs at day 2 (75.1 ± 6.3 breaths/min) and week 1 (64.1 ± 5.2 breaths/min) were higher than those in OC lambs (44.4 ± 5.0 and 40.8 ± 4.7 breaths/min, respectively). Body weight-adjusted Vi was not different between TLH and OC lambs; values declined from 442.0 ± 40.5 ml·min⁻¹·kg⁻¹ at day 2 to 185.7 ± 11.1 ml·min⁻¹·kg⁻¹ at week 8, after which there were no further age-related changes in Vi.

Respiratory compliances (Fig. 6). Body weight-adjusted values of Crs, Cw, and Cl (measured at Paw of 30 cmH₂O) were not different between TLH and OC lambs. Values of Crs and Cw declined with age from 1.00 ± 0.05 and 3.72 ± 0.43 ml·cmH₂O⁻¹·kg⁻¹ at day 2, to 0.88 ± 0.07 and 2.01 ± 0.15 ml·cmH₂O⁻¹·kg⁻¹ at week 8, respectively; Cl did not change with age (1.50 ± 0.06 ml·cmH₂O⁻¹·kg⁻¹). When Cl (measured at an Paw of 10 cmH₂O) was expressed in relation to FRC, values in TLH lambs were lower (P = 0.07) than in OC lambs at day 2 but were normal at other age points.

Postmortem Data (Table 1)
Newborn lambs (0–1 days). Body weights at postmortem were not different among C, TLH, and ULH lambs. Ratios of lung weights to body weights (both wet and dry) were not different between C and TLH lambs; however, ratios in ULH lambs were 15% (wet) and 35% (dry) lower than in C lambs. Pulmonary tissue concentrations of DNA (3.6 ± 0.1 mg/g; n = 10) and protein (130.1 ± 3.7 mg/g; n = 10) were not different between OC and TLH lambs. Pulmonary DNA (185.4 ± 31.7 mg/kg body wt; n = 10) and protein contents (2.6 ± 0.4 g/kg body wt; n = 10) were also not different between OC and TLH lambs.

Postnatal lambs (8 wk). Body weights and lung weights at postmortem were not different between OC and TLH lambs at 8 wk after birth. Ratios of lung weights (wet or dry) to body weights were also not significantly different between the two groups. Pulmonary tissue concentrations of DNA (2.5 ± 0.1 mg/g; n = 10) and protein (109.7 ± 4.3 mg/g; n = 10) were not different between OC and TLH lambs.
achieved by measuring fundamental aspects of postnatal pulmonary function between birth and 8 wk in treated and untreated animals. Our results showed that continuous drainage of fetal lung liquid leads to lung hypoplasia and lethal respiratory insufficiency soon after birth, whereas prenatal treatment of pulmonary hypoplasia by a short period of tracheal obstruction prevents death at birth and restores most aspects of pulmonary function to normal within 1–2 wk after birth. Specifically, we found that DLCO, pulmonary gas volumes (FRC, IC, and TLC), Crs (Cw and CL), pulmonary weights, and DNA and protein contents were normal in postnatal lambs that had undergone a period of tracheal obstruction.

Untreated Lung Hypoplasia

To study the postnatal consequences of in utero correction of lung hypoplasia by tracheal obstruction, we induced pulmonary hypoplasia by the continuous drainage of fetal lung liquid (1, 19, 20). On the basis of previous studies of lung liquid composition in fetal sheep (15), we are confident that only lung liquid, and not amniotic fluid, was drained. We have confirmed that prolonged lung liquid drainage induces pulmonary hypoplasia, as indicated by a 35% reduction in dry lung weight-to-body weight ratio, and a 58% reduction in pulmonary DNA content (n = 2) in our ULH group at birth, leading to respiratory insufficiency at birth and the subsequent death of these animals.

Our ULH lambs can be considered analogous to infants with fatal CDH in that fetal lung development was compromised and lungs were unable to sustain effective gas exchange after birth (27). As in infants with severe lung hypoplasia, ULH lambs were unable to maintain adequate PaO2 and PaCO2 values despite ventilatory support and O2 supplementation. Other investigators have reported similar arterial blood-gas tensions in term ventilated lungs with lung hypoplasia (7, 13, 22, 23). The poor gas-exchange capacity in our ULH lambs, and in animals from studies on experimental CDH (7, 13, 22, 23), may be attributed to 1) reduced alveolar surface area, 2) increased air-blood barrier thickness, or 3) reduced

Table 1. Body weights, lung weights, lung-to-body weight ratios, and pulmonary DNA and protein contents of C, TLH, and ULH lambs at birth and of OC and TLH lambs at 8 wk after birth

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<tr>
<th></th>
<th>Body Weight at Postmortem, kg</th>
<th>Lung Weight, g</th>
<th>Lung-to-Body Weight Ratio, g/kg</th>
<th>Pulmonary DNA Content, mg/kg body wt</th>
<th>Pulmonary Protein Content, g/kg body wt</th>
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<td>Wet</td>
<td>Dry</td>
<td>Wet</td>
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<td>Newborn (0–1 days)</td>
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<td>C</td>
<td>5.0 ± 0.5</td>
<td>98.6 ± 13.2</td>
<td>19.7 ± 2.5</td>
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<td>TLH</td>
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<td>21.65 ± 2.1</td>
<td>3.6 ± 0.6</td>
<td>191.0 ± 59.6</td>
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<tr>
<td>ULH</td>
<td>4.1 ± 0.4</td>
<td>68.2 ± 8.6†</td>
<td>10.7 ± 1.1†</td>
<td>2.6 ± 0.1*</td>
<td>46.1 &amp; 103.4</td>
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<td>OC</td>
<td>16.1 ± 1.5</td>
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<td>53.0 ± 2.4</td>
<td>3.1 ± 0.3</td>
<td>70.1 ± 8.0</td>
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Values are means ± SE for 5 lambs in each group. C, control lambs that had no prenatal surgery; OC, control lambs that had undergone surgery but had no tracheal drainage or tracheal obstruction; TLH, lambs with prenatal treatment of tracheal hypoplasia (10 days of tracheal obstruction); ULH, lambs with untreated lung hypoplasia. Pulmonary DNA and protein contents obtained from 2 ULH lambs; individual values presented. *Values in ULH lambs that are significantly different from values in C lambs, P < 0.05. †Values in ULH lambs that are significantly different from TLH lambs, P < 0.05.
pulmonary vascularization, all of which have been reported in experimental models of pulmonary hypoplasia (1, 8).

Treated-Lung Hypoplasia: Resting Arterial Blood-Gas Parameters

In TLH lambs, we found that PaCO₂ was elevated and that PaO₂ was reduced for 2 wk after birth. In contrast, in ventilated near-term fetal sheep it was found that in utero tracheal obstruction (31 days) in the presence of initial lung hypoplasia led to normal PaCO₂ and PaO₂ values (23). However, in that study higher peak inspiratory pressures were required for lambs that underwent in utero tracheal obstruction compared with control animals, presumably because of their lower pulmonary compliance (23). We did not mechanically ventilate our lambs at high lung volumes, which would be expected to increase alveolar ventilation and influence arterial blood-gas parameters. Our lambs spontaneously breathed room air (without O₂ supplementation) when arterial blood samples were collected, and this may explain the difference in results between our study and those of O'Toole et al. (23).

DlCO

Although DlCO was unaffected in our TLH lambs, it tended to be lower than in controls at 3 days after birth and may have contributed to the lower PaO₂ and elevated PaCO₂ values of TLH lambs. It is also possible that increased dead space ventilation in our TLH lambs soon after birth (i.e., elevated f and VT values) may have affected arterial blood-gas values in the early neonatal period.

Pulmonary Gas Volumes (TLC and FRC)

Tracheal obstruction in the normal fetus accelerates pulmonary growth and maturation (increased alveolarization, decreased septal wall thickness) leading to increased lung luminal volume (1, 5, 8, 19). Our results show that 10 days of in utero tracheal obstruction, in the presence of initial lung hypoplasia, provides a sufficient stimulus to lung growth to restore postnatal pulmonary gas volumes to normal soon after birth. We have confirmed the earlier findings of O'Toole et al. (23), who demonstrated that in utero tracheal obstruction (31 days) in the presence of lung hypoplasia returned IC₃₀ to normal at term. However, it is becoming apparent that extended periods of tracheal obstruction (>2 wk) may not be necessary to restore pulmonary mass and volume because the lung growth response after tracheal obstruction is so rapid; as little as 6 days of tracheal obstruction in fetal sheep with lung hypoplasia caused a 48% increase in pulmonary DNA content and a 98% increase in protein content (21). It has been shown that the increases in lung DNA and protein contents and lung weights do not continue after 7–14 days of tracheal obstruction in fetal sheep with normally grown lungs, possibly because of the physical limitations imposed by the chest wall. Therefore, once lung volume and/or mass has been restored in fetuses with lung hypoplasia, there do not appear to be further benefits of continuing the period of tracheal obstruction. There is also evidence to suggest that periods of tracheal obstruction beyond 4–6 wk may induce degeneration of type II cells (6).

Previous studies have shown that tracheal obstruction leads to a decrease in type II cell density (1, 3, 9, 25) and their subsequent ability to synthesize surfactant (23), which would be expected to reduce C₁ in the postnatal period. Results from our laboratory have shown that tracheal obstruction for 10 days in fetal sheep with normal lungs reduces 1) type II cell density, probably because of accelerated differentiation of type II into type I cells (14), and 2) surfactant protein A, B, and C gene expression (18). These results prompt the question as to why our TLH lambs survived the newborn period. Recent evidence indicates that tracheal obstruction for 14 days in the presence of fetal lung hypoplasia does not cause a total loss of alveolar type II cells as it does in fetuses with normally grown lungs (24). It is possible that, because type II cell density is increased in hypoplastic lungs (2), type II cell density would be expected to be lower in normal lungs than in hypoplastic lungs after tracheal obstruction.

It is now evident that a period of unimpeded tracheal flow after a period of tracheal obstruction is important for restoration of type II cell density and their ability to synthesize surfactant (3, 9, 24). It is possible that type II cell number and their ability to synthesize surfactant was reduced in our TLH fetuses after 10 days of tracheal obstruction. Indeed, specific C₁ measured at Paw of 10 cmH₂O was reduced at day 2 in TLH lambs. However, there may have been sufficient time between the release of the tracheal obstruction (147 days in all fetuses) and the first measurement of C₁ at postnatal day 2 (a period of 4 ± 1 days) to allow recovery of type II cell number and function. Other investigators have found that restoration of tracheal flow for as little as 2 days after tracheal obstruction for 14 days leads to an increase in type II cell density and the surfactant protein C mRNA content per type II cell (3).

Resting Ventilatory Parameters and Respiratory Compliances

Although TLH lambs had normal values, they had reduced VT and increased f compared with controls. These findings are consistent with a reduced Crs (26). Although body weight-adjusted C₁ measured at Paw of 30 cmH₂O was not different between OC and TLH lambs we did find that mean values of FRC-adjusted C₁ at 10 cmH₂O (i.e., a lung volume closer to that which occurs during tidal breathing) tended to be lower at day 2 (P = 0.07) and week 1 (P = 0.11) by 47 and 33%, respectively; these postnatal ages correspond to the times at which abnormal breathing patterns were observed (i.e., low VT, high f) in TLH lambs. We believe that expressing C₁ in relation to lung volume (i.e., FRC) is more appropriate than expressing it in relation to body weight as body weight includes body compartments that have no influence on properties of the lung. In view of these results, it appears possible that 10 days
of tracheal obstruction in TLH lambs may have impaired type II cell density and/or function. Analysis of pulmonary surfactant protein content and gene expression would be required to confirm this.

Effects of Prenatal Surgery on Postnatal Lung Function

To eliminate the possibility that prenatal surgery affects postnatal pulmonary function, we have compared results from the OC lambs in this study with data from lambs we have previously studied that had not undergone prenatal surgery (4). Postnatal pulmonary function was essentially the same in both groups. DL\textsubscript{CO} (adjusted for both body weight and FRC) in OC lambs was lower than that in C lambs at 2 days after birth; all other aspects of postnatal pulmonary function were normal in OC lambs. Body weights and gestational ages at birth were not different between the two control groups.

Conclusions

It is now evident that prenatal treatment of pulmonary hypoplasia by tracheal obstruction has both beneficial and unwanted consequences for fetal lung development. We have shown that tracheal obstruction in the presence of lung hypoplasia prevents death at birth and returns most aspects of pulmonary function to normal within 1–2 wk after birth. However, other studies have shown that in utero tracheal obstruction reduces type II cell density (1, 3, 9, 25) and pulmonary compliance (23) and impairs pulmonary blood flow (24). We have not quantified pulmonary surfactant in this study; however, our data on respiratory function suggest that CL was reduced soon after birth in lambs that had undergone 10 days of in utero tracheal obstruction, possibly because of a reduced pulmonary surfactant content. Although specific CL was reduced in TLH lambs, it did not cause fatal respiratory insufficiency at birth. However, given that the reduction in type II cell density (1, 3, 9, 25) and pulmonary compliance (23) and impairs pulmonary blood flow (24). We have not quantified pulmonary surfactant in this study; however, our data on respiratory function suggest that CL was reduced soon after birth in lambs that had undergone 10 days of in utero tracheal obstruction, possibly because of a reduced pulmonary surfactant content. Although specific CL was reduced in TLH lambs, it did not cause fatal respiratory insufficiency at birth. However, given that the reduction in type II cell number appears to be proportional to the duration of tracheal obstruction, it is likely that longer periods of in utero tracheal obstruction (6) would lead to even greater surfactant deficiency. Therefore, if prolonged periods (>4 wk) of in utero tracheal obstruction are necessary to restore lung growth in fetuses with pulmonary hypoplasia, recovery of the type II cell population before birth would require removal of the tracheal obstruction for several days to ensure adequate respiratory function at birth.

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REFERENCES