Fetal lung growth after short-term tracheal occlusion is linearly related to intratracheal pressure

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Kitano, Yoshihiro, Daniel von Allmen, Masaki Kanai, Theresa M. Quinn, Paul Davies, Yukie Kitano, and Alan W. Flake. Fetal lung growth after short-term tracheal occlusion is linearly related to intratracheal pressure. J Appl Physiol 90: 493–500, 2001.—Prenatal tracheal occlusion (TO) has been shown to accelerate fetal lung growth, yet the mechanism is poorly understood. The goal of this study was to determine the relationship between fetal intratracheal pressure (Pitr) and fetal lung growth after TO. Fetal lambs underwent placement of an intratracheal catheter and a reference catheter at 115–120 days gestation (term, 145 days). Fetal Pitr was continuously controlled at three levels (high, 8 mmHg; moderate, 4 mmHg; low, 1 mmHg) by a servo-regulated pump. The animals were killed after 4 days, and the parameters of lung growth were compared. Lung volume (136.0 ± 16.7, 94.9 ± 9.7, 55.5 ± 12.4 ml/kg), lung-to-body weight ratio (6.31 ± 0.70, 4.89 ± 0.38, 3.39 ± 0.22%), whole right lung dry weight (3.01 ± 0.29, 2.53 ± 0.15, 2.07 ± 0.24 g/kg), right lung DNA (130.0 ± 11.3, 116.7 ± 8.6, 97.5 ± 10.9 mg/kg), and protein contents (1,865.5 ± 92.5, 1,657.6 ± 106.8, 1,312.0 ± 142.5 mg/kg) in high, moderate, and low groups, respectively, all increased in the moderate compared with the low group and increased further in the high compared with the moderate group. Morphometry confirmed a stepwise increase in the volume of respiratory region and alveolar surface area. We conclude that lung growth in the animals that had their Pitr controlled by TO during the initial 15–24 h could have affected the lung growth measured 4 days later. In addition, the TO group was not connected to the servo-pump, which may have affected lung growth by undefined alterations of fetal breathing. The present study was designed to exclude these possibilities and to document the pure effects of different mean Pitr values on fetal lung growth in the animals that had their Pitr controlled by this system. We hypothesized that more lung expa-
sion secondary to increased Pitr would result in more rapid lung growth over a defined period of time.

METHODS

All experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee at the Children’s Hospital of Philadelphia and followed guidelines set forth in the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Surgical Procedure

Time-dated, pregnant Western-Cross ewes (115–120 days gestation; term = 145 days; Thomas D. Morris, Upperco, MD) were anesthetized with intramuscular ketamine (20 mg/kg) injection followed by halothane inhalation (1–2.5%). The maternal abdomen was entered by a midline laparotomy incision, and the fetal head was exteriorized through a small hysterotomy. A vertical midline neck incision was made in the fetus, and the trachea was exposed. A tracheotomy was made, and an arterial pressure tubing (0.28 cm OD; Abbott, North Chicago, IL) was inserted 5 cm caudally at the level of thyroid isthmus. The trachea was tied over this tubing with 1–0 Prolene sutures, and the fetal skin was closed. A fluid-filled 5-cm latex cylindrical balloon was secured on the anterior chest wall of the fetus for amniotic pressure measurement. The tips of the two catheters were secured at the same level to minimize measurement errors due to the relative position of the two catheters. Antibiotics were given to the ewe (2 g cefazolin and 160 mg gentamycin) and injected into the amniotic space (50,000 IU penicillin and 10 mg gentamycin). The twin fetuses underwent placement of catheters in a similar fashion without the trachea being tied over the tubing. In this nonservo group, Pitr was simply recorded without manipulation by the servo system.

Maintenance of Fetal Pitr

The four fluid-filled catheters were connected to disposable transducers (Abbott, North Chicago, IL) and the pressure monitor/servo-control pump system (PM/4 and FS/20/Q, Living Systems Instrumentation, Burlington, VT). The pressure sensitivity of the sensor was ~0.5 mmHg, and the response time was <1 s. The maximal flow rate of the pump was 2.5 ml/min. The system was connected to a personal computer equipped with data-acquisition software (WINDAQ/EX, DATAQ, Akron, OH). Pitr was measured relative to the atmosphere minus amniotic pressure relative to the atmosphere.

After measurement of baseline Pitr for 30 min, Pitr was controlled at three different levels: high, ~8 mmHg (n = 5); moderate, ~4 mmHg (n = 5); and low, ~1 mmHg (n = 5). Moderate and low levels were chosen to mimic the Pitr in trachea-occluded and nonoccluded fetuses, respectively. The pressure data were continuously recorded at 1 Hz for the entire duration of the experiment. The animals were killed after 4 days, and parameters of lung growth were compared among the three groups.

Tissue Preparation

After 4 days, the catheters were capped off, and the fetuses were delivered by cesarean section under the same anesthesia used for catheterization (intramuscular ketamine injection followed by halothane inhalation). The fetal body weights were measured after euthanasia by injection of potassium chloride (10 meq). The ewes were killed after cesarean section by cardiac injection of potassium chloride (20–40 meq) under deep halothane anesthesia. The trachea and the lungs were excised en bloc, and the volume of the lungs was measured before lung fluid drainage. After lung fluid drainage by gravity, the lung wet weights were measured. The trachea and any remaining mediastinal connective tissue were removed, and the left bronchus was cannulated. The left lung was inflated fixed at a pressure of 25 cmH2O with 10% buffered formalin for 3–4 days. The entire right lung was chopped into small pieces and lyophilized. Whole right lung dry weights were determined by measuring the weight daily until it plateaued after 4–7 days. Approximately 20 mg of lyophilized tissue were sonicated in 5 ml of phosphate-buffered saline to make a crude suspension for the measurement of DNA and protein. DNA was measured with a commercially available kit (QLIAGEN tissue kit, QLIAGEN, Santa Clarita, CA), and protein was measured by the modified Lowry method (DC protein, Bio-Rad, Hercules, CA).

Lung Morphometry

Lung was divided into parenchyma and nonparenchyma. Nonparenchyma was defined as airways and vessels with >1 mm diameter, and parenchyma was defined as the rest of the lung tissue. Parenchyma was divided into the respiratory region (acinar air spaces and their intervening tissues) and the nonrespiratory region (extra-acinar airways and vessels <1 mm diameter). Finally, the respiratory region was divided into respiratory air spaces and respiratory tissues. Volume density (Vv, the volume of a tissue compartment per unit volume of reference compartment) for each compartment was determined by point counting of a three-level sampling cascade (levels I–III).

The volume of inflation-fixed left lung was measured by the water displacement method (32). The left lung was then cut in an approximately coronal plane into 1-cm-thick slices. For level I, a transparent square test lattice in which the points were spaced 5 mm apart was laid on the cut surface of each slice, and points overlying nonparenchyma and parenchyma were counted macroscopically to determine the Vv of parenchyma in lung (Vv). Five blocks were then randomly

Fig. 1. The results of intratracheal pressure (Pitr) measurement in the high (~8 mmHg Pitr), moderate (Mod; ~4 mmHg Pitr), and low (~1 mmHg Pitr) groups. Values are means ± SD. After baseline measurements for 30 min, the Pitr was controlled by a servo-regulated pump. The results from the control (Co) animals, which had catheters placed without tracheal occlusion or pressure manipulation, are also depicted. Significant difference, *P < 0.05.
sampled and embedded in paraffin. A 5-μm-thick section was cut from each block and stained with hematoxylin and eosin. A coherent multipurpose test lattice consisting of 42 test points and a discontinuous series of line probes (KR-821, Klarmann Rulings, Litchfield, NH) were fitted in the eyepiece of a light microscope (Leica DMRBE, Leica, Allendale, NL) and used for levels II and III. In level II, four randomly chosen fields in each section were point counted at a magnification of 350, and Vv of the respiratory region in parenchyma (VVp) was determined. In level III, Vv of respiratory air spaces (VVra) and respiratory tissues (VVrt) in the respiratory region were determined by using a magnification of 3200. Surface density of the alveolar epithelium in the respiratory region was estimated by counting the number of intersects of the test line with alveolar air space-epithelial interface in the same field that was used to measure VVra and VVrt. Absolute values for all features were calculated by using the volume of the respiratory region (volume of inflation fixed left lung × VVp × VVp).

Statistical Analysis

Data were expressed as means ± SD. Statistical analysis was performed among the three (high, moderate, and low) groups using one- or two-way ANOVA with Bonferroni adjustment. The nonservo group was compared with the low group by unpaired Student’s t-test. Pearson’s correlation coefficient was calculated where appropriate. Statistical significance was confirmed at P < 0.05.

RESULTS

Animal Survival

A total of 26 fetuses were prepared for this study. Five fetuses did not survive the experiment (4 fetuses were confirmed dead by ultrasound on postoperative day 1, and 1 fetus died from a sudden failure of transducer, which infused an excessive amount of lactated Ringer solution overnight). Two additional fetuses were excluded from the study due either to severe hydrops with massive ascites or abnormally small body weight (<1.4 kg), leaving 19 fetuses for the following analyses.

Fetal Pitr

Daily mean Pitr was calculated for each animal, from which the daily mean Pitr for each group (high, moderate, low, and control) was obtained. The results are shown in Fig. 1. There were no differences in the Pitr on days 0–1, 1–2, 2–3, and 3–4 by two-way ANOVA within each group. A mean Pitr for the entire experimental period was calculated for each group as follows: high group, 8.0 ± 0.3 mmHg; moderate group, 4.3 ± 0.6 mmHg; low group, 1.3 ± 0.7 mmHg. The difference between each group was statistically significant. The mean Pitr of the nonservo group (1.6 ± 0.4 mmHg) was not different from that of the low group. The volume of lactated Ringer solution infused or that of lung fluid withdrawn is depicted in Fig. 2.

Comparison of Lung Growth

Gross appearance. All animals in the high group had mild signs of hydrops (a small amount of ascites and posterior mediastinal tissue edema). Lungs from the high group were grossly larger than those from the moderate group, which were grossly larger than those from the low group. Figure 3 shows representative lungs from a set of twins: one in the high group and the other in the moderate group.

Fig. 3. Gross appearance of lungs from a representative set of twins. Lungs of a fetus in the high group (A) are larger than those of a fetus in the moderate group (B). Body weights of fetuses were 2.21 kg (A) and 1.91 kg (B).
Volume and weight measurements. The results are summarized in Table 1. Fetal body weight tended to be higher in the high group, partly due to the edema from lactated Ringer infusion. Statistical significance was confirmed between the high and the low group. Lung volume per body weight, drained lung fluid per body weight, lung-to-body weight ratio (after lung fluid drainage), and whole right lung dry weight per body weight all significantly increased in the moderate group compared with the low group and increased significantly further in the high group. The nonservo group had similar measurement as the low group, except in drained lung fluid. The lung fluid was significantly smaller in the nonservo group.

DNA and protein content. The results are summarized in Table 2. The right lung DNA and protein contents increased significantly in the moderate group compared with the low group. They increased further in the high group compared with the low group, but statistical significance was confirmed only in the protein content. Protein-to-DNA ratio was constant among the three groups. The nonservo group had a significantly larger amount of total protein compared with the low group.

Lung morphometry. A representative set of lung specimens after inflation fixation is shown in Fig. 4. The alveolar air space appeared more prominent, and the septa appeared thinner with increased Pitr. This was confirmed by morphometry (Table 3); V_{V,r} tended to increase and V_{V,t} tended to decrease with the increasing levels of Pitr (P < 0.05 only between the high and the low groups). V_{V,r} also increased with increasing levels of Pitr, but statistical difference was confirmed only between the low and the other two groups. V_{V,t} were not different among the three groups. Surface density of the alveolar epithelium in the respiratory region tended to decrease with increased Pitr, but we did not see statistical significance. The absolute volumes of respiratory region and respiratory air spaces, as well as gas exchanging surface area, all significantly increased in the high group compared with the other two groups (Table 4). Despite a gradual decrease in V_{V,t}, absolute volume of the respiratory tissue increased in lungs with higher Pitr. No difference was observed between the nonservo and the low group in any of the morphometric measurements.

Correlation Between Pitr and Lung Growth

Mean Pitr for the entire experimental period was electronically calculated for each animal, and they were correlated with right lung dry weight per body weight (g/kg), right lung DNA content per body weight (mg/kg), and right lung protein content per body weight (mg/kg). As shown in Fig. 5, there was a strong correlation between mean Pitr and the three parameters. Pearson’s correlation coefficients were 0.865, 0.781, and 0.881, respectively.

DISCUSSION

Although the mechanism of TO-induced lung growth remains poorly understood, most investigators agree that the major stimulus is mechanical (6, 21, 26, 28). The relationships between fetal lung growth, lung volume, and Pitr have been explored in chronically catheterized fetal sheep (6, 26). These studies suggest that volume is a key determinant of fetal lung growth. In this study, we examined the effects of different degrees of lung expansion on lung growth by developing an in vivo system to control mean Pitr at predetermined levels in fetal sheep. With the use of this system, fetal Pitr was successfully maintained at three different levels in unanesthetized animals. After 4 days, there was a linear relationship between lung pressure and Pitr. Our results demonstrate that fetal lung growth is closely correlated with fetal Pitr, at least at 4 days after TO, and provide additional evidence that one of the major stimulants of TO-induced fetal lung growth is mechanical.

The pressure levels for the moderate and the low groups were chosen so that they corresponded to the pressures in trachea-occluded and normal animals,
respectively. By selecting the Pitr of ~4 mmHg, the actual lung fluid exchange observed in the moderate group was negligible. As a result, the moderate group had similar values of lung volume, lung-to-body weight ratio, and whole right lung dry weight per body weight as the animals whose trachea were simply occluded with the same preparation (95.2 ± 14.8 ml/kg, 5.33 ± 0.77%, 2.63 ± 0.20 g/kg, respectively) (22). In the low group, ~100 ml per day of lung fluid were withdrawn, suggesting the lung fluid production rate in this group to be ~1.1–2.5 ml·kg⁻¹·h⁻¹. This is in agreement with previous reports that estimated fetal lung fluid production rate to be 2–4 ml·kg⁻¹·h⁻¹ (18). Although most of the parameters of the low group were not different from those of the nonservo group, lung fluid was significantly increased and the total protein was significantly reduced. Because the mean Pitr in the low group tended to be lower than that of the nonservo group, this small decrease in Pitr could have resulted in decreased lung growth. However, the reason why lung fluid was reduced in the nonservo group (which had higher Pitr) is unknown. It is possible that our measurement was not sensitive enough to measure those small differences. Alternatively, the servo-regulation of Pitr itself could have affected fetal lung fluid production through an unknown mechanism.

Although an Pitr value of 8 mmHg is well beyond a physiological range, this group was included in the study to allow comparison of the effects of a wider range of Pitr. In preliminary studies, we determined that an Pitr value of up to 8 mmHg was well tolerated in fetal sheep without evidence of morphological damage to the fetal lung, whereas higher levels resulted in some incidence of pleural rupture and fetal death.

Lung liquid secretion rate is known to decrease to undetectable levels after TO, when Pitr remains constant at 4–5 mmHg. It is highly likely that the infused lactated Ringer solution was reabsorbed from the pulmonary epithelium in the high group. This is supported by our observation that animals in the high group had some degree of hydrops and a trend toward higher body weight. A direct effect of the absorbed lactated Ringer solution on lung growth, independent of the effect of increased Pitr, is unlikely but cannot be dismissed. However, increased body weight due to fluid retention in this group would have had a negative bias on the growth measurements when corrected for body weight.

### Table 3. Morphometric results: relative values

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Vv of Parenchyma, %</th>
<th>Vv of Respiratory Region, %</th>
<th>Vv of Respiratory Air Spaces, %</th>
<th>Vv of Respiratory Tissues, %</th>
<th>Sv of Alveolar Surface, no./cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>5</td>
<td>87.7 ± 1.2 *</td>
<td>92.7 ± 1.0</td>
<td>74.0 ± 2.4</td>
<td>26.1 ± 2.4</td>
<td>389.5 ± 23.4</td>
</tr>
<tr>
<td>Moderate</td>
<td>5</td>
<td>86.2 ± 1.3 *</td>
<td>93.7 ± 1.0</td>
<td>69.9 ± 2.6</td>
<td>30.1 ± 2.6</td>
<td>397.1 ± 30.2</td>
</tr>
<tr>
<td>Low</td>
<td>5</td>
<td>80.9 ± 2.3 *</td>
<td>92.5 ± 1.8</td>
<td>66.9 ± 4.1</td>
<td>34.0 ± 4.1</td>
<td>431.8 ± 32.0</td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>79.1 ± 2.0</td>
<td>92.2 ± 0.8</td>
<td>64.4 ± 3.3</td>
<td>35.6 ± 3.3</td>
<td>442.4 ± 27.7</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = no. of animals. Vv, volume density; Sv, surface density. *P < 0.05 by 1-way ANOVA.
weight and would, therefore, further strengthen our interpretation of the results of the study.

The volume of infused lactated Ringer in the high group showed an interesting trend. On day 0–1, 228.0 ± 64.7 ml were necessary, which decreased to 57.8 ± 37.1 ml for day 1–2, 103.2 ± 28.8 ml for day 2–3, and 79.2 ± 15.6 ml for day 3–4. We speculate that a significant portion of fluid infused on day 0–1 was necessary to inflate the lungs to their maximum before any lung growth took place. This is not surprising given the high respiratory system compliance of 16.4 ml/cmH₂O in fetal sheep (11). The decrease in infused fluid on day 1–2, we believe, reflects the time lag for the lungs to detect the stretch signal and initiate proliferative lung growth. A similar trend has been reported by Nardo et al. (26), who found that lung liquid volumes increased after 1 day of TO, did not increase on day 2, and increased further through days 4–7. They speculated that the initial expansion could be limited by lung tissue and that the secondary expansion may result from restructuring of the extracellular matrix. De Paepe et al. (10) also found a lag phase in lung growth after TO in fetal rabbits. However, in their study, TO was performed during the pseudoglandular stage of lung development when lung fluid production is not as active as in later stages (14, 18). Therefore, it could be that the lag phase they observed was due to a small lung fluid production rate resulting in relatively minimal lung expansion.

The relationship among fetal lung growth, lung volume, and Pitr has been investigated by others. Nardo et al. (26) demonstrated that TO-induced lung growth is most rapid within 2 days after TO and is essentially complete within the first 7 days and that the lung DNA content is closely related to the lung liquid volume, not to fetal Pitr. Boland et al. (6) reported that cortisol pretreatment before TO resulted in higher lung volume and lung growth but not a higher Pitr compared with TO alone. From these results, they concluded that lung volume rather than Pitr is the major determinant of fetal lung growth. However, from these studies, it is difficult to conclude that the increase in lung volume was the cause and not the result of lung growth. Although we have found a linear relationship between Pitr and lung growth, we cannot conclude that pressure is the major stimuli of lung growth. In our study, we maintained pressure by infusion or withdrawal of lung fluid; therefore, volume was not a constant. Similarly, lung compliance in the three groups may have varied over the time course of our study due to restructuring of the extracellular matrix. In addition, when epinephrine is continuously infused into the fetal lamb after TO, lung volume and lung fluid are increased, but not lung dry weight, DNA, or protein contents, suggesting the presence of a certain threshold of lung volume beyond which acceleration of lung growth is initiated (24). Thus, in agreement with previous studies, it appears that lung expansion is a better, more comprehensive concept to describe the most important parameter of fetal lung growth. However, lung expansion is related to transpulmonary pressure,

### Table 4. Morphometric results: absolute values

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Fixed Left Lung Volume, ml</th>
<th>Volume of Left Lung Respiratory Region, ml</th>
<th>Volume of Left Lung Respiratory Air Spaces, ml</th>
<th>Volume of Left Lung Respiratory Tissues, ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>5</td>
<td>155.0 ± 23.4</td>
<td>126.0 ± 19.1</td>
<td>93.4 ± 16.5</td>
<td>32.6 ± 3.4</td>
</tr>
<tr>
<td>Moderate</td>
<td>5</td>
<td>103.6 ± 28.9</td>
<td>83.8 ± 23.8</td>
<td>58.6 ± 17.3</td>
<td>25.2 ± 7.2</td>
</tr>
<tr>
<td>Low</td>
<td>5</td>
<td>62.0 ± 14.7</td>
<td>46.1 ± 12.3</td>
<td>30.7 ± 9.9</td>
<td>15.4 ± 2.9</td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>51.8 ± 11.0</td>
<td>37.7 ± 8.0</td>
<td>24.3 ± 5.5</td>
<td>13.4 ± 2.8</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = no. of animals. *P < 0.05 by 1-way ANOVA.

![Fig. 5. Correlation between mean Pitr during the entire period of experiment and right lung dry weight per body weight (A), DNA content per body weight (B), and protein content per body weight (C). Pearson’s correlation coefficients were 0.865 (A), 0.781 (B), and 0.881 (C) (n = 15 animals).](http://jap.physiology.org/DownloadedFromhttp://jap.physiology.org/).
and we speculate that transpulmonary pressure, which is the pressure difference between the intra-alveolar pressure and the pleural pressure, may serve as a useful parameter for estimation of lung expansion and may correlate more accurately with lung growth.

The concept that tissue stretch affects cell growth has been described in multiple models and organs, including compensatory lung growth after partial pneumonectomy (14, 30), skeletal muscle (13, 34), bladder smooth muscle (4), vascular endothelial cells (2), and skin (33). Although expression of genes such as insulin-like growth factor I (21, 27), insulin-like growth factor II (17), platelet-derived growth factor (25), parathyroid hormone-related peptide (35), mitogen-activated protein kinase (20), and immediate early genes (c-fos and junB) (12) have been independently reported to be altered in lung by mechanical stimuli, the molecular mechanisms of mechanotransduction are only beginning to be explored (3).

Although we have measured multiple parameters of lung growth, we have not fully determined the physiological effect of increased lung mass. Because TO is usually performed after the completion of airway development, the components of the lung that grow in response to TO should be distal to the terminal bronchiole, resulting in a "polyalveolar lung." Polyalveolar lung is a term first applied by Hislop and Reid (16) to a newborn with an enlarged lobe in which several segments of the lung had approximately five times the normal alveolar number and demonstrated the clinical features of lobar emphysema. Our morphometric data confirm that the major lung growth in response to increased Pitr occurs in the respiratory region, resulting in increased gas-exchanging surface area. However, an increase in alveolar number without an increase in airway branching is known to occur in compensatory lung growth after pneumonectomy (19), in patients with CDH (5), and in cases of unilateral pulmonary aplasia (31). Emphysema does not necessarily accompany the polyalveolar lung in these circumstances. We speculate that TO may accelerate the same process and are optimistic about the long-term function of the enlarged lungs. Davey et al. (9) reported that, in a fetal sheep model of pulmonary hypoplasia induced by lung fluid drainage, subsequent TO prevented death at birth and restored most aspects of pulmonary function by 2 wk after birth. Harrison et al. (15) reported that the oxygen requirement after birth was low in human CDH survivors after TO. As a result, seven out of eight survivors did not require extracorporeal membrane oxygenation. These results suggest that the enlarged lungs do function after birth, although the achieved lung growth must be balanced against the adverse effects of TO on surfactant production (29).

In summary, we have shown that lung growth achieved during the first 4 days after TO is closely correlated with fetal Pitr. These results offer additional evidence that an increase in lung expansion is one of the major factors responsible for TO-induced lung growth. Clinically, these results suggest that lung growth after TO could potentially be augmented by increasing lung expansion or, on the other hand, could be inadequate if lung expansion does not occur. The latter is possible with a decreased lung fluid production rate, low lung compliance, high pleural pressure, decreased chest wall compliance, or an increased lung fluid absorption rate. Clinical application of TO in selected CDH patients may be hindered by 1) severe lung hypoplasia and relative immaturity resulting in decreased lung fluid production and decreased lung compliance, 2) decreased lung fluid production or increased absorption induced by medication (23), 3) decreased lung fluid production due to hypoxia and/or acidemia (36), or 4) physical barriers to lung expansion caused by liver and visceral herniation. Alternatively, it may be possible to increase lung expansion by enhancing Cl−-led lung fluid secretion with substances such as prolactin (8), by decreasing Na+−led absorption with a Na+−channel blocker, or by increasing lung compliance with steroids. Understanding the molecular regulation of fetal lung growth may ultimately allow for pharmacological or genetic manipulation of lung expansion and subsequent lung growth with or without the need for TO.

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