Altered Lung Development after Prenatal Nicotine Exposure in Young Lambs

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ABSTRACT

There is compelling evidence that prenatal nicotine exposure permanently alters lung development and airway function. The aim of this study was to determine how prenatal nicotine exposure alters proximal and distal airway function. Thirteen lambs were continuously exposed during the last fetal trimester to low-dose nicotine (LN) and 12 to a moderate dose (MN) (maternal s.c. dose: 0.5 and 1.5 mg/kg/d, respectively). Ten lambs served as controls (C). Proximal airway function was measured by lung mechanics. A multiple-breath N₂ washout technique was used to measure lung volume (functional residual capacity) and efficiency of gas mixing in distal airways, *i.e.* terminal respiratory units (moment ratio and nitrogen clearance). In comparison with C, both LN and MN had significantly reduced specific airway conductance to the same extent at a median study age of 12, 25, and 51 d, indicating signs of proximal airway obstruction. Distal airway function showed significant improvement in LN. Ventilation and functional residual capacity were unaffected. In summary, prenatal nicotine exposure induced airway obstruction in proximal airways and improved gas mixing in distal airways, possibly reflecting restriction in proximal airway growth and

accelerated maturation of the acinar part of the lung, respectively. We speculate that prenatal nicotine exposure has a disparate impact on airway development and function. The effect on the distal airways seemed to be inversely related to dose, which was not the case in the large airways. The altered airway function persisted during the study period, indicating that the effects of prenatal nicotine exposure might be permanent. (*Pediatr Res* 56: 432–439, 2004)

Abbreviations

C, control
M₀, zero moment
C_{dyn}, dynamic lung compliance
M₁, first moment
FRC, functional residual capacity
NC, nitrogen clearance
LN, low-dose nicotine
R_l, lung resistance
MN, moderate-dose nicotine

Environmental tobacco smoke is a severe health problem, not only for the whole pediatric population but also for the fetus *in utero* (1). There is compelling evidence that maternal smoking is associated with premature birth, low birth weight, and increased fetal and neonatal morbidity as well as mortality (2, 3). Epidemiologic studies have shown that environmental tobacco smoke is associated with a significantly increased incidence of wheezing, bronchitis, lower respiratory illness, and increased number of hospital admissions during infancy and childhood (4, 5). These

studies further suggest that there is a stronger correlation between prenatal, rather than postnatal, exposure and lower respiratory illness in the offspring of smoking mothers.

Altered mechanical properties of the lungs have also been found in these infants and children (6–12). These abnormalities in lung function are dominated by signs of airway obstruction with reduced forced expiratory flow rates at FRC, increased airway resistance, and a decrease in the ratio of time to peak tidal expiratory flow to total expiratory time (T_{ptef}/T_e). Low FRC and decreased lung compliance have also been found in infants exposed to environmental tobacco smoke. Studies of sudden infant death victims have shown that infants of mothers who smoked during pregnancy have abnormal airway pathology in the form of greater inner airway wall thickness and higher proportions of airway smooth muscle compared with infants of mothers who did not smoke (13, 14).

The relationship between prenatal nicotine exposure and altered lung development and function has been studied in

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several animal models (15–19). These studies have shown altered alveolar structure and lung hypoplasia, as well as changes in the airways, such as increased wall dimensions and increased collagen deposition. Decreased expiratory flow rates and increased pulmonary resistance have also been found in these animal studies.

Apart from the detrimental effects of prenatal nicotine exposure on lung development and function, it is known that premature infants of smoking mothers have a decreased risk to develop neonatal respiratory distress syndrome (20–22). These paradoxical findings have been attributed to an enhancement of lung maturation as evidenced by a more mature lecithin/sphingomyelin ratio in amniotic fluid from smoking compared with nonsmoking mothers. In addition, it has been shown that nicotine stimulates branching morphogenesis and surfactant gene expression in embryonic mouse lung culture (23). It has been demonstrated in rhesus monkeys that prenatal nicotine exposure increases the relative number of type 2 cells in developing alveoli and alveolar ducts, as defined by increased expression of type 2 cell SP-B mRNA together with increased levels of SP-B in amniotic fluid (16). As an explanation for the apparent differences in nicotinic effects on proximal compared with distal airways, it has been proposed that prenatal nicotine exposure exerts an anatomically dysanaptic effect on the development of the fetal lung, meaning that two components of an organ grow discordantly (17). In the case of the nicotine-exposed fetal lung, this could result in inhibition of proximal airway growth and simultaneous stimulation of the alveolar part of the lung.

The aim of this study was to determine the effects of a moderate- and a low-dose prenatal nicotine exposure on both proximal and distal (terminal respiratory units) airway function in young lambs during the first 2 mo of life.

METHODS

Subjects. Thirty-five term lambs of mixed breed delivered by spontaneous vaginal delivery were studied three times during their first 2 mo of life. Twenty-five of these lambs were exposed to nicotine prenatally, 13 with a low dose (LN), and 12 with a moderate dose (MN). Ten lambs served as control animals (C). An adaptive randomization procedure was used to randomly allocate ewes to the three study groups (24). The study was blinded and the investigators were unaware of the grouping of the lambs until the study was completed.

There were no major differences in birth weight or gestational age between the groups. The lambs were studied at the median (range) ages of 12 (5–17), 25 (22–34), and 51 (45–61) d, *i.e.* the earliest possible time after postoperative recovery, as late as practical with this chronic preparation, and once in between. [Median (range) study ages for each group were 12 (5–17), 26 (24–34), and 51 (45–61) d for LN lambs; 12 (7–16), 24 (22–27), and 51 (47–58) d for MN lambs; and 12 (8–13), 25 (23–28), and 52 (49–54) d for the control group.] The research protocol was approved by the Vanderbilt University Animal Care Committee.

Prenatal nicotine exposure. The fetal sheep were exposed to nicotine during the last third of gestation, when differenti-

ation of the peripheral respiratory unit is very intense. This third trimester exposure minimizes the risk for fetal growth restriction and enables a comparison with previous studies of the breathing pattern in young lambs after prenatal nicotine exposure (25). Osmotic pumps (Alzet, Model 2ML4, DURECT Corp., Cupertino, CA, U.S.A.) were implanted subcutaneously in the ewe on approximately d 98 of the 147-d gestation. The pumps delivered nicotine bitartrate of approximately 0.5 mg/kg/d in the LN group and 1.5 mg/kg/d in the MN group. The LN dose was comparable to the dose we previously used (25) and results in nicotine levels obtained by light smokers (<10 cigarettes/d), whereas the MN dose corresponds to the dose obtained by moderate smokers (approximately 20 cigarettes/d) (26). The ewes of the control lambs received normal saline. The pumps were replaced after 28 d and removed after delivery of the lamb. Maternal plasma concentrations of nicotine and cotinine were determined by gas chromatography with nitrogen-phosphorus detection (27, 28) from samples obtained 14 d after the first implantation of the capsules, *i.e.* on approximately d 112 of gestation.

Instrumentation. The lambs were instrumented 3–7 d after delivery using anesthesia with Telazol (Fort Dodge Animal Health, Fort Dodge, IA 50501) (tiletamine HCl and zolazepam HCl) followed by isoflurane. Polyvinyl chloride catheters were placed in a cranial tibial artery and a lateral saphenous vein. Two pleural balloons (Sil-Tec envelopes (Technical Products Inc. of Georgia, Decatur, GA 30035)) for measurements of pleural pressure were placed in the right pleural space through a small thoracotomy. A tracheostomy with placement of a tracheal window was also performed. When the lambs were not studied, tracheal patency was reestablished with a piece of endotracheal tube (Portex, Keene, NH, U.S.A.) (25). At least 48 h postoperative recovery was allowed before the first study. Antibiotics (gentamicin and ticarcillin) were given daily from the day of instrumentation until completion of the last study.

Lung function measurements and calculations. On each study occasion, the lamb was standing in a loose-fitting sling (Alice King Chatham Medical Arts, Hawthorne, CA, U.S.A.). The lambs were unanesthetized and unsedated during the first two studies. Because older unsedated lambs can be very restless during these studies, they were lightly sedated on the third study occasion with chloral hydrate using a 50 mg/kg bolus injection followed by an infusion of 25 mg/kg/h. Measurements before and after sedation did not reveal any significant effect on lung function (data not presented). The lambs were breathing air spontaneously and the studies were performed during quiet breathing while awake. The technique for measurements and calculations of lung function used in this study has been evaluated previously and described in detail both in lambs (29) and in newborn infants (30). Lung mechanics, ventilation, FRC, and indices of gas mixing efficiency in distal airways were assessed.

Ventilation flow in the cuffed endotracheal tube inserted through the tracheostomy was measured by a heated pneumotachograph (model 3700, Hans Rudolph Inc., Kansas City, MO, U.S.A.) connected to a Sensortech pressure transducer (model 103LP, Sensortech GmbH, Puchheim, Germany) and a carrier demodulator. Flow was integrated to volume. Transpulmonary pressure was measured between the

Table 1. Lung function testing results

Study groups	Postnatal age 12 d			Postnatal age 25 d		
	LN	MN	C	LN	MN	C
Number of lambs	13	12	10	13	12	10
Study weight, kg	7.9 ± 1.6	7.8 ± 1.5	8.4 ± 1.8	12.4 ± 2.4	11.6 ± 1.5	11.7 ± 1.9
FRC, mL·kg ⁻¹	35 ± 6.6	32 ± 5.0	34 ± 4.2	31 ± 6.6	29 ± 5.4	30 ± 5.6
Compliance, mL·cm H ₂ O ⁻¹	14.8 ± 5.3	13.4 ± 5.3	16.1 ± 7.4	16.2 ± 3.8	19.5 ± 8.3	15.9 ± 4.0
Specific compliance, cm H ₂ O ⁻¹	0.054 ± 0.01	0.053 ± 0.01	0.055 ± 0.02	0.045 ± 0.02	0.054 ± 0.02	0.046 ± 0.01
Resistance, cm H ₂ O·L ⁻¹ ·s	11.0 ± 4.2	10.7 ± 4.4	7.2 ± 4.1	10.1 ± 6.7	9.9 ± 3.6	8.4 ± 3.7
Specific conductance, cm H ₂ O ⁻¹ ·s ⁻¹ ·L ^{†*}	0.38 ± 0.1	0.44 ± 0.2	0.81 ± 0.7	0.36 ± 0.2	0.37 ± 0.2	0.44 ± 0.2
Moment ratio, M ₁ /M ₀ **	2.55 ± 0.2	2.87 ± 0.5	2.70 ± 0.2	2.40 ± 0.2	2.61 ± 0.3	2.70 ± 0.3
Nitrogen Clearance Index**	7.2 ± 1.2	9.3 ± 3.1	8.1 ± 1.2	6.8 ± 0.7	8.4 ± 1.5	8.5 ± 1.8
Tidal volume, mL·kg ⁻¹	9.5 ± 1.2	8.8 ± 1.0	9.0 ± 1.4	8.0 ± 1.3	7.7 ± 1.1	7.9 ± 1.5
Breathing frequency, min ⁻¹	47 ± 13	56 ± 13	48 ± 9.9	39 ± 13	38 ± 8.1	37 ± 8.8
Minute ventilation, mL·kg ⁻¹ ·min ⁻¹	435 ± 75	493 ± 184	425 ± 78	297 ± 63	292 ± 59	296 ± 45

Values are mean ± SD.

† MN different from C at $p < 0.01$ over the whole study period.

* LN different from C at $p < 0.05$ over the whole study period.

** LN different from C at $p < 0.01$ over the whole study period.

airway (measured at the end of a 15-gauge blunt needle inserted perpendicularly and located in the center of the endotracheal tube connector) and the pleural balloon (Sil-Tec envelope) in the right pleural space by a pressure transducer (Sensortek model 140PC) and a carrier demodulator. Nitrogen concentration in the breathing gas was measured continuously. The probe of the nitrogen analyzer (Model 505, Med Science, Division of Advanced Instruments, Inc., St. Louis, MO 63152) was mounted at the outlet of the endotracheal tube, which was connected to a system of tubing with a continuous bypass flow of breathing gas. A nitrogen washout test was performed by changing breathing gas instantaneously to 100% oxygen during an expiration. The nitrogen washout test lasted until 1/40th of the end-tidal nitrogen concentration before the start of 100% oxygen breathing was reached. The washout test was performed two to three times in each lamb with at least 5 min in between to allow gas equilibration within the lung (31).

Before the study, the equipment measuring lung mechanics was tested for its frequency response up to 20 Hz. No deviations in phase or amplitude were noted, and the pneumotachometer was linear for the actual flow range.

All signals were digitized with 12-bit accuracy at a frequency of 250 Hz and stored to a disc. For the analysis, the time scale of the nitrogen signal was corrected for the measured transit time to the meter.

Lung mechanics were calculated from sampled ventilation flow and transpulmonary pressure. Assuming a single-compartment lung model, C_{dyn} and R_l were determined from the equation of motion by means of multiple linear regression analysis. Specific compliance was calculated as C_{dyn}/FRC and specific conductance as $1/R_l/FRC$. The methods for lung mechanics calculations have been described previously (32).

The total exhaled N₂ volume was calculated as the sum of the N₂ volumes exhaled in each breath during the N₂ washout test. This volume was obtained by summing the product of simultaneously measured flow and N₂ concentration signals. No correction was made for tissue derived nitrogen. FRC was calculated as the total expired nitrogen volume during wash-

out, divided by end-tidal nitrogen concentration preceding the washout.

Efficiency of gas mixing was assessed by calculating the nitrogen clearance (NC) index and moment ratio from the course of nitrogen elimination (33). NC index was defined as the ventilation volume necessary to dilute the nitrogen lung volume from 90 to 10% of its total value, divided by FRC. The zero (M_0) and first (M_1) moments of end-tidal nitrogen concentration, with accumulated expired volume normalized by FRC as the independent variable, were calculated and the ratio of (M_1/M_0) was determined as previously described (31). Tidal volume, breathing frequency, and minute ventilation were calculated from the recording of ventilatory flow.

Statistics. Data are reported at each time period as mean ± SD except in the figures where data are presented as mean ± SEM. Since each lamb was studied three times over the study period during their first 2 mo of life, response features analyses were performed. For each lamb, the area-under-the-curve (AUC) was computed for each outcome (34). In addition, rate-of-change over time was estimated for each lamb and each outcome using linear regression. One-way ANOVA was used to compare mean AUC and rate-of-change across the three groups, and two-sample t tests were used to compare the LN and MN groups to the control group. If normality of these response features was suspect, the Kruskal-Wallis and Mann-Whitney U tests were used. A p value < 0.05 was regarded as statistically significant.

RESULTS

Nicotine levels. Maternal plasma concentrations for nicotine and the major nicotine metabolite, cotinine, were 5.8 ± 2.8 and 16.8 ± 5.1 ng/mL for LN and 19.8 ± 2.3 and 70.9 ± 25.4 ng/mL for MN, respectively.

Body weight. There were no significant differences in weights of the lambs between the groups on any study occasion, and there were no differences in postnatal weight gain (Table 1).

Table 1. (Continued)

Study groups	Postnatal age 51 d		
	LN	MN	C
Number of lambs	13	12	10
Study weight, kg	19.5 ± 3.1	18.3 ± 2.7	18.6 ± 2.6
FRC, mL·kg ⁻¹	29 ± 5.6	28 ± 4.2	26 ± 7.7
Compliance, mL·cm H ₂ O ⁻¹	27.1 ± 8.6	21.9 ± 9.4	25.9 ± 8.2
Specific compliance, cm H ₂ O ⁻¹	0.049 ± 0.02	0.042 ± 0.01	0.054 ± 0.02
Resistance, cm H ₂ O·L ⁻¹ ·s	8.7 ± 1.9	11.8 ± 5.5	7.0 ± 2.2
Specific conductance, cm H ₂ O ⁻¹ ·s ⁻¹ ·kg ⁻¹	0.22 ± 0.1	0.20 ± 0.1	0.36 ± 0.2
Moment ratio, M ₁ /M ₀ **	2.26 ± 0.1	2.22 ± 0.3	2.45 ± 0.4
Nitrogen Clearance Index**	6.4 ± 1.1	6.6 ± 1.2	6.8 ± 2.4
Tidal volume, mL·kg ⁻¹	7.7 ± 1.6	7.0 ± 0.7	7.5 ± 1.7
Breathing frequency, min ⁻¹	28 ± 2.9	27 ± 4.0	28 ± 5.8
Minute ventilation, mL·kg ⁻¹ ·min ⁻¹	216 ± 44	187 ± 29	206 ± 43

Lung volume. The increase in FRC during the study period (the studies at 12, 25, and 51 d) differed between the groups. Compared with the control lambs, the gain in FRC in the LN lambs was 7.0 ± 1.8 mL/d, whereas the corresponding FRC gain in the C lambs was 4.5 ± 2.0 mL/d ($p < 0.01$). The corresponding value for the MN lambs was 6.4 ± 2.4 mL/d, which also differed from the C lambs ($p < 0.05$). The difference in FRC between LN and C lambs was confirmed by a significant difference in mean regression slope over time in FRC (FRC change per day) between these groups ($p < 0.01$). The corresponding slope difference between MN and C lambs was of borderline significance ($p = 0.07$).

Normalized to body weight, there were no significant differences in FRC/kg between the study groups on any study occasion (Table 1).

Mechanical properties of the lungs. Results from lung mechanics measurements on all three study occasions are presented in Table 1. There was a similar increase over time in C_{dyn} in all study groups, and there were no significant differences between the groups (Fig. 1A). C_{dyn} normalized to lung volume (specific compliance, C_{dyn}/FRC), did not differ between the groups during the whole study period, and there was no significant change over time in any of the nicotine or control groups (Fig. 1B).

Airway resistance was higher in the nicotine-exposed lambs compared with controls, although the difference was not statistically significant between the groups during the whole study period overall ($p = 0.06$) (Fig. 2A). However, when airway resistance was normalized to lung volume (FRC), specific conductance ($1/R_i/FRC$) was statistically different between the groups during the whole study period overall ($p < 0.01$). Both the LN and the MN groups had significantly lower $1/R_i/FRC$ when compared with the C group during the whole study period (LN versus C: $p < 0.05$ and MN versus C: $p < 0.01$) (Fig. 2B). The differences in specific conductance seemed to persist over time and the nicotine groups did not differ from each other.

Gas-mixing efficiency. Results of moment analysis and NC index are presented in Table 1. In the moment analysis, there was an overall statistically significant difference among the three groups during the whole study period ($p < 0.05$). The

Figure 1. Dynamic lung compliance (A) and specific compliance (B) in 13 low-dose nicotine (LN, filled triangles), 12 moderate-dose nicotine (MN, filled squares), and 10 control (C, filled diamonds) lambs studied at a median age of 12, 25, and 51 d. Values are mean $\pm$ SEM. No statistically significant differences were found between the groups.

Figure 2. Airway resistance (A) and specific conductance ($1/R_f/\text{FRC}$) (B) in 13 low-dose nicotine (LN, filled triangles), 12 moderate-dose nicotine (MN, filled squares), and 10 control (C, filled diamonds) lambs studied at a median age of 12, 25, and 51 d. Values are mean $\pm$ SEM. *Specific conductance was different from C over the study period at $p < 0.01$. **Specific conductance was different from C over the study period at $p < 0.01$.

M_1/M_0 ratio was significantly lower in the LN group when compared with controls during the whole study period ($p < 0.01$) (Table 1 and Fig. 3). This difference means that gas mixing in the lungs was more efficient in the LN-exposed lambs compared with the controls. This difference seemed to persist over the study period. Moment ratio in the MN lambs, however, did not differ significantly from the controls when tested over time.

There was an overall statistically significant difference between the groups for the NC index during the study period ($p < 0.01$). The LN lambs compared with the controls showed a significantly lower NC index (improved gas mixing) during the whole study period ($p < 0.01$), whereas the MN lambs did not differ significantly from the control lambs (Fig. 4).

Ventilation. Respiratory rate as well as tidal volume and minute ventilation, normalized to body weight, were higher on the first study occasion compared with the second and third

Figure 3. Moment analysis in 13 low-dose nicotine (LN, filled triangles), 12 moderate-dose nicotine (MN, filled squares), and 10 control (C, filled diamonds) lambs studied at a median age of 12, 25, and 51 d. Values are mean $\pm$ SEM. **Different from C over the study period at $p < 0.01$.

Figure 4. Nitrogen clearance index in 13 low-dose nicotine (LN, filled triangles), 12 moderate-dose nicotine (MN, filled squares), and 10 control (C, filled diamonds) lambs studied at a median age of 12, 25, and 51 d. Values are mean $\pm$ SEM. **Different from C over the study period at $p < 0.01$.

(Table 1). There were highly significant differences between the first and last study for respiratory rate, tidal volume, and minute ventilation in all three study groups ($p < 0.01$ by paired t test). There were no differences in ventilation between the study groups on any study occasion. Comparison of the rate of change in ventilation parameters using mean regression slopes for each study group showed no significant differences between the groups.

DISCUSSION

This study in young lambs has demonstrated that continuous prenatal exposure to either a low or a moderate dose of nicotine during the third trimester induces reduced specific airway conductance, indicating an obstruction in the proximal airways. Indices of distal airway function (moment ratio and nitrogen clearance) were significantly lower after a low dose of prenatal nicotine exposure, indicating increased gas mixing efficiency. These results support the concept that nicotine might have a different impact on different parts of the airways. Disparity of the nicotinic effect on proximal *versus* distal airway development and function and persistence of these changes during the whole 7-wk study period are the main results in our study that have not been reported by other investigators.

Proximal airway function. Measurement of airway resistance mainly reflects resistance to gas flow in proximal large airways (35, 36), where the gas flow is high due to a relatively narrow total cross-sectional area of the airways compared with the distal part of the lung. Factors limiting total cross-sectional area of the large airways alter the resistive part of the mechanical properties of the lung to a higher extent than airway changes in small peripheral airways, where gas flow is comparatively low due to larger total cross sectional airway diameter. Jones *et al.* (12) reported in a recent study that infants of mothers who smoked during pregnancy had reduced forced expiratory flow when examined by the rapid thoracic compression technique. This technique is assumed to measure flow resistance in the small airways between alveoli and the flow limiting segment (wherever the disease process is located) proximal to the alveoli (36). The forced expiratory flow technique also differs from the gas dilution technique used by us, which predominantly measures function of the most distal part of the airways (acinus with respiratory bronchioles) where the gas mixing takes place.

In the present study, measurements of lung mechanics showed a significant difference in airway conductance between nicotine-exposed lambs and controls. Specific conductance was significantly reduced to the same extent during the whole study period by both the low and the moderate nicotine dose. Although the maternal nicotine doses may appear high compared with human cigarette smokers, the achieved maternal plasma nicotine concentrations of the LN and MN group correspond to those observed in human smokers smoking <10 and approximately 20 cigarettes per day, respectively (26). Concentrations of cotinine, the major metabolite of nicotine that is thought to be pharmacologically inactive (37), were considerably lower than those observed in human smokers (26). These nicotine and cotinine concentrations mean that prenatal exposure to even a fairly low dose of nicotine affects proximal airway function with increased resistance to gas flow. The change in airway conductance prevailed during the whole study period, indicating a possible permanent alteration of the proximal airways.

Airway obstruction after prenatal nicotine exposure is in agreement with previous studies both in infants (6–12) and in rhesus monkeys (17). A possible explanation for this obstruction is provided by Sekhon *et al.* (16), who showed that

prenatally administered nicotine increased $\alpha 7$ nicotine acetylcholine receptor subunit expression and binding in the fetal lung. They subsequently reported that $\alpha 7$ nAChR co-localized with collagen in the same airway wall cells, suggesting that nicotine directly interacted with $\alpha 7$ nAChR to increase collagen deposition and, therefore, airway wall dimensions in the fetal lung (19). In addition, increased airway smooth muscle may contribute to excessive airway narrowing, as has been found in intrauterine smoke-exposed infants who died from SIDS (14).

Decreased airway dimensions may also be a result of reduced forces opposing airway narrowing as a result of decreased alveolar attachment points, as demonstrated in human infants and neonatal guinea pigs exposed to cigarette smoke *in utero* (18, 38), but it is not known whether this would affect airway resistance.

The elasticity of the lung (compliance and specific compliance) seemed to have been unaffected in the present study. The unchanged compliance is consistent with the results in prenatally nicotine-exposed monkeys (17). In contrast, parenchymal elastic tissue was reduced after prenatal maternal smoke or nicotine exposure in rat pups (15, 39).

Distal airway function. Effects of prenatal nicotine exposure on the terminal respiratory unit (the acinus, including the alveolar space and respiratory bronchioles, where gas mixing takes place mainly by diffusion) have been contradictory. In the rat, nicotine induced severe damage to normal alveolar structure (39, 40), whereas improvement of the surfactant system has been reported in the rhesus monkey (16). Enhancement of small airway branching morphogenesis and surfactant gene expression has been found in embryonic mouse lung culture (23). It is also well known that the incidence of respiratory distress syndrome in premature infants of smoking mothers is reduced due to assumed acceleration of lung maturation in these infants (20–22).

To study distal airway function, we used a multiple-breath nitrogen washout technique, measuring gas-mixing efficiency in the lung. In previous studies, we and others have found that measures of gas-mixing efficiency by means of gas dilution techniques are highly sensitive to changes in the terminal respiratory units of the lung (41, 42). The gas mixing of inspired and remaining alveolar gas during breathing is thought to occur in the preacinar and acinar part of the airways by means of a complex interaction between diffusive and convective forces (43). The efficiency of this process is influenced by several factors. According to model studies of gas mixing in terminal respiratory units, the efficiency in this process is highly influenced by airway structure (44). Factors such as alveolar size and number, alveolar septation, and airway branching points all affect the gas-mixing efficiency in the lung. During growth and development, the acinus structure becomes more “complex,” facilitating the gas-mixing efficiency during breathing. Factors influencing the normal development of acinus structure also affect gas-mixing efficiency in the lung.

Analysis of gas-mixing efficiency in distal airways showed significantly reduced moment ratio and nitrogen clearance index in the group exposed to a low dose of nicotine compared

with controls. We interpret these results as an improvement in the ability of the distal airways to mix inspired gas with remaining alveolar gas during breathing. The morphologic structure behind this improvement in gas mixing is not known, inasmuch as we did not examine the structural changes in the lung in this study. One could, however, speculate that the low dose of prenatal nicotine exposure specifically enhanced maturation of the acini in these lambs. An accelerated lung growth (increase in FRC) during the study period in the low-dose nicotine-exposed lambs also speaks in favor of enhanced lung maturation in this group. These results seen in the LN group are in agreement with the clinical findings of accelerated fetal lung maturation in infants of smoking mothers, *i.e.* earlier appearance of surfactant production (19–21), as well as the findings by Wuenschell *et al.* (23) in embryonic mouse lung culture showing stimulation of airway branching morphogenesis and increased surfactant gene expression after nicotine exposure. Stimulation of the surfactant-producing cells in the acini (16) in combination with accelerated small airway branching might be the structural background to the improved gas-mixing efficiency found in the low-dose nicotine-exposed lambs. Gas-mixing efficiency in distal airways did not improve in the lambs exposed to a moderate dose of nicotine, indicating that the effect of nicotine on the distal airways might depend on the dose of prenatal exposure.

Our finding of functional evidence of accelerated maturation of the acinar part of the nicotine-exposed lung is in contrast to findings in the rhesus monkey (16) and the rat (38, 39) models. In those studies, animals exposed to nicotine prenatally showed morphologic damage and alteration of the normal alveolar structure. The functional consequences of such changes should have been a deterioration of the gas mixing ability in the lung. Besides differences in species susceptibility to nicotine, the most plausible explanation for the discrepancy between our study and those in rhesus monkeys and rats is the difference in the dose of nicotine exposure. The LN lambs in our study were exposed to 0.5 mg/kg/d of nicotine, whereas, in the baboon model, the animals were exposed to 1–1.5 mg/kg/d. In fact, the MN-exposed lambs (1.5 mg/kg/d) in our study showed considerably less effective gas mixing on the first study occasion compared with the LN group and less effective mixing in comparison with the control animals (Fig. 3 and 4). Even though this difference in gas mixing between the nicotine-exposed groups did not prevail during the whole study period, it might further support the hypothesis that the nicotinic effect on the developing acinus depends on the dose of exposure.

Ventilation and lung volume. In this study, we did not find any significant differences in tidal volume, respiratory rate, or minute ventilation between the nicotine-exposed lambs and the controls on any study occasion. In the MN lambs at 12 d, there was a tendency toward increased minute ventilation, mainly due to an increase in respiratory rate. This difference was, however, not statistically significant. The absence of a difference in breathing pattern between nicotine-exposed and control lambs is in contrast to our previous study in low-dose nicotine-exposed lambs, where we found a reduced tidal volume and an increased respiratory rate in comparison with nonexposed lambs (25). However, in that study, the largest effect from

prenatal nicotine exposure was seen in lambs studied at an average age of 5 d instead of 12 d as in the current study. In the previous study, we found a progressive reduction in the difference in breathing pattern between nicotine-exposed and control lambs after 10 d of age. Therefore, prenatal nicotine exposure might have its dominating effect on breathing regulation and breathing pattern during the first week after birth, which we could not confirm in our current study because these lambs were not examined until 1 wk later.

The normal FRC found in the nicotine-exposed lambs in this study differs from some previous studies in infants (8) and rats (15) showing a reduction of the lung volume. However, there were differences in FRC gain during the whole study period between the study groups, probably reflecting different lung growth, where LN lambs showed most increase in FRC. We speculate that these differences in FRC gain also might reflect differences in lung maturation between the groups. In comparison with changes in gas-mixing efficiency, however, we found no simple correlation between FRC change and changes in M_1/M_0 or NC over time, indicating that there is no simple relationship between factors influencing lung volume growth and differentiation of peripheral airways.

SUMMARY

This study showed that prenatal nicotine exposure has complex influences on lung function in young lambs with different effects on proximal and distal airways. The lung mechanics measurements showed signs of airway obstruction in proximal airways, whereas the gas dilution technique showed signs of improved gas mixing in distal airways, which might reflect restriction in large airway growth and accelerated maturation of the acinar part of the lung, respectively. The effect on peripheral airways seemed to be dose related because only the low dose of nicotine improved distal airway function (gas-mixing efficiency). However, changes in proximal airway function (specific conductance) seemed to be unrelated to dose. The altered airway function persisted during the whole 51-d-study period, indicating that prenatal nicotine exposure might have a permanent impact on lung development and function with possible long-term health consequences.

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