Growth of the Lung Parenchyma Early in Life

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Rationale: Early in life, lung growth can occur by alveolarization, an increase in the number of alveoli, as well as expansion. We hypothesized that if lung growth early in life occurred primarily by alveolarization, then the ratio of pulmonary diffusion capacity of carbon monoxide (DL_{CO}) to alveolar volume (V_A) would remain constant; however, if lung growth occurred primarily by alveolar expansion, then DL_{CO}/V_A would decline with increasing age, as observed in older children and adolescents.

Objectives: To evaluate the relationship between alveolar volume and pulmonary diffusion capacity early in life.

Methods: In 50 sleeping infants and toddlers, with equal number of males and females between the ages of 3 and 23 months, we measured DL_{CO} and V_A using single breath-hold maneuvers at elevated lung volumes.

Measurements and Main Results: DL_{CO} and V_A increased with increasing age and body length. Males had higher DL_{CO} and V_A when adjusted for age, but not when adjusted for length. DL_{CO} increased with V_A ; there was no gender difference when DL_{CO} was adjusted for V_A . The ratio of DL_{CO}/V_A remained constant with age and body length.

Conclusions: Our results suggest that surface area for diffusion increases proportionally with alveolar volume in the first 2 years of life. Larger DL_{CO} and V_A for males than females when adjusted for age, but not when adjusted for length, is primarily related to greater body length in boys. The constant ratio for DL_{CO}/V_A in infants and toddlers is consistent with lung growth in this age occurring primarily by the addition of alveoli rather than the expansion of alveoli.

Keywords: pulmonary diffusion capacity; alveolar volume; lung development

The lung, which provides surface area for gas exchange, begins alveolarization of the lung parenchyma in late gestation; however, the number of alveoli present at birth is estimated to be less than 20% of the number in adults (1–3). In addition, alveolar size of an infant is smaller than the alveolar size of an adult (1–3). Therefore, postnatal growth and development of the lung parenchyma includes increases in number as well as size of alveoli. Lung volume early in life is thought to occur primarily by the addition of alveoli during the rapid phase of somatic growth, and then, following alveolarization of the lung parenchyma, lung volume increases by the expansion of existing alveoli. However, from the limited number of morphometric studies of relatively few autopsied lungs from infants and toddlers, which have used differing morphometric techniques to estimate alveolar number, it remains

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Current understanding of early lung growth and development is based largely on small numbers of autopsied lungs from infants and toddlers; *in vivo* physiologic measurements in this age group are scarce.

What This Study Adds to the Field

Pulmonary diffusing capacity and alveolar volume increases with increasing age among infants and toddlers, but their ratio remains constant. This suggests that, in this age range, lung volume increases by alveolarization rather than expansion.

unclear whether the addition of alveoli is complete by 6 months, 2 to 3 years, or 8 years of age (2, 4–6).

In vivo physiologic measurements of alveolar volume (V_A) and pulmonary diffusing capacity for carbon monoxide (DL_{CO}) can provide a functional assessment of the volume and surface area available for gas exchange, which indirectly reflects the cumulative effects of alveolar number and size. In older subjects from 8 years of age to adulthood, DL_{CO} and V_A increase with somatic growth; however, the ratio (DL_{CO}/V_A) declines in this age range (7, 8). These physiologic results are consistent with morphometric data that parenchymal lung growth occurs in this age range primarily by the expansion of the existing number of alveoli, and thus alveolarization is complete by 8 years of age. The aim of our study was to use physiologic measurements of pulmonary diffusing capacity and alveolar volume in infants and toddlers to evaluate the relationship between surface area and lung volume during this rapid period of lung growth. We hypothesized that if lung growth in infants and toddlers occurs primarily by the addition of alveoli, then the ratio of carbon monoxide diffusion capacity to alveolar volume (DL_{CO}/V_A) would remain constant or increase, but not decline in this young age range. (This data has been presented in abstract format [9].)

METHODS

Subjects were recruited from advertisements in local publications in Indianapolis, Indiana. All subjects were born at a gestational age greater than 37 weeks, had no cardio-respiratory malformations, and their respiratory history was negative for wheezing, asthma, treatment with asthma medications, or hospitalization for a respiratory illness. History of exposure to tobacco smoking was obtained from the parents. Subjects were recruited between 2007 and 2008; they were all evaluated in the Infant Pulmonary Function Laboratory at James Whitcomb Riley Hospital for Children, Indiana University Medical Center, while sleeping in a supine position with chloral hydrate sedation (50–100 mg/kg). Oxygen saturation and heart rate were monitored during testing as recommended by ATS/ERS guidelines (10). The study was approved by the institutional Review Boards at Indiana University and informed consent was obtained from infants' parents.

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Alveolar volume and pulmonary diffusing capacity were measured as previously described (11). In sleeping infants, the respiratory system was inflated twice to a lung volume defined by an airway pressure of 30 centimeters H₂O (V_{30}), which inhibited inspiratory effort and induced a respiratory pause at functional residual capacity (FRC). The inspiratory gas was switched from room air to the test gas (0.3% C¹⁸O, 5% He, 21% O₂ and balance N₂) and inflation to V_{30} with the test gas induced a respiratory pause at V_{30} , which was maintained for 4 seconds (breath-hold time), and then followed by passive exhalation to FRC. The helium and carbon monoxide concentrations were continuously measured with a respiratory gas mass spectrometer (Perkin Elmer MGA-1100, Waltham, MA). Analog signals for flow, pressure, and gas concentrations were sampled at 100 Hz, amplified, digitized, displayed on the computer monitor in real time, and adjusted for phase differences between the signals before analysis. Alveolar volume at an airway pressure of 30 centimeters H₂O (V_{A30}) was calculated by the dilution technique. The inspiratory volume of test gas was the measured inflation volume of the test gas containing 4% helium and the alveolar concentration was calculated as the mean helium concentration between 60 and 80% of the passive expiratory volume following the 4-second breath-hold. Following 60% of expired volume, the helium concentration remained constant, which reflects that alveolar equilibration had been achieved. DL_{CO} was calculated from the inspired volume of test gas (0.3% C¹⁸O) and the alveolar concentration of carbon monoxide, which was calculated as the average C¹⁸O concentration between 60 and 80% of the passive expired volume following the breath-hold, and the calculated DL_{CO} was corrected to an Hb of 13.4 using the subjects' measured Hb, as recommended by ATS standards (12, 13). Following the completion of measurements of V_{A30} and DL_{CO} , a finger-stick was used to obtain a blood sample for determining Hb concentration (Hemacue Hb201, Lake Forest, CA). Values for V_{A30} and DL_{CO} were expressed as the means of 2 or 3 measurements that were within 10% of each other.

Statistical Analysis

Statistical analysis was performed using SAS software version 9.1 (SAS, Cary, NC). Simple linear regression models were used to determine whether there were significant relationships between V_A , DL_{CO} or DL_{CO}/V_A and length, age, or sex of the children. The effects of race and exposure to tobacco smoke as predictors were also evaluated. Stepwise regression was used to determine candidate multiple regression models. R^2 values and significance levels (P value ≤ 0.1) were considered in selecting the best model for presentation. Sex was also included in selected models to determine if there was a difference in the outcomes between boys and girls after adjusting for other covariates. Values are expressed as mean $\pm$ SD. For all analyses, the α level for statistical significance was set at 0.05.

RESULTS

We evaluated 54 subjects ranging in age from 3 to 23 months. Two of the subjects did not sleep adequately to obtain measurements, and two subjects did not have at least two measurements within 10% of each other. The individual data for the 50 subjects and the results are listed in the on-line supplement (Table E1). There were equal numbers of boys and girls evaluated and they were equally distributed over the age range. For a given age, males were longer than females (see Figure E1 in the online supplement). There were a greater number of Caucasians ($n = 32$) than non-Caucasians ($n = 18$); the latter were primarily African American.

Alveolar volume increased with increasing age; boys had higher values than girls for alveolar volume when adjusted for age (Table 1). However, when alveolar volume was adjusted for body length, there was no longer a significant difference in alveolar volume for boys and girls (Figure 1). Pulmonary diffusing capacity increased with increasing age; boys had higher values than girls when adjusted for age (Table 1). When pulmonary diffusion capacity was adjusted for body length, there was no significant difference between boys and girls (Figure 2). Pulmo-

TABLE 1. LINEAR REGRESSIONS FOR ALVEOLAR VOLUME (V_A) AND PULMONARY DIFFUSING CAPACITY (DL_{CO})

Outcome	Parameter	Estimate	P-value	R ²	Root MSE
V_{A30} (ml, BTPS)	Intercept	235	<0.0001	0.741	89.52
	Age (mo)	29.5	<0.0001		
	Female	-62	0.019		
V_{A30} (ml, BTPS)	Intercept	-1111	<0.0001	0.841	70.05
	Length (cm)	22.9	<0.0001		
	Female	-20.1	0.3160		
DL_{CO} (ml/min/mm Hg; STPD)	Intercept	1.44	<0.0001	0.795	0.432
	Age (mo)	0.17	<0.0001		
	Female	-0.41	0.0020		
DL_{CO} (ml/min/mm Hg; STPD)	Intercept	-5.84	<0.0001	0.852	0.368
	Length (cm)	0.125	<0.0001		
	Female	-0.171	0.1070		
DL_{CO} (ml/min/mm Hg; STPD)	Intercept	0.399	0.0170	0.901	0.300
	V_{A30} (ml)	0.005	<0.0001		
	Female	-0.069	0.4220		

Definition of abbreviations: BTPS = body temperature and pressure, saturated; MSE = mean squared error; STPD = standard temperature and pressure, dry.

nary diffusing capacity and alveolar volume were not related to race or exposure to tobacco smoke (see Table E2).

Pulmonary diffusing capacity increased linearly with increasing lung volume, as illustrated in Figure 3A; this relationship was not affected by sex, race, or exposure to tobacco smoking. The ratio of pulmonary diffusion capacity to alveolar volume remained constant with body length ($P = 0.34$); the mean was 5.82 ± 0.58 and did not differ for sex ($P = 0.95$) (Figure 3B). If DL_{CO}/V_A was not corrected for Hb, the values still remained constant with body length (5.58 ± 0.54).

DISCUSSION

Our study of healthy infants born at full term demonstrated that in the first 2 years of life, alveolar volume, as well as pulmonary diffusing capacity, increased with age and somatic growth. More importantly, pulmonary diffusing capacity increased linearly with increasing alveolar volume, and the ratio of pulmonary diffusing capacity to alveolar volume remained constant in this age group. Our *in vivo* physiologic assessment of parenchymal lung growth early in life is consistent with morphometric findings of autopsied

Figure 1. Alveolar volume at lung volume defined by 30 centimeters H₂O (V_{A30} ; ml) versus body length (cm) for males (triangles; $n = 25$) and females (circles; $n = 25$). There was a significant linear relationship between alveolar volume and body length ($R^2 = 0.84$; $P < 0.0001$); after adjusting for body length, males did not differ from females for alveolar volume ($P = 0.316$). BTPS = body temperature and pressure, saturated.

Figure 2. Pulmonary diffusing capacity (DL_{CO} ; ml/min/mm Hg) versus body length (cm) for males (triangles; $n = 25$) and females (circles; $n = 25$). There was a significant linear relationship between pulmonary diffusing capacity and body length ($R^2 = 0.85$; $P < 0.0001$); after adjusting for body length, there was no significant differences for males and females ($P < 0.107$). STPD = standard temperature and pressure, dry.

lungs that suggest that the number of alveoli increase with increasing lung volume until at least 2 years of age.

Our study assessed pulmonary diffusion capacity and alveolar volume in this age group using the single breath-hold technique, which obtains measurement at an elevated lung volume, similar to those obtained in older cooperative children. Using the criteria of accepting values within 10%, which is the criteria used for older cooperative subjects performing this maneuver, we successfully obtained measurements in 50 of 52 infants and toddlers that fell asleep. We found high correlations for increasing alveolar volume with increasing age as well as increasing body length. When adjusted for age, boys had higher alveolar volumes; however, this age-related difference could be accounted for by the greater somatic size of boys than girls. Previous studies of lung volumes in infants and toddlers also found no sex differences when volumes are adjusted for body size (14, 15). Our physiologic data is also consistent with morphometric data of autopsied lungs, which found no sex differences from subjects less than 2 years of age (2).

Pulmonary diffusion capacity increased with age, and males had higher values than females when adjusted for age. After adjusting for body length, DL_{CO} did not differ significantly for males and females. Although a sex difference in DL_{CO} has been reported for older children when DL_{CO} was adjusted for body length, our study, as well as those in older children (7, 8), did not find a sex difference when pulmonary diffusing capacity was adjusted for lung volume. In addition, for our infants and toddlers, DL_{CO} had a higher correlation with alveolar volume than with age or body length. Cumulatively, these findings strongly suggest that sex differences in pulmonary diffusing capacity are primarily related to sex differences in somatic size, rather than sex differences in the structure of the lung parenchyma. Our physiologic results agree with morphometric results that males and females have a similar number of alveoli per unit volume as well as similar mean linear intercepts (2).

Among our infants and toddlers, the ratio of pulmonary diffusing capacity to alveolar volume remained constant with age and body length, and did not differ between sexes. Our physiologic measurements in infants and toddlers suggest that the ratio of alveolar surface area to alveolar volume remains constant in this very young age group, which is consistent with rapid lung growth early in life occurring by increasing alveolar number rather than increasing alveolar size. In contrast to our findings in infants and toddlers, children between 8 and 18 years of age demonstrate a decline in DL_{CO}/V_A with increasing age (7, 8). In this older age group, the decline in the ratio of surface area to volume occurs during a period of slower parenchymal growth and

Figure 3. (A) Pulmonary diffusing capacity (DL_{CO} ; ml/min/mm Hg) versus alveolar volume (V_A) at lung volume defined by 30 centimeters H_2O (V_{A30} ; ml) for males (triangles; $n = 25$) and females (circles; $n = 25$). There was a significant linear relationship between pulmonary diffusing capacity and alveolar volume ($R^2 = 0.90$; $P < 0.0001$); after adjusting for alveolar volume, there was no significant difference in pulmonary diffusing capacity for males and females ($P < 0.422$). (B) Ratio of pulmonary diffusing capacity to alveolar volume at lung volume defined by 30 centimeters H_2O (DL_{CO}/V_{A30} ; 1/min/mm Hg) versus body length for males (triangles; $n = 25$) and females (circles; $n = 25$). There was no significant relationship between the ratio of pulmonary diffusing capacity to alveolar volume and body length ($P = 0.339$), and there was no significant difference for males and females ($P = 0.950$). BTPS = body temperature and pressure, saturated; STPD = standard temperature and pressure, dry.

it is consistent with morphometric data that there is an expansion of a fixed number of alveoli (16).

Only one previous study reported measurements of pulmonary diffusing capacity in infants and toddlers (17). In contrast to our single breath-hold maneuver at an elevated lung volume, which is similar to that used in older cooperative children, these investigators evaluated sleeping infants using steady-state tidal breathing measurements, whereas functional residual capacity was measured separately by helium dilution. Although measurements of pulmonary diffusing capacity obtained at functional residual capacity should result in lower values than measurements obtained at an elevated lung volume (18), Boule and colleagues (17) reported much higher values for DL_{CO} than we observed for our subjects. Differences in the results for the studies are most likely secondary to methodological differences.

We used a 4-second breath-hold maneuver in our study because it was not practical to achieve a 10-second maneuver with our sleeping infants. In our infants we observed a plateau concentration for helium during expiration from V_{30} to FRC, which suggests that a 4-second maneuver was adequate to achieve

alveolar equilibration in these healthy infants. In addition, studies in healthy adults reported no difference in the values for pulmonary diffusing capacity measured for breath-hold times of 3, 5, and 10 seconds (19). Therefore, we believe that our 4-second maneuver accurately measured diffusing capacity in this young age group. We also adjusted the pulmonary diffusion capacity for the subjects' hemoglobin, as recommended by ATS. All of our healthy subjects had Hb values within normal limits. We did not partition DL_{CO} into the proportion related to membrane diffusion and pulmonary capillary volume. Therefore, we were unable to determine how each of these components changed with somatic growth. Morphometric data suggest that there may be a small decrease in alveolar septal thickness in the age range we evaluated, which could contribute to an increase in membrane diffusion and DL_{CO} (3).

In conclusion, we found that pulmonary diffusing capacity and alveolar volume increase with increasing age and somatic size among infants and toddlers. Sex differences are primarily related to somatic size; there are no sex differences when pulmonary diffusing capacity is related to alveolar volume. In addition, the ratio of pulmonary diffusion to alveolar volume remains constant in the first 2 years of life, which is consistent with lung growth in this age occurring as the result of an increase in the number of alveoli rather than an expansion of the same number of alveoli.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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