

# Alveolarization Continues during Childhood and Adolescence

## New Evidence from Helium-3 Magnetic Resonance

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**Rationale:** The current hypothesis that human pulmonary alveolarization is complete by 3 years is contradicted by new evidence of alveolarization throughout adolescence in mammals.

**Objectives:** We reexamined the current hypothesis using helium-3 (<sup>3</sup>He) magnetic resonance (MR) to assess alveolar size noninvasively between 7 and 21 years, during which lung volume nearly quadruples. If new alveolarization does not occur, alveolar size should increase to the same extent.

**Methods:** Lung volumes were measured by spirometry and plethysmography in 109 healthy subjects aged 7–21 years. Using <sup>3</sup>HeMR we determined two independent measures of peripheral airspace dimensions: apparent diffusion coefficient (ADC) of <sup>3</sup>He at FRC (n = 109), and average diffusion distance of helium ( $\bar{X}_{rms}$ ) by q-space analysis (n = 46). We compared the change in these parameters with lung growth against a model of lung expansion with no new alveolarization.

**Measurements and Main Results:** ADC increased by 0.19% for every 1% increment in FRC (95% confidence interval [CI], 0.13–0.25), whereas the expected change in the absence of neoalveolarization is 0.41% (95% CI, 0.31–0.52). Similarly, increase of ( $\bar{X}_{rms}$ ) with FRC was significantly less than the predicted increase in the absence of neoalveolarization. The number of alveoli is estimated to increase 1.94-fold (95% CI, 1.64–2.30) across the age range studied.

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**Author Contributions:** M.N. was responsible for the practical conduct of the research including planning and coordination, preliminary analyses, and interpretation of the data, and wrote the first draft of the manuscript. J.O.-B. had overall responsibility for the <sup>3</sup>He MR measurements. C.S.B. had overall responsibility for the physiologic measurements. M.M. set up the protocols for hyperpolarized gas production and collection and for the <sup>3</sup>He MR measurements using the rapid acquisition with refocused echoes sequence. I.B. and R.G. performed the <sup>3</sup>He MR measurements and analyzed the <sup>3</sup>He data. K.S.P. programmed data collection using the MR spectroscopy sequence and contributed to many of the measurements. C.E.K. and B.D.S. were responsible for the selection of subjects, the statistical analysis, and model-fitting. S.E.W. performed many of the physiologic measurements and had responsibility for much of the study administration. M.S., J.O.-B., C.S.B., and C.E.K. planned the study. All authors contributed to the different drafts of the manuscript and approved the final version. M.S. had the overall responsibility for the project and a major input into study design, analysis, and manuscript. M.S. acts as the guarantor.

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### AT A GLANCE COMMENTARY

#### Scientific Knowledge on the Subject

It is believed that human pulmonary alveolarization ceases in early childhood and that new alveoli do not form after about 2–3 years of life.

#### What This Study Adds to the Field

We provide direct evidence that supports ongoing alveolarization throughout childhood and adolescence in humans.

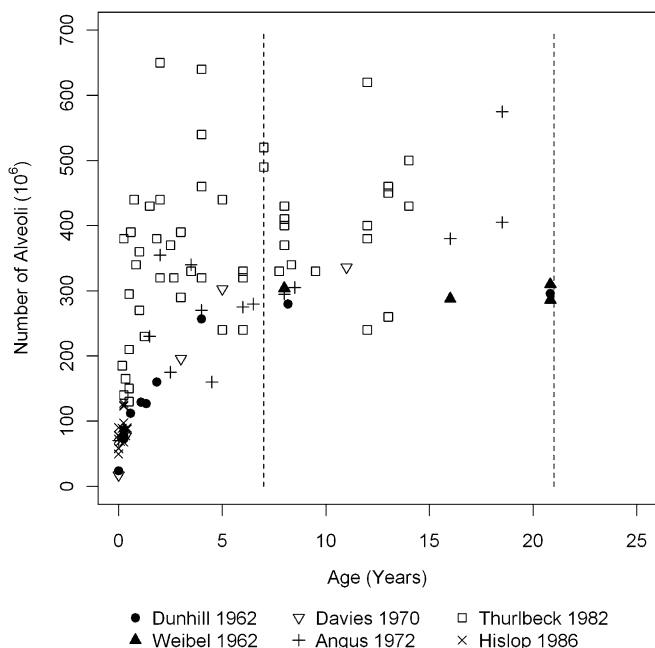
**Conclusions:** Our observations are best explained by postulating that the lungs grow partly by neoalveolarization throughout childhood and adolescence. This has important implications: developing lungs have the potential to recover from early life insults and respond to emerging alveolar therapies. Conversely, drugs, diseases, or environmental exposures could adversely affect alveolarization throughout childhood.

**Keywords:** growth and development; lung development; alveolarization

The process and timing of postnatal development of alveoli (alveolarization) in the human lung is poorly understood (1, 2) despite a number of studies in recent decades (3, 4). Apart from clarifying the prognosis of diseases of early childhood, accurate knowledge of alveolarization can contribute to understanding the factors affecting lung growth and development and lung structure–function relationships. Further, it can provide the basis for the development and evaluation of new treatment modalities designed to restore damaged alveolar function.

Currently, pulmonary alveoli are thought to stop multiplying by 2–3 years of age in humans (5, 6), although some have suggested 7–8 years as the limit (7, 8). This hypothesis is based on studies using older morphometric histologic techniques to analyze whole lungs from autopsies of children. However, there is a disparity between these studies both in the measured alveolar number and in the estimated age at which alveolarization is complete (Figure 1) (3, 4, 7–10). Repeating these studies with modern techniques of morphometry is impractical because of severe ethical constraints in access to adequate specimens from children (11). However, new evidence based on modern techniques has shown that alveolarization continues beyond early life and through maturity in some mammals (12–14). Post-pneumonectomy alveolarization can occur in mature animals (13). Although there is increasing acceptance of the possibility of late alveolarization in humans (1), there is no direct evidence for this.

*In vivo* measurement methods are currently the only realistic options to study human alveolarization. One such novel method depends on the measurement of self-diffusion of hyperpolarized



**Figure 1.** Number of alveoli in the developing human lung estimated by morphometry from previously published studies. *Dotted lines* (7 yr and 21 yr) represent the age range reported in the present study.

helium-3 ( $^3\text{He}$ ) in the lung periphery during a brief breath-hold using magnetic resonance (MR). A parameter known as apparent diffusion coefficient (ADC) can be determined, which is related to the dimensions of the enclosing structure, in this case the lung peripheral airspaces including alveolar ducts and alveoli (15, 16). Recently, it has also become possible to determine the average linear dimensions of the peripheral airspaces and alveoli by  $^3\text{HeMR}$  (17).

Using  $^3\text{HeMR}$ , we tested the hypothesis that human alveolarization stops by 3 years of age. Because human lung volume increases three- to fourfold between 7 years of age and adulthood (18), the volume of individual alveoli should increase to the same extent over this period of growth if no more alveoli form.  $^3\text{HeMR}$  can reliably detect such an increase in alveolar size. Some of the results of these studies have been previously reported in the form of an abstract (19).

## METHODS

### Subjects

Children and young adults (7–21 yr of age) were recruited from the Leicester respiratory cohorts (20) and a community database. Of the 173 who attended, we excluded 53 born before 36 weeks of gestation, admitted to a neonatal unit or with significant respiratory illnesses, and 11 because data did not meet validity criteria, leaving 109 subjects. The study had Research Ethics Committee approval. Written informed consent was obtained from all subjects and, for minors less than 18 years, from their parents or legal guardians.

### Physiologic Measurements

We measured height, weight, and at least three technically acceptable recordings of spirometry and plethysmography (Jaeger Masterscreen Body, Wuerzburg, Germany) and recorded the highest values of  $\text{FEV}_1$  and FVC and the mean value of FRC (21, 22). Z-scores for height and weight (23) and spirometry and plethysmography (18, 24) were based on United Kingdom reference values.

### Hyperpolarized $^3\text{HeMR}$

$^3\text{HeMR}$  was undertaken in a 0.15-T permanent magnet system (Inter-magnetics General Corp., New York, NY), where the subjects lie supine and breath-hold after inhalation of 600 ml of a mixture of  $^3\text{He}$  in  $^4\text{He}$  from FRC. We used two techniques of MR.

First, we obtained the global ADC using a modified rapid acquisition with refocused echoes MR sequence (25). At least three technically satisfactory ADC values were obtained in all subjects and the mean was taken as the raw ADC. Separate measurements done with constant bolus volume but different concentrations of helium were used to adjust the raw ADC for differences in  $^3\text{He}$  concentration: the concentration-corrected value is  $\text{ADC}_0$ . We also conducted measurements with different bolus sizes in volunteers (bolus effect study) to determine the effect of lung inflation on ADC.

The second technique (q-space technique) (17) was used in 46 subjects. The mean displacement ( $\overline{X_{rms}}$ ) of  $^3\text{He}$  atoms is derived from the displacement probability profile of the  $^3\text{He}$  atoms obtained from this technique. This gives a direct measure of mean linear dimensions of peripheral airspaces. We also applied the Yablonskiy acinar model (26) to the q-space data and obtained values for mean alveolar duct diameter,  $R$  (including alveolar sleeve), and mean alveolar sleeve depth,  $h$ . The effect of helium concentration and bolus size on these parameters was estimated.

### Statistical Analysis

We fitted a multilevel linear regression model of log-transformed  $\text{ADC}_0$  against log-transformed FRC and relative inflation volume combining the data from single measurements from the main study with measurements at varying bolus volumes from the bolus effect study. The first level of the model represents subjects and the second level different measurements on a given subject. The null hypothesis of no neoalveolarization is satisfied if the regression slopes of ADC against FRC and inflation volume are equal. We tested this using the Wald test.

The model was also used to estimate the fully concentration- and volume-corrected ADC:  $\text{ADC}_{\text{corr}}$ . We assessed associations of log-transformed  $\text{ADC}_{\text{corr}}$  with age, height, weight, and log FRC separately using linear regression models with and without adjusting for confounding factors. All tests were two-sided with a significance threshold of 0.05. Similarly, we tested for trends in mean peripheral airspace dimension with height, weight, age, and FRC. For this outcome, we did not adjust for potential confounding because of limited sample size. Data were analyzed using Stata Version 11 (StataCorp, College Station, TX). Details of methods are provided in the online supplement.

## RESULTS

### Demographics and Potential Confounders

The study population consisted of 109 subjects (50 males) aged 7–21 years (median, 12.8 yr): 79 (72.4%) whites; 22 (20.2%) of south Asian origin; and 8 (7.3%) of other or mixed ethnicity. Forty six participants (42.2%) had current or previous passive exposure to tobacco smoke (before birth or during childhood). Seven (6.4%) had tried cigarette smoking but none were current smokers. Previous inhaled corticosteroid treatment was recorded in 18 (16.5%).

### Physiologic and Lung Function Measurements

All subjects were appropriately grown (Table 1) and measurements of  $\text{FEV}_1$ , FVC, and FRC (Table 1) were approximately normally distributed. The range of the measured parameters was as expected given the wide age range. Linear regression of FRC on age gave a 3.4-fold increase in mean estimated FRC from 7 (1.02 L) to 21 years (3.50 L).

### Hyperpolarized $^3\text{HeMR}$

Within-subject values of ADC were internally consistent (mean coefficient of variation, 3.1%). The concentration-corrected

**TABLE 1. MEAN AND DISTRIBUTION OF MEASUREMENTS INCLUDING LUNG FUNCTION TESTS AND HELIUM MAGNETIC RESONANCE DATA**

Measurement	N*	Mean (SD)	Range
<b>Anthropometry</b>			
Height, cm	109	154.7 (14.9)	122.6 to 184.5
Weight, kg	109	49.7 (14.8)	22.5 to 99.7
<b>Lung function</b>			
FEV <sub>1</sub> , L	108	2.8 (0.89)	1.34 to 5.16
FVC, L	108	3.2 (1.02)	1.51 to 5.85
FRC, L	109	1.98 (0.7)	1.02 to 3.95
FEV <sub>1</sub> z-score	108	0.2 (0.94)	-2.5 to 2.9
FVC z-score	108	0.09 (1.00)	-2.4 to 2.6
FRC z-score	109	-0.55 (0.95)	-3.2 to 1.9
<b><sup>3</sup>HeMR data</b>			
ADC <sub>corr</sub> , cm <sup>2</sup> sec <sup>-1</sup>	109	0.094 (0.012)	0.070 to 0.139
$\bar{X}_{rms}$ , mm	45†	0.418 (0.039)	0.354 to 0.515
Alveolar duct radius including alveolar sleeve (R)‡, mm	46†	0.426 (0.040)	0.318 to 0.503
Alveolar sleeve height (h)‡, mm	46†	0.244 (0.043)	0.164 to 0.345

Definition of abbreviations: ADC = apparent diffusion coefficient of <sup>3</sup>He;  $\bar{X}_{rms}$  = mean peripheral airspace diameter.

\* Number of technically acceptable observations.

† Measurements were done only in a subset of the volunteers.

‡ Alveolar duct radius (R) and alveolar sleeve height (h) derived from application of the Yablonskiy model of alveolar duct (26).

ADC (ADC<sub>0</sub>) increased by 0.19% (95% confidence interval [CI], 0.13–0.25) for every 1% increment in FRC in the study population (slope  $\alpha$ ) but by 0.41% (95% CI, 0.31–0.52) for every 1% inflation in the bolus-effect study (slope  $\beta$ ) ( $P$  value for difference between  $\alpha$  and  $\beta < 0.001$ ). The change of ADC<sub>0</sub> with FRC remained less than in the predicted scenario of “no alveolarization” after adjusting for confounders ( $P = 0.02$ ). From  $\alpha$  and  $\beta$ , the rate of neoalveolarization was estimated as 0.54 (95% CI, 0.41–0.68). Based on these results, we estimated that the observed 3.4-fold increase in FRC between 7 and 21 years of age was accompanied by a 1.94-fold (95% CI, 1.64–2.30) increase in alveolar number and a 1.75-fold (95% CI, 1.48–2.07) increase in alveolar volume (see online supplement for derivation).

The slope  $\beta$  was used to calculate ADC<sub>corr</sub>, the fully concentration- and volume-corrected ADC. Mean (SD) ADC<sub>corr</sub> by <sup>3</sup>HeMR was 0.094 (0.012) cm<sup>2</sup>s<sup>-1</sup>, and was similar in males and females ( $P = 0.90$ ). ADC<sub>corr</sub> increased significantly with most measures of growth including age (slope of log[ADC<sub>corr</sub>] vs. age = 0.013; 95% CI, 0.006–0.021;  $r^2 = 0.10$ ;  $P < 0.001$ ) (Figure 2, top panel); FRC (slope of log[ADC<sub>corr</sub>] vs. log[FRC] = 0.16; 95% CI, 0.09–0.23;  $r^2 = 0.18$ ;  $P < 0.001$ ) (Figure 2, bottom panel); and height (slope of log[ADC<sub>corr</sub>] vs. height = 0.0031; 95% CI, 0.0016–0.0047;  $r^2 = 0.13$ ;  $P < 0.001$ ). Adjustment for potential confounders had negligible effects on the measured relationships between ADC with age or FRC.

The mean peripheral airspace dimension ( $\bar{X}_{rms}$ ; range and mean reported in Table 1) did not change with helium concentration. There was no significant increase with age (slope of log( $\bar{X}_{rms}$ ) vs. age = 0.005; 95% CI, -0.003 to 0.012;  $r^2 = 0.04$ ;  $P = 0.2$ ) (Figure 3, top panel) and with FRC (slope of log( $\bar{X}_{rms}$ ) vs. log[FRC] = 0.053; 95% CI, -0.021 to 0.128;  $r^2 = 0.05$ ;  $P = 0.16$ ) (Figure 3, bottom panel). Because measurements of  $\bar{X}_{rms}$  with different bolus sizes changed as the cubic root of volume, in the absence of neoalveolarization the slope of log( $\bar{X}_{rms}$ ) against log[FRC] should be 0.33.  $\bar{X}_{rms}$  data are compatible with an even higher rate of alveolarization than estimated from ADC data (see online supplement and Figure 3, bottom panel). Similarly, both parameters derived from the Yablonskiy acinar model,  $R$  and  $h$ , changed significantly less with increasing FRC (slope of log[ $R$ ] vs. log[FRC] = 0.105; 95% CI, -0.03 to 0.18;  $r^2 = 0.159$ ;  $P = 0.006$ ) (slope of log[ $h$ ] vs. log[FRC] = 0.16; 95% CI, -0.013 to 0.30;  $r^2 = 0.09$ ;  $P = 0.03$ ) than expected from the

scenario of no new alveolarization. These results did not alter after adjusting for potential confounders.

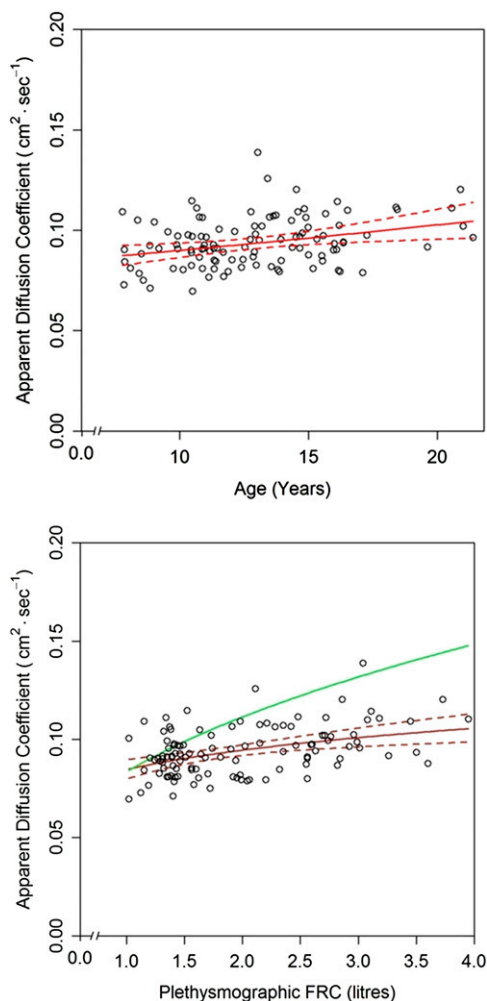
## DISCUSSION

We have shown that dimensions of alveoli determined by <sup>3</sup>HeMR increase with age and lung size during childhood and adolescence at a rate much less than would be expected if lung growth occurred only by expansion of the preexisting airspaces. This is best explained by postulating that lung grows largely by neoalveolarization through childhood and adolescence. This contradicts the prevailing hypothesis that alveolarization is restricted to fetal life and early childhood. If confirmed by future studies, our findings have important clinical implications for the prognosis of lung disease and for the impact of drugs and environmental exposures during childhood.

The reliability of the <sup>3</sup>HeMR technique is critical to this interpretation. Our measurements were highly repeatable, with a within-subject coefficient of variation of ADC of 3.1%. The validity of ADC as a measure of pulmonary alveolar dimensions has been demonstrated by morphometry in animal models (27, 28) and in human lungs (29, 30). These studies demonstrate the use of ADC in noninvasively assessing peripheral airspace dimensions. Parameters derived from the second technique of MR ( $R$  and  $h$ ) have also been validated against human lung morphometry (30).

We considered the possibility that our results could be explained by changes in geometry of lung acinus with growth rather than neoalveolarization. However, our q-space data, analyzed using the acinar model of Yablonskiy and coworkers (26), show that both alveolar sleeve diameter and alveolar duct diameter increased less than expected with lung growth. In addition, because ADC and the parameters derived from q-space MR are measured with diffusion times of 14 and 5 milliseconds, respectively, they measure different geometric aspects of the peripheral airspaces and changes in relative geometry should be reflected in different relationships of these parameters with growth. However, both increase with FRC at a rate considerably slower than expected in the absence of neoalveolarization. Therefore, it is unlikely that changes in geometry occur with growth.

In the only other report of ADC in childhood, Altes and coworkers (31) measured ADC in 29 healthy subjects ranging from 4–30 years. They also report an increase of ADC with age. However, they did not measure lung size by independent

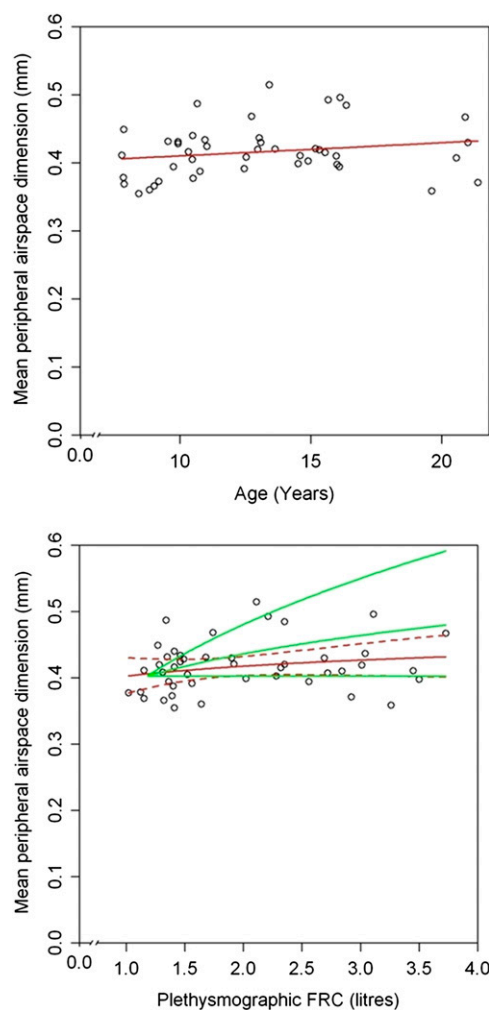


**Figure 2.** Scatterplot of apparent diffusion coefficient ( $ADC_0$ ) against (*top panel*) age and (*bottom panel*) resting lung volume (FRC). Values are weighted mean ADC from three measurements in each individual child and corrected to zero helium (He) concentration ( $ADC_0$ ) and FRC. The *solid red lines* indicate back-transformed linear fits of  $\log(ADC)$  on (*top panel*) age and on (*bottom panel*)  $\log(FRC)$  and the areas between the *dotted red lines* are 95% simultaneous confidence bands (Scheffe method). The *green curve* in the *bottom panel* shows the estimated change in ADC for a child with an initial FRC of 1 L assuming that the growth of the lung occurs only by proportional enlargement of preexisting alveoli without new alveolarization. This curve was estimated from the multilevel model (*see METHODS*).

means and there was no attempt to determine an expected line for increase of ADC with age. Shanbhag and coworkers (17) measured  $\overline{X_{rms}}$  in five children aged 6 years and in adults and found that  $\overline{X_{rms}}$  was lower in children than adults. There was no assessment of lung volume in this study. The mean age of adult subjects was 49 years, when effects of alveolar enlargement caused by senescence (25, 32) may have begun. In support of this,  $\overline{X_{rms}}$  reported for the five children was very close to the values in the two youngest adults (both 28 yr) in their study.

The belief that alveolarization in humans was complete by 3 years was based on studies using older methods of morphometry, the drawbacks of which have been discussed previously (33). New methods of measuring alveolar number and size have since been developed (34, 35). However, human alveolarization has not yet been studied using these new techniques, largely because of ethical constraints that preclude the acquisition of suitable postmortem lung tissue.

Recent studies in mammals using new morphometric techniques support continued alveolarization to adulthood in rabbits (14), rhesus monkeys (12), and after pneumonectomy in mature dogs (13). Massaro and coworkers (36) showed evidence of calorie intake-related alveolar gain in adult mice refed after starvation. Schittny and coworkers (37) observed an increase in alveolar number after completion of microvascular maturation in rats using design-based stereology. Using three-dimensional synchrotron radiation X-ray tomography, they showed local duplication of single capillary layers in areas of postmaturity septal growth, which indicated a potential mechanism for postmature alveolarization. This dispelled the notion that the immature double capillary layer in alveolar walls is a prerequisite for new septation. Thus, there is emerging evidence of alveolarization in maturing lungs in other mammals. There is indirect evidence that



**Figure 3.** Scatterplot of mean peripheral airspace dimension  $\overline{X_{rms}}$  against (*top panel*) age and (*bottom panel*) FRC. The *red lines* indicate back transformed log-linear fits in the *top panel* and log-log fits in the *bottom panel*. *Dotted red lines* in the *bottom panel* indicate 95% confidence intervals. *Green lines* in the *bottom panel* indicate the following in a child with an initial FRC of 1 L: *top line*, predicted change in  $\overline{X_{rms}}$  with FRC if lung growth was accomplished only by expansion of preexisting alveoli (scenario of no alveolarization); *middle line*, predicted change if rate of neoevaluarization was 0.54 (predicted from apparent diffusion coefficient vs. FRC multilevel model); *lower line*, predicted change if all growth of lung was by neoevaluarization. The data suggest that the rate of neoevaluarization may be even higher than predicted from the apparent diffusion coefficient versus FRC model (Figure 2, *bottom panel*).

this also happens in humans. Brown and coworkers (38) used electrical impedance tomography to determine an average alveolar number of 90 million at age 2–3 years compared with 300 million in adults, implying that alveolarization continues to take place after 3 years of age. Using a new unbiased morphometric technique, Ochs and coworkers (35) showed that alveolar number was closely related to adult lung volume and that mean alveolar size was almost constant between subjects. If alveolarization were completed by 2–3 years, the final number of alveoli, and by extrapolation the final size of the lung, would have to be set by then, which is implausible. This provides more indirect evidence in favor of human alveolarization beyond early childhood.

The strengths of the study include the large number of volunteers from a wide age range spanning most of the period of lung growth. Prospectively collected data on early life exposures and preexisting lung disease were available (20), which allowed selection of volunteers known to be healthy. Repeated MR measurements at varying inflation volumes were performed in some subjects, which facilitated the statistical test of the hypothesis of no new alveolarization. Independent measurement of lung size by plethysmography enabled the calculation of expected slope in the models. Finally, the application of two different techniques of MR permitted evaluation of lung geometry.

Studies of alveolar size and number in subjects at different ages, including our own, share the common assumption that the cross-sectional data are representative of longitudinal changes. We found wide interindividual variability in ADC, but our large number of volunteers and the repeatability of ADC measurements (within subjects) minimize the effect of this problem. Also, preliminary longitudinal data (not shown) corroborate the evidence from this study. Further longitudinal measurements of ADC will help refine the results of this study and begin to explain the wide population scatter of ADC values. The assumptions underpinning the multilevel regression model are transparent and the model fits the data well, suggesting that the assumptions are realistic (see online supplement).

There are important implications to our observation that alveolarization is not confined to early life. Children who die after extremely preterm birth have been shown to have larger, simpler, and fewer alveoli (39), based on histologic studies of fatal cases. There have been no studies of alveolarization in survivors of neonatal chronic lung disease and it is possible that there is catch-up alveolarization. There may be hope of recovery from diseases that result in diminished alveolar number at birth (e.g., pulmonary hypoplasia caused by severe oligohydramnios or congenital diaphragmatic hernia) or later in life (e.g., surgical lung resection). Adverse environmental exposures can affect alveolar structure. Systemic corticosteroids in early postnatal life can inhibit alveolarization (40, 41). If alveolarization is not confined to early life, it is a possible that inhaled corticosteroids may be deleterious throughout childhood. Passive tobacco smoke exposure in childhood is linked to adult chronic obstructive pulmonary disease (42). Impaired alveolarization may be a potential mechanism linking passive smoking during childhood to increased lung senescence and emphysema, and therefore chronic obstructive pulmonary disease. Finally, the advent of potential “alveolar therapy” to restore damaged alveolar structure (43) requires safe, noninvasive repeatable measurements to study the outcome of future therapeutic trials. Hyperpolarized  $^3\text{HeMR}$  provides such a method.

We conclude that there is evidence for continuing alveolarization through childhood and adolescence in humans. The exciting possibility of late alveolarization implies that the lung may be able to recover from damage occurring in early life. Emerging therapies have the potential to enhance this process. Conversely,

environmental exposures during childhood could have a longer window of opportunity to impair the process of alveolarization, with potential adverse consequences throughout later life.

**Author disclosures** are available with the text of this article at [www.atsjournals.org](http://www.atsjournals.org).

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