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In Vivo Lung Morphometry With Hyperpolarized ^3He Diffusion MRI: Reproducibility and the Role of Diffusion Sensitizing Gradient Direction

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Abstract

Purpose—Lung morphometry with hyperpolarized gas diffusion MRI is a highly sensitive technique for the non-invasive measurement of acinar microstructural parameters traditionally only accessible by histology. The goal of this work is to establish the reproducibility of these measurements in healthy volunteers and their dependence upon the direction of the applied diffusion-sensitizing gradient.

Methods—Helium-3 lung morphometry MRI was performed on a total of five healthy subjects. Two subjects received duplicate imaging on the same day and three after a four or twenty-seven month delay to assess reproducibility. Four subjects repeated the measurement during the same session with different diffusion-sensitizing gradient directions to determine the effect on the parameter estimates.

Results—The helium-3 lung morphometry measurements were reproducible over the short and long term (e.g. % coefficient of variation (CV) of mean chord length, $L_m = 2.1\%$ and 2.9% respectively) and across different diffusion gradient directions (L_m % CV = 2.6%). Results also show independence of field inhomogeneity effects at 1.5T.

Conclusion—Helium-3 lung morphometry is a reproducible technique for measuring acinar microstructure and is effectively independent of the choice of diffusion gradient direction. This provides confidence for the use of this technique to compare populations and treatment efficacy.

Keywords

MRI; hyperpolarized gas; helium; lung morphometry; diffusion; reproducibility

INTRODUCTION

Lung morphometry with hyperpolarized gas diffusion MRI (1,2) provides localized in vivo measurements of both standard lung morphological parameters (mean chord length (L_m), surface-to-volume ratio (S/V) and alveolar density (N_v)) as well as architectural parameters of the acinar ducts and sacs - alveolar depth (h) and acinar duct radii (R), introduced by

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Weibel and colleagues (3,4). This technique was validated against direct histological measurements in human (1) and mouse (5) lungs and demonstrated strong correlations with literature data (1,6). It was also shown that the method provides a very sensitive measure of changes in lung microstructure at early stages of emphysema (7). A detailed theoretical analysis of the accuracy of the lung morphometry with hyperpolarized ^3He gas was provided in (8). While most applications to date have used hyperpolarized ^3He gas, recently, both a theoretical analysis (9) and initial results of in vivo lung morphometry with hyperpolarized ^{129}Xe gas were reported in humans (10,11) and rats (12).

As we look towards using this technique to detect changes during longitudinal studies and therapeutic trials, it is important to further establish the precision of the measurements and the sensitivity of the technique to experimental conditions. Herein, we study the short term and long term reproducibility of the technique, the influence of the RF magnetization consumption and the role of the diffusion-sensitizing gradient direction. We demonstrate that for our experimental protocol in human studies (7) the data are reproducible and there is a minimal influence of diffusion gradient direction and RF signal depletion on the lung morphometry measurements.

METHODS

Subject Selection

All subjects provided informed consent and procedures were performed with approval from the local Institutional Review Board. Helium-3 MRI was conducted under a FDA IND exemption (IND 59,269). Five healthy never-smokers were recruited from the local community (mean age = 31 ± 19 , range = 19–63, 3 male/2 female) for helium-3 MRI. Recruitment criteria included no known pre-existing pulmonary, cerebrovascular, hematologic, or cardiac disease, the ability to perform a six-minute walk test without significant blood oxygen level desaturation by pulse oximetry, and no contra-indications for MRI.

^3He Lung Morphometry

Helium-3 gas was hyperpolarized to approximately 40% polarization using a commercial Nycomed Amersham Imaging IGI.9600. He polarizer (GE Healthcare, Durham, NC). Axial gradient echo diffusion ^3He MRI images were acquired on a Siemens 1.5T Avanto scanner (Siemens Medical Systems, Iselin, NJ) using a custom ^3He 48 cm diameter rigid transmit and flexible 8-channel phased array receiver coil set (Stark Contrast MRI Coils Research, Erlangen, Germany) with $7\text{ mm} \times 7\text{ mm}$ resolution over three, 30-mm axial slices, (flip-angle $\theta = 5.5^\circ$, TR/TE = 13/8.3 ms, b = 0, 2, 4, 6, 8, 10 s/cm²) (13). The flip angle for helium imaging was calculated from the proton calibration voltage following a previously described technique (14). The diffusion gradient pair (1.8 ms duration each, 0.3 ms rise times, no gap between lobes) (15) was oriented along the readout direction (left-right), unless otherwise specified. Subjects inhaled 1 liter of a 40/60 mixture of hyperpolarized ^3He gas in nitrogen from functional residual capacity (FRC) and held their breath for nine seconds. During ^3He imaging, each subject's ECG and oxygen saturation were monitored for potential adverse effects.

A total of seventeen lung morphometry measurements were performed on the five subjects. Four subjects repeated helium imaging during the same session with the diffusion gradient oriented along the readout (RO, Right-Left) and slice selection (SS, Superior-Inferior) directions. In one of these subjects, an additional dataset was acquired with the diffusion gradient oriented along the phase encoding direction (PE, Anterior-Posterior). In two of these subjects, the diffusion acquisitions along both the RO and SS directions were repeated after a four month delay to assess long-term reproducibility. During their return visit, these subjects also repeated the RO direction scan to assess short-term reproducibility. One additional subject returned for longitudinal scanning after a 27 month delay.

To verify our RF calibration and measure the magnitude of signal depletion due to RF consumption, we acquired two gradient echo images of the lung ($28 \times 28 \times 10 \text{ mm}^3$ resolution, 20 2D slices, TR/TE = 4.1/2.04 ms, flip-angle $\theta = 15.5$ or 25°) during a single two second breath hold on three separate subjects. Acquisition of the two images was interleaved such that each line of k-space was acquired twice before moving to the next line. The cosine of the flip angle was calculated as the ratio of the two signal intensities for each voxel.

Semi-automated segmentation of each lung was performed using custom software in MATLAB (R2012b, Mathworks, Natick, MA). For each image voxel, the data from all channels in the receiver array were jointly analyzed using Bayesian probability theory (16–18) and a previously developed mathematical model of ^3He gas diffusion in lung acinar airways (1) was used to determine the dimensions of the lung acinar airways and alveoli.

Statistical Analysis

Two-way repeated measures analysis of variance (ANOVA) was conducted on all seventeen datasets using the R software package (version 3.01, R Foundation for Statistical Computing, Vienna, Austria) to determine the contribution of subject, diffusion direction, and scanning repeats for each parameter value. Equivalence across repeated measurements was calculated using a paired TOST (two one-sided t-tests). For both tests, $p < 0.05$ was considered significant. The %CV was calculated from the mean and standard deviations of the parameters across the lung for each subject. Due to the small number of subjects, the four and twenty-seven month reproducibility experiments were both considered long-term for these analyses.

RESULTS

All helium inhalations were well tolerated and no adverse events occurred, consistent with our previous report (19).

Short and long term reproducibility

The values of all lung morphometry parameters demonstrate a high degree of reproducibility both during different inhalations on the same day and months apart and ANOVA analysis did not detect any statistically significant effects from repeated measures (short or long term) for any of the measurements studied. The images in Figure 1a show the reproducibility of parameter maps for L_m , R , h , and N_v over the short term (same imaging session) and long term (four months) in a healthy subject. The median values of these parameters over all

slices are included in Table 1 for each subject, along with the % coefficient of variation (% CV) averaged across subjects. Equivalence testing indicates that the long-term repeated measurements of Lm , h , and R were all statistically indistinguishable to within 13 μm .

Diffusion direction

The parameter maps in Figure 1b show the values of the helium lung morphometry parameters when the diffusion gradients are oriented along three different axes. ANOVA analysis did not detect any statistically significant effects from diffusion direction for any of the measurements studied. The median parameter values for the diffusion gradients oriented along the different directions are given in Table 1 along with the % CV averaged across subjects. For the subject with data using diffusion gradients along all three directions, the average pixel-wise fractional anisotropy of the mean chord length (Lm) was 0.09 and a histogram of the Lm values across the lung for the different diffusion gradient orientations is shown in Figure 2. Equivalence testing indicates that the repeated measurements across diffusion directions of Lm , h , and R were all statistically indistinguishable to within 12 μm .

RF Calibration

The standard deviation of the flip angle across the lung for the RF calibration experiments was 20%, corresponding to 1.1 degrees for the 5.5 degree flip angle typically used in our diffusion measurements. The distribution of flip angles across the lung for one of our calibration experiments is shown in Figure 3.

DISCUSSION

A comparison of parameter maps indicates that the lung morphometry measurements are reproducible across repeated scans and diffusion direction and the median values across the lung are statistically indistinguishable between scans. While there are subtle differences in the parameter maps in Figure 1, these effects are small compared to natural variation across the lung.

Table 1 includes the observed standard deviation of parameter values across the lungs, which are consistent across scans and with our previous lung morphometry measurements (20). This variation can be attributed to two sources: true heterogeneity in structure and uncertainty in the parameter estimates due to noise. Spatial variability in lung parenchyma microstructure is well known in the histology literature. For example, Haefeli-Bleuer and Weibel (4) reported the intra-acinar variation on the order of 17–18% in parameters R and h . Such spatial variations have also been identified in a number of helium MRI studies using ADC (21–23).

Our Bayesian analysis calculates uncertainties, σ_{fit} , for each parameter estimate, which are inversely proportional to the effective SNR in each voxel as discussed in detail in our prior publications (13,17). For the current study, a single-channel SNR of 100–150 was typical in sensitive regions of the $b=0$ images. The contribution of true structural heterogeneity, σ_{true} , to a given parameter's variability across the lung can thus be estimated as:

$$\sigma_{true}^2 = \sigma_{observed}^2 - \sigma_{fit}^2. \text{ The percent true structural heterogeneity and fit uncertainty averaged}$$

across scans are shown at the bottom of Table 1 for each parameter. Based upon the fit uncertainty, Lm is the most precisely determined parameter from our data and model whereas h is the least well determined. In all cases, the fit uncertainty is smaller than the true structural variation of parameter values across the lung, indicating that most of the observed heterogeneity is the result of true structural differences. Also we note that the regions of increased parameter variability do not directly correlate with lower SNR regions on the combined ventilation images in Figure 1.

There are a number of potential sources of variability in the repeated measurements. Small localized differences in parameter values could potentially result from variability in inflation levels between scans acquired during different breath hold maneuvers. Techniques to dynamically monitor breathing patterns during imaging (24) should improve the reproducibility of the inflation level and therefore the parameter maps obtained. Subject movement between scans (same day) or offsets in slice positioning (long term) could affect the measurements. We also assume that the subjects are in the same physiological state for all scans and their lung microstructure is preserved. While this is likely a good assumption in young healthy control subjects and on same-day measurements, there is still the potential for changes in the overall health or environmental factors (e.g. seasonal variations) between visits that could alter pulmonary function and structure, hence the values obtained by our measurements. We would expect that the variability of repeated measurements may be slightly larger in emphysema patients, due to disease heterogeneity across the lung and the influence of ventilation defects, and air trapping.

The reproducibility of the helium ADC (apparent diffusion coefficient) measurements has been established in healthy subjects both on the same day (25,26) and a week later (22,25). These groups found that the ADC was highly reproducible with intra-subject variations much smaller than the inter-subject variations. The reproducibility of helium lung morphometry measurements in the current study is comparable to these values.

The helium-3 lung morphometry technique is based on the geometrical model of acinar structure established by Weibel and colleagues (3,4) that describes the acinar airways as anisotropic cylindrical air passages lined with a sleeve of alveoli. With the imaging resolution currently achievable, our model assumes that these acinar airways are isotropically distributed in direction over a given imaging voxel (15) – a microscopically anisotropic but macroscopically nearly isotropic model. This simplification is supported by the random appearance of alveoli on histology sections (27) and assumes that the MRI signal has a negligible contribution from the 5% of gas that lies in the conducting airways (28). While we manually segment out the large bronchi, smaller conducting airways still make a small contribution to the diffusion signal. By acquiring lung morphometry data with the diffusion gradients oriented along different anatomical directions, we are able to probe the validity of these assumptions. While we detected small local differences in the parameter values obtained along different diffusion directions, the consistency of the overall parameter estimates across the lungs suggests that the model is reasonable. This independence of lung morphometry parameters to the direction of the diffusion gradient allows us to acquire data along only a single diffusion direction, thereby significantly decreasing the imaging time required.

Such an ‘isotropy’ of our lung morphometry technique is consistent with prior measurements of the helium ADC with the diffusion gradient oriented along different directions (29,30). The helium ADC averaged across the lung was consistent across different diffusion gradient directions in human (29) and animal studies (30), and the variation in human ADC across different directions was less than 4%.

Hyperpolarized gas imaging experiments are also modulated by the signal loss from RF sampling that can appear similar to diffusion attenuation and this effect is commonly minimized by the utilization of small flip angle imaging. We measured the RF pulse accuracy and homogeneity across the lung of our helium transmission coil to validate our calibration procedure.

To examine the effects of RF signal depletion on our parameter estimates, we simulated lung morphometry data, including the effects of RF signal depletion on the acquired MR signal, using a typical SNR (200:1) and the average parameter values for healthy subjects. These simulated data (not shown) were then analyzed without including the effect of RF pulses in the model. The simulated data indicates that RF signal loss from our standard 5.5 degree flip angle introduces a bias of 1.4% in R and h if not accounted for. Even in lung regions with the strongest RF pulse (2 standard deviations greater than the requested flip angle based upon the calibration experiment in Figure 3), the simulated data indicates that the bias would only be 2.4% in R and 2.1% in h . These effects are biologically insignificant and negligible compared to the variability of parameter values across the lung, the reproducibility of the measurement, and the observed changes associated with emphysema (7). Future studies may correct for the RF depletion from the known flip angle to remove this small effect.

It was previously suggested (31) that magnetic field inhomogeneities could affect our lung morphometry measurements. However, we have demonstrated an excellent agreement between our ^3He -based measurements and direct morphometry (1), suggesting that this is not a significant factor at 1.5 T. The strongest magnetic field gradients appear at the interfaces between lung airspaces and lung tissue, specifically alveolar septa and blood vessels. Theoretical considerations in (8) demonstrated that the effects from alveolar septa are minor at this field strength. The effects from blood vessels can be evaluated in Figure 1. While the ventilation images (Figure 1, first column) demonstrate regions of substantial signal loss due to the presence of blood vessels (red arrows), the voxels within and surrounding these regions (with sufficient signal to noise to be modeled) appear no different on the morphometric parameter maps compared to areas not affected by these field inhomogeneities.

This study used a small number of subjects for each measurement, due to the expense and limited availability of helium-3 gas. Although this limits the power of the study, it still serves as a demonstration of the validity and reproducibility of our helium lung morphometry measurements, providing confidence for its use in longitudinal studies and clinical trials. While we cannot rule out the possibility that a more highly powered study might find statistically significant differences between repeated scans and different directions, the equivalence testing and small % CV in the current data indicate that any such

differences would be biologically insignificant and smaller than the heterogeneity in values across the lung.

CONCLUSIONS

This study demonstrates the short and long-term reproducibility of helium-3 lung morphometry measurements and its independence on the diffusion gradient orientation. The latter is significant as it allows for a substantial reduction of imaging time – a measurement with only one gradient orientation direction is necessary and sufficient. Together, this provides confidence for the use of helium-3 lung morphometry to detect changes during longitudinal studies and clinical trials.

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Figure 1.

a) Example of short-term (same day) and long-term (four month) reproducibility of helium lung morphometry measurements over the central slice for subject 4. The red arrows on the ventilation image indicate two examples of areas with significant signal loss due to blood vessel induced magnetic susceptibility effects.

b) Comparison of parameter maps from subject 1 over the central slice acquired with the diffusion gradient oriented along the readout (RO), slice select (SS), and phase encoding (PE) directions.

Figure 2. Histogram of Lm over the whole lung from subject 1 when the diffusion gradient is oriented along the three different directions.

Figure 3. Histogram of the flip angle across the lung of a single subject from the calibration experiment. Requested flip angle = 15.5 degrees; measured flip angle (Median $\pm$ SD) = 14.7 $\pm$ 2.9.

Parameter values for healthy subjects illustrating the reproducibility of lung morphometry measurements and the dependence on diffusion gradient direction. Values are Median $\pm$ SD across the lung. CV = coefficient of variation. The last two rows decompose the observed heterogeneity (SD) across the lung into the contributions from fitting uncertainty and true parameter variation across the lung

Table 1

Subject	Age	Visit	Diffusion Direction	L_m (μm)	R (μm)	h (μm)	N_r (mm^{-3})
1	19	Baseline	Phase	210 $\pm$ 30	300 $\pm$ 30	130 $\pm$ 40	120 $\pm$ 40
			Read	220 $\pm$ 30	310 $\pm$ 30	120 $\pm$ 40	120 $\pm$ 40
			Slice	210 $\pm$ 30	310 $\pm$ 40	130 $\pm$ 40	110 $\pm$ 40
2	34	Baseline	Read	210 $\pm$ 30	320 $\pm$ 30	140 $\pm$ 40	110 $\pm$ 40
			Slice	200 $\pm$ 20	310 $\pm$ 30	140 $\pm$ 40	120 $\pm$ 30
			Read	180 $\pm$ 20	300 $\pm$ 30	150 $\pm$ 40	130 $\pm$ 40
3	20	4 months prior	Slice	190 $\pm$ 20	300 $\pm$ 20	150 $\pm$ 30	130 $\pm$ 30
			Read	200 $\pm$ 20	300 $\pm$ 40	140 $\pm$ 40	120 $\pm$ 50
		Baseline	Slice	190 $\pm$ 20	300 $\pm$ 30	150 $\pm$ 30	130 $\pm$ 40
			Read	190 $\pm$ 20	300 $\pm$ 40	150 $\pm$ 40	120 $\pm$ 50
		Same Day	Read	210 $\pm$ 30	310 $\pm$ 30	130 $\pm$ 40	110 $\pm$ 40
			Slice	200 $\pm$ 20	300 $\pm$ 30	130 $\pm$ 40	120 $\pm$ 30
4	19	4 months prior	Read	220 $\pm$ 40	320 $\pm$ 50	140 $\pm$ 40	100 $\pm$ 50
			Slice	210 $\pm$ 30	310 $\pm$ 40	130 $\pm$ 40	110 $\pm$ 40
		Baseline	Read	220 $\pm$ 30	320 $\pm$ 40	130 $\pm$ 40	100 $\pm$ 40
			Read	250 $\pm$ 50	330 $\pm$ 30	110 $\pm$ 40	100 $\pm$ 30
5	64	27 months later	Read	240 $\pm$ 50	330 $\pm$ 30	120 $\pm$ 40	90 $\pm$ 30
		Same Day		2.1%	0.8%	2.0%	2.0%
		Long Term		2.9%	1.6%	3.1%	3.5%
Average % CV				2.6%	1.7%	1.7%	1.9%
Average % Parameter Fit Uncertainty (σ_{fit})				3.0%	5.0%	15.6%	14.0%
Average % True Heterogeneity (σ_{true})				13.9%	9.1%	21.4%	30.7%