In vivo measurement of lung volumes in mice

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Mitzner, W., R. Brown, and W. Lee. In vivo measurement of lung volumes in mice. Physiol Genomics 4: 215–221, 2001.—We describe longitudinal measurements of functional residual capacity (FRC) in breathing mice using a clinical computed tomography (CT) scanner. Lungs of anesthetized mice from the A/J and C3H/HeJ strains were scanned over a 10-s period. Using a fixed threshold for CT density, we could accurately and reproducibly obtain the amount of air in the lungs at FRC, with a 10% coefficient of variation. Total lung volume, and the fractions in left and right lungs, were measured in the two strains from 4 to 12 wk of age. Results show that in both strains the FRC increases only up to 6 wk of age and then remains stable despite a steady increase in body weight. Over this time period, FRC was consistently about 50% greater in the C3H/HeJ strain compared with the A/J strain. The C3H/HeJ strain also has a significantly smaller fraction of the total lung volume in the left lung. We conclude that accurate measurements of FRC in breathing mice can be made using a standard clinical CT scanner. This method may be useful for repeated noninvasive assessment of both structural and functional changes in the lungs of experimental and genetically manipulated mice.

functional residual capacity; lung volume distribution; respiratory mechanics; computed tomography

AS AN EXPERIMENTAL MODEL of lung disease, the mouse provides unique opportunities to study genetic alterations. To maximize the interpretation of genetic and pathological changes, it is important to know basic anatomic and physiological conditions. To this end, we have evaluated the use of high-resolution computed tomography (HRCT) to quantify lung volumes in two common mouse strains. Use of CT to measure lung gas volumes has been successfully applied in normal and diseased human lungs (3, 4), but the method has not been previously used in mice.

METHODS

Our study protocol was approved by the Johns Hopkins Animal Care and Use Committee. Four A/J and four C3H/HeJ mice all starting at 4 wk of age were used for this study. They were anesthetized with 0.2 ml ip Etomidate, a short-acting nonbarbiturate hypnotic. The mice were then placed prone in a 13 × 13-cm plastic housing with nine equally spaced partitions (3 rows of 3). The anesthetized animals were all placed with their noses in the same vertical plane. This mouse hotel was then placed on the CT gantry for lung scanning as described next. The mice were spontaneously breathing during the scanning procedure. Following the scan, the mice were placed in their cages and recovered from the anesthesia within 30 min.

Lung volume. HRCT scans were obtained with a Somatom IV Scanner (Siemens, Iselin, NJ) using a spiral mode to acquire 24 contiguous images in 15 s at 137 kVp and 165 mA. The images were reconstructed as 1-mm slice thickness and a 512 × 512 matrix using a 12-cm field of view and a high spatial frequency (resolution) algorithm that enhanced edge detection, at a window level of ~450 Hounsfield units (HU) and a window width of 1,350 HU. In larger animals, these settings have been shown to provide accurate measurement of airway lumen size in airways approaching 1 mm (6, 14), and since the mouse thorax is of comparable size to conducting airways in larger species, these settings provide good visualization of the lung and thoracic wall boundary. The pixel dimension in these images was 0.23 × 0.23 mm.

The CT images were then transferred to a Macintosh computer for analysis using NIH Image software. The following procedure was used. The Hounsfield unit scale was first converted to percent air by selecting areas with pure vascular tissue (the heart) as 0% and pure air (outside the thorax) as 100%. A fixed threshold was selected and used for all images. This threshold (29% air) was chosen to ensure that all of the air in the lung was incorporated, without adding additional pixels with low radiodensity. The total thresholded area of each section was then measured and stored along with the measure of the percent air in this area. The percent air per thresholded slice was multiplied by the thresholded area to get the total amount of lung air in that slice. The total lung air volume was then determined by adding the air volumes of all 1-mm thick slices. This procedure of measuring lung air volume was described by Olson and Hoffman (11), who used it to study volume changes in pneumonectomized rabbits. The voxel resolution of current scanners, however, is over an order of magnitude greater than that of the Imatron scanner used in that study. In addition to total lung volume, we also calculated the left and right individual lung volumes.

Calibration protocol. Five mice were used to validate the accuracy of the CT measurement system for lung air volume. The anesthetized mice were tracheostomized with an 18-
gauge needle and connected to a mouse ventilator as previously described (5). They were ventilated with 100% oxygen for 10 min, and then the lungs were sealed. This procedure allows all the gas in the lung to be absorbed to bring the lung air volume to zero (13). The dead animals were then placed in the CT scanner, and lung volumes were measured after sequentially injecting known air volumes in each animal.

Lung volume measurement protocol. In each of the experimental mice, the lung volumes were measured at weekly intervals from 4 to 12 wk of age. C3H/HeJ and A/J strains were statistically compared using ANOVA with repeated measures.

RESULTS

Figure 1A shows examples of grey scale CT images, and Fig. 1B shows the same images used for quantitative analysis after thresholding. Eight different animals are positioned in individual slots of the animal holder and placed in the scanner in the following order: bottom row (left to right), A/J-1 to A/J-3; middle row, A/J-4, C3H-5, and C3H-6; top row, C3H-7 and C3H-8. Scale bar = 10 mm.

Fig. 1. Example of a grey scale computed tomography (CT) images (A) and the same image used for quantitative analysis after thresholding (B). Eight different animals are positioned in individual slots of the animal holder and placed in the scanner in the following order: bottom row (left to right), A/J-1 to A/J-3; middle row, A/J-4, C3H-5, and C3H-6; top row, C3H-7 and C3H-8. Scale bar = 10 mm.

Fig. 2 shows the results from known air volumes injected into the five mice used for calibration. The coefficients of variation at each known volume (100, 200, 400, and 800 μl) were 0.3, 0.1, 0.1, and 0.09, respectively. The larger coefficient at 100 μl partially reflects there being only three measurements at that volume. In addition to this calibration procedure, we also made repeated measurements at the initial 4 wk time point. Ten minutes after the first CT scans were done, a second set was acquired. The repeated functional residual capacity (FRC) measurements for each of the eight experimental mice are given in Table 1. The reproducibility in these eight breathing mice was excellent.

![Fig. 2. Correlation between known and CT measured air volumes in 5 mice.](http://physiolgenomics.physiology.org)
Figure 3 shows the body weights of individual animals and a comparison of the means in the two strains. There were no significant differences in the body weights over time. The body weight is significantly greater in the C3H/HeJ strain compared with the A/J ($P = 0.013$).

Figure 4 shows the FRC in individual animals and a comparison of the means in the two strains. The FRC appears to increase slightly from 4 to 6 wk, then remains stable after 6 wk. The FRC is significantly greater in the C3H/HeJ strain compared with the A/J ($P = 0.011$). Averaged over all time points, the mean FRC is 52% ($\pm 10.1$ SE) higher in the C3H/HeJ strain.

Figure 5 shows the FRC normalized to animal body weight. After 6 wk, the normalized lung volumes are stable in the C3H/HeJ strain and decrease in the A/J strain. The FRC/body weight is significantly greater in the C3H/HeJ strain compared with the A/J ($P = 0.021$).

Figure 6 shows the percentage of FRC in the left lung in individual animals and a comparison of the means in the two strains. Averaged over all time points, the A/J strain has 40.4% ($\pm 1.4$ SE) of lung volume in the left lung, and the C3H/HeJ strain has 36.9% ($\pm 1.2$ SE) in the left lung ($P = 0.040$). There was no tendency for this lung volume partitioning to change over the 4–12 wk age span in either strain.

DISCUSSION

The representative CT images in Fig. 2 show good clarity of the boundary between the lung and chest wall. In the dead mice used for calibration, the accuracy of the CT method to measure injected air was excellent, with a coefficient of variation about 10%. This is not nearly as good as the 2–3% accuracy reported by Hoffman et al. (7), who used CT to measure lung volumes in dogs. However, they were measuring lung volumes that were two to three orders of magnitude larger, and, given that their voxel size was only about 40 times greater than what we used, a greater accuracy in dogs would be expected. Recently, develop-

Table 1. Repeat measurements of FRC taken from CT scans done 10 min apart

<table>
<thead>
<tr>
<th>Animal</th>
<th>1st Measurement</th>
<th>2nd Measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/J-1</td>
<td>216</td>
<td>215</td>
</tr>
<tr>
<td>A/J-2</td>
<td>150</td>
<td>140</td>
</tr>
<tr>
<td>A/J-3</td>
<td>170</td>
<td>170</td>
</tr>
<tr>
<td>A/J-4</td>
<td>149</td>
<td>140</td>
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<tr>
<td>C3H-5</td>
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<td>369</td>
</tr>
<tr>
<td>C3H-6</td>
<td>162</td>
<td>163</td>
</tr>
<tr>
<td>C3H-7</td>
<td>282</td>
<td>289</td>
</tr>
<tr>
<td>C3H-8</td>
<td>223</td>
<td>270</td>
</tr>
</tbody>
</table>

All values are volumes in $\mu l$. FRC, functional residual capacity; CT, computed tomography.
A method of micro-CT methodology was described (2), with voxels up to 500 times smaller than what is currently available with clinical CT scanners. If such technology becomes more readily available, then not only would the volume accuracy improve, but it would make possible the resolution of airway and vascular structure within the parenchyma of mice. We did not attempt any calibration in the living animals. Prior to making these measurements, there was some concern regarding the potential effect of respiratory motions associated with normal breathing, and we were initially surprised to find that repeat measurements in the same animal showed excellent reproducibility (Table 1). It is also clear that these respiratory motions do not lead to significant image degradation. We believe that the main reasons for this finding is that the respiratory motion in mice is on the same scale as the resolution limit of the clinical CT scanner. That is, a nominal mouse tidal volume is 0.2 ml (10, 12). Since mice breathe primarily with diaphragmatic motion, and since the area of the diaphragm at FRC is on the order of 2 cm² (unpublished observations), the expected linear displacement of the lung during tidal breathing is thus only about 1 mm. With the mouse breathing at a rate of about 2 Hz, this respiratory motion leads to a blurring of only the most caudal CT scans, with minimal distortion of the boundary between the lung and rib cage. This boundary appears sharper in scans from a dead mouse, but since we are using our method only to measure lung air volume, the effect of this slight blurring in vivo is lessened. However, the reproducibility of the lung volume measurement is likely affected somewhat by this slight boundary blurring in the caudal scans. Its manifestation may contribute to the observed variability in lung volume measurements over time in individual animals (Fig. 4), where the curves generally show steady lung growth with fluctuations up to 25%. This level of variability in the lung volume measurement in an individual mouse is larger than what one normally expects in humans. Reasons for the larger variability in mice with this technique likely reflect a combination of technical and physiological considerations, including noise associated with respiratory motion, partial volume effect of varying mouse orientation in the scanner, and a variable amount of inspiratory muscle tone as discussed below. We do not believe that varying anesthesia levels affected the level of variability. Although we used a short-acting anesthetic, whose effect was likely changing over the time course of experiments, the excellent reproducibility shown in Table 1 with repeated measurements taken 10 min apart suggests that the anesthetic level of etomidate had negligible effect.

We should also note that our use of the term FRC is not precisely analogous to what it normally indicates in humans, i.e., the lung volume determined solely by the

![Fig. 4. Functional residual capacity (FRC) in individual animals and a comparison of the means in the two strains. Mean FRC is 52% higher in the C3H/HeJ strain (P = 0.011), although there is some variability with age. The FRC increases slightly from 4 to 6 wk, then remains stable after 6 wk.](http://physiolgenomics.physiology.org/)

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passive recoil of the lung and thoracic wall, for convenience. The mice we studied are breathing, so the mean lung volume we measure is slightly higher than the end-expiratory lung volume. For a mouse breathing with a nominal inspiratory-to-expiratory (I:E) ratio of 1:2 and tidal volume of 200 $\mu$l, the lung volume we measure would be expected to be 67 $\mu$l (20–25%) greater than the actual end-expiratory lung volume.

Our results demonstrate substantial differences in FRC between the two strains studied. This was clearly apparent even with the limited sample used to evaluate the method. We found FRC values averaging 350 $\mu$l in the C3H/HeJ animals and 270 $\mu$l in the A/J. There was considerably more variability in the C3H/HeJ strain, and we do not know of any physiological reasons for this. Given the small number of animals, however, this may just reflect normal statistical variation. Using a gas dilution method, Lai and Chou (8) reported FRC measurements from C57BL/6 mice (weighing 22 g) to average 250 $\mu$l, a value close to that in the A/J strain.

The roughly 35% larger FRC volumes in the C3H/HeJ animals, compared with the A/J animals persisted over the 8-wk study period. We know of no other studies where similar measurement or comparison of in vivo lung volumes was made in different mouse strains. Recent work by Tankersley et al. (13) compared the pressure-volume curves in these strains. Their results showed that total lung capacity (TLC; defined as the lung volume at 30 cm$H_2O$) in the C3H/HeJ animals was 44% larger than that in the A/J animals, a magnitude consistent with the differences in FRC that we found here. They also defined an FRC in the dead mice as the lung volume at 0 transthoracic pressure, and found FRC in the C3H/HeJ and A/J mice to be 370 and 270 $\mu$l, respectively. These values are in the range of what we found over time in the same strains in vivo, even considering the slight overestimation that results from tidal breathing.

This correspondence between the FRC measurements in living and dead mice was not entirely expected. Although FRC in most mammals is thought to result from a balance between the inward recoil of the lungs and outward recoil of the thoracic cages, in mice it was suggested by Leith (9) that FRC might be determined by active tone in the inspiratory musculature, that is, because mice have evolved with extremely compliant chests that provide them the ability to squeeze through tiny holes. This high thoracic wall compliance thus essentially eliminates any outward recoil of the relaxed chest wall. Indeed, recent measurements of chest wall compliance in mice have confirmed that there is negligible recoil in the physiological range of thoracic volumes (13). It thus seems likely that mice would need to regulate their FRC with some active inspiratory tone, but the relative consistency we observed between FRC values in anesthetized
spontaneously breathing mice and that in mice post-mortem suggests that only small amounts of inspiratory tone might be required. In mechanically ventilated mice with exogenous muscle paralysis, one might expect the FRC to be lower than in the intact situation in vivo. We have observed this anecdotally in other experiments using anesthetized ventilated mice (1, 5). In such animals, even with an intact chest wall, dynamic lung compliance increases as the level of end-expiratory pressure is increased up to about 3 cmH2O (unpublished observations). This observation not only supports the idea that some active inspiratory muscle tone sets FRC in vivo, but also suggests that experimentally ventilated intact mice with no added positive end-expiratory pressure (PEEP) or periodic deep inspirations may become progressively stiffer with increasing atelectasis or airway closure.

We also found that the A/J strain has an additional 3.5% of total lung volume in the left lung, compared with the C3H/HeJ strain. We know of no functional significance to this observation, and there is scant information in the published literature with which this finding can be compared. Our measured quantitative volume fractions at FRC in the mouse left lung of 36.9 and 40.4% in C3H/HeJ and A/J, respectively, can be compared with that at TLC in 14-wk-old rabbits, where Yee and Hyatt (15) found 43% of total lung volume in the left lung. In two male human subjects scanned with an Imatron electron beam CT scanner, Hoffman found 45 and 46% of the lung air volume in the left lung at FRC (Eric Hoffman, personal communication). Thus it appears that mice may have a smaller fraction of the lung volume on the left, perhaps reflecting a larger heart or more leftward location. Why different mouse strains would have lung volume partitioned differently between left and right lungs is not clear, but if the airway branching at the carina were different, there could conceivably be some functional effect of air pollutants in the left and right lungs. Cardiac size might also be expected to be larger in the strain with a smaller fraction of lung volume on the left side.

Although the animals of both strains were continually growing during the 8-wk study period, the lung growth (as evidenced by lung volume) appears to be nearly complete by 6 wk of age. Thus the lung volumeto-body weight ratio rises slightly from 4 to 6 wk in both strains, then is stable or falls slightly with age. Because the body weights of the two strains were not very different, the higher lung volumes in the C3H/HeJ strain persist even when normalized to body weight.

In conclusion, we have shown that accurate, reproducible measurements of FRC in breathing mice can be made using a standard clinical CT scanner. This method may be useful for noninvasively assessing both structural and functional changes in the lungs of experimental mice.

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REFERENCES


