

Airway distension during lung inflation in healthy and allergic-sensitised mice *in vivo*

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ARTICLE INFO

Article history:

Accepted 6 December 2012

Keywords:

Airway kinematics
Airway compliance
Hyperresponsiveness
Synchrotron radiation
Computed tomography

ABSTRACT

We evaluated the airway distention during lung inflation of varying size in healthy and allergic-sensitised mice *in vivo*. Computed tomography (CT) images of healthy and ovalbumin-treated mice were acquired using a synchrotron *in vivo* CT system when lung pressures was 0 and 20 cmH₂O, and the morphometric distension (diameter, length, and volume) and the compliance of airway segments (to as small as ~150 μm internal diameter) were calculated. With respect to airway size, in healthy mice, the changes in airway diameter and compliance were larger in the small-airway group. In contrast, in allergic-sensitised mice, there were no significant differences in the changes in airway distension or compliance. Airway wall thickness in allergic-sensitised mice increased significantly in all airway groups, but the change was much larger in the small than in the large-airway group. Compared with healthy airways, the changes in diameter and airway compliance of the allergic-sensitised mice were significantly smaller in the small-airway group.

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1. Introduction

During ventilation of the lung, both the bronchial tree and alveolar regions of the lung expand and contract in volume, but the manner in which this occurs is dependent on the local mechanoelastic properties of the structures. Due to the complexity of the arrangement of the airways and vascular structures within the parenchyma, the airway geometry—both its shape and volume—changes markedly and in a complex manner during respiration. From measurements of excised rat lungs using micro-computed tomography (micro-CT) (Sera et al., 2005), for smaller airways (diameter < 300 μm), the diameter and length increased dramatically at end tidal inspiration and total lung capacity compared with the values at functional residual capacity, and the airway volume for the smaller airways did not change linearly with those of lung volume. In addition, smaller airways were generally more compliant than larger airways with increasing airway generation.

The intrapulmonary airways *in situ* are surrounded by lung parenchyma and thorax, and the effective microscopic airway

deformations during ventilation are influenced by the surrounding support. It is particularly important to evaluate airway compliance in the lung parenchyma and thorax because the alveolar membrane and the thorax act as a spring and a wall, respectively (Hyatt and Flath, 1966; Takishima et al., 1975; Sera et al., 2004, 2005). Previously, the airway compliance in humans (airway diameter > 2.5 mm) was evaluated *in vivo* from the relationship between the cross-sectional area and transmural pressure during forced expiration (Brackel et al., 2000). In addition, a new technique has been developed to measure the airway compliance of humans *in vivo* and to evaluate the bronchodilator response in those with asthma (Kelly et al., 2010, 2011). This method is based on the combination of airway diameter measurements by high-resolution computed tomography (HRCT) with pressure–volume recording. In particular, HRCT scans were obtained during voluntary breath-holding at various lung volumes (Kelly et al., 2010, 2011). The diameter–pressure relationship for airways of varying size could be obtained from the results of these studies.

Asthma is characterised by chronic inflammation of the airway walls. Structural changes reported in the airways of asthmatic patients include epithelial fragility, goblet cell hyperplasia, enlarged submucosal mucus glands, angiogenesis, increased matrix deposition in the airway wall, increased airway smooth muscle mass, wall thickening and elastin abnormalities (Bai and Knight, 2005). These structural changes within the airway wall alter the

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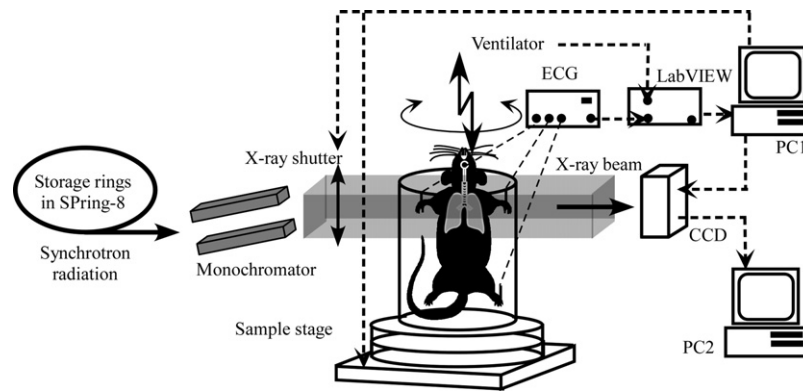


Fig. 1. Four-dimensional *in vivo* synchrotron CT system.

mechanical properties of the airway. Further, asthma is recognised as a disease of both the large and small airways (Roche, 1998; Tashkin, 2002; Bai and Knight, 2005; Tulic and Hamid, 2006; Usmani and Barnes, 2012). In particular, decreases in small airway diameter lead rapidly to increased airflow resistance (Wiggs et al., 1992; Hamid et al., 1997), because resistance is inversely related to the fourth power of the airway radius (Tashkin, 2002; Corren, 2008; van den Berge et al., 2011; Johnson and Hamid, 2012). The diameter may be reduced by an increase in the volume of tissue encroaching into the space and contraction of the smooth muscle.

In asthma, structural changes of airway components occur in both the large and small airways but predominantly in the small airways (Carroll et al., 1993). We hypothesised that airway distension during lung inflation and airway compliance in hyperresponsiveness were small, and that the decrements would be greater in peripheral airways. Airway lumen is covered with lung surfactant, and airway distension and compliance are determined by airway pressure, tissue elasticity, and surface tension. Previously, we showed that airway distension and compliance in hyperresponsiveness were small and that the decrements would be greater in peripheral airways (Sera et al., 2007). However, the previous evaluation was based on the measurement of euthanised mice during quasi-static inflation. Moreover, the total scan time was over 3 h.

In the present study, we calculated the airway distension during inflation (morphometric changes in diameter, length and volume) and airway compliance of varying size in healthy and ovalbumin (OVA)-treated mice as a model of hyperresponsiveness *in vivo*. Recently, we developed a four-dimensional *in vivo* computed tomography (CT) system at SPring-8 (Super Photon ring-8 GeV) to analyse small airway mechanics in mice (Sera et al., 2008). SPring-8 is a third-generation synchrotron radiation source in Japan, and provides a much higher X-ray flux than a typically laboratory X-ray source (Goto et al., 2001). The synchrotron CT images of lung fixed sample depict the peripheral structures including peripheral airways, airspaces, and alveolar walls individually, and these findings in normal and diseased lung are correlated well with the microscopic images of the corresponding histological section (Ikura et al., 2004). In our system, anaesthetised mice are connected to a custom mechanical ventilator that can induce iso-lung pressure holding intermittently and arbitrarily during ventilator cycles. Projections are also acquired prospectively in synchrony with airway pressure, ECG, X-ray shutter and a Charge Coupled Device (CCD) shutter during iso-pressure hold to reduce motion artefacts and radiation dose. Consequently, 3D changes in airway geometry during lung inflation can be evaluated in four-dimensions *in vivo*.

2. Methods

2.1. Animals

All experimental protocols were approved by the SPring-8 Experimental Animal Care and Use Committee. Five-week-old specific pathogen-free male A/J mice ($n = 10$; SLC Japan Inc., Shizuoka, Japan) were divided into two groups: healthy ($n = 5$) and allergic-sensitised mice ($n = 5$). Anaesthetised mice were instilled with OVA (1 mg/mL) intranasally using a micropipette (50 μ L) (Shinagawa and Kojima, 2003). OVA was administered three days per week for 10 weeks. In the healthy group, the mice received no treatment for 10 weeks. Twenty-four hours after the last administration of OVA, mice were anaesthetised with 50 mg/kg of sodium pentobarbitone by intraperitoneal (ip) injection and the lungs were imaged using 4D *in vivo* CT (Sera et al., 2008). Anaesthesia was maintained during the CT scan with 1% isoflurane. After imaging, mice were exsanguinated to allow measurement of total IgE.

2.2. X-ray imaging

The 4D *in vivo* CT system was constructed in medical imaging beamline (BL20B2) at SPring-8 (Sera et al., 2008). Briefly, the X-ray beam was monochromated using a double-crystal monochromator, and then transported into the experimental hutches. We placed a precision-rotation stage (Kouzu, Kawasaki, Japan), a high-resolution CCD camera (ORCAII-HR; Hamamatsu Photonics K.K., Japan), an X-ray shutter, a custom mechanical ventilator and an ECG (BMA-200; CWE, Inc., Ardmore, PA) in the experimental hutch (Fig. 1). In our CT system, the lung pressure was held at the same level when each projection was acquired. The programme that controlled synchronisation between the ventilator, X-ray exposure, X-ray shutter, and physiological motion was developed using LabVIEW (National Instruments, Austin, TX). So that exposures were made at a specific phase of the ventilator and ECG cycle, the programme generated triggers for the X-ray shutter and CCD detector from the airway pressure.

In this study, to analyse the morphometric distension in the same branching airway networks, images were acquired at two points in the pressure cycle: 0 and 20 cmH₂O, between T and next P wave of the ECG cycle (T-P segment, diastolic phase; Fig. 2). The triggers for the X-ray shutter were generated after 20 ms of the first R peak of the ECG signal during iso-pressure hold (300 ms). The X-ray energy was 20 keV, and the exposure time was 40 ms. In this protocol, iso-pressure was maintained prospectively during a respiratory cycle, and a projection image was acquired at each constant airway pressure. First, the lungs at 0 cmH₂O were imaged while the ventilator parameter was kept at the normal settings

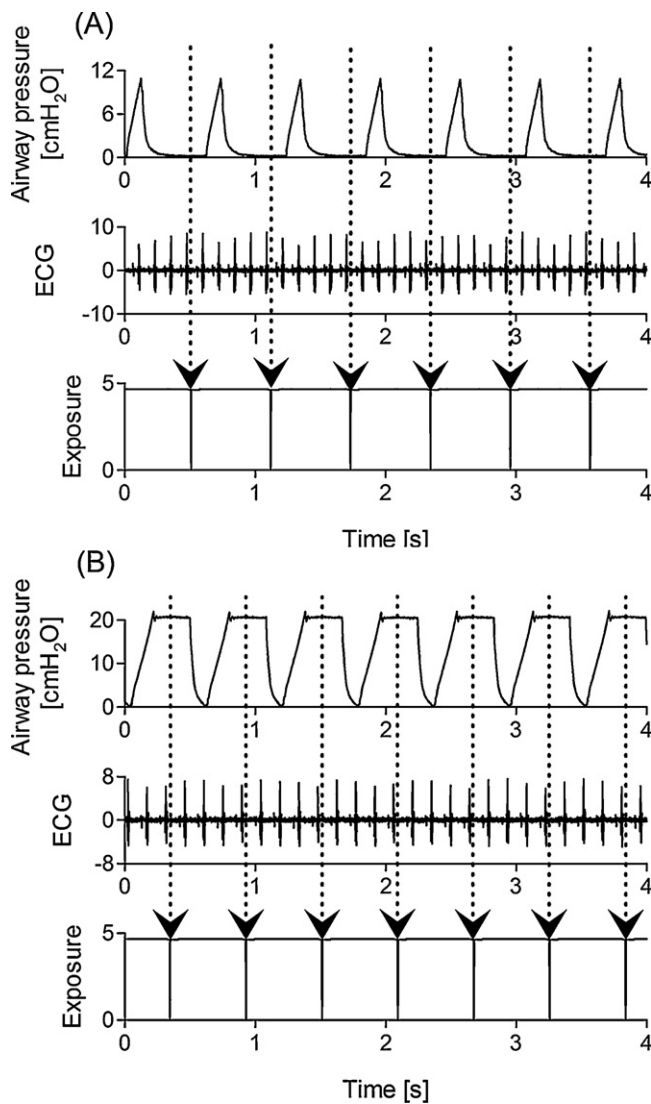


Fig. 2. Acquisition sequences at 0 cmH₂O (A) and 20 cmH₂O (B).

(Fig. 2(A)). Then, the lungs at 20 cmH₂O were imaged with the deep-breath settings of the ventilator (Fig. 2(B)). Thus, a dataset was acquired in one rotation (scan time: 7.5 min), and the total scan time (two data sets) was 15 min.

These conditions were repeated sequentially over a circular orbit of 180°. 900 projections of the sample were recorded with ECG and respiratory gatings during 180° rotation of the sample. The volume data were 4000 × 4000 × 944 voxels (voxel = 5.6 μm). Projections were normalised against a flat-field image with no specimen after being corrected for the dark current. Reconstruction of the tomographic images was carried out using a conventional convolution back-projection algorithm.

2.3. Analysis

The length (*Law*), diameter (*Daw*) and volume (*Vaw*) of healthy and allergic-sensitised airway segments were measured at each pressure using Amira software (Visage Imaging, Berlin, Germany), which detected the skeleton and branching voxels automatically (Fig. 3). The Euclidian distance to the nearest boundary was stored at each skeleton voxel as local diameter; i.e., the diameter of a sphere superimposed on the airway. *Law* was defined as the voxel numbers between branching voxels, and *Daw* was the averaged

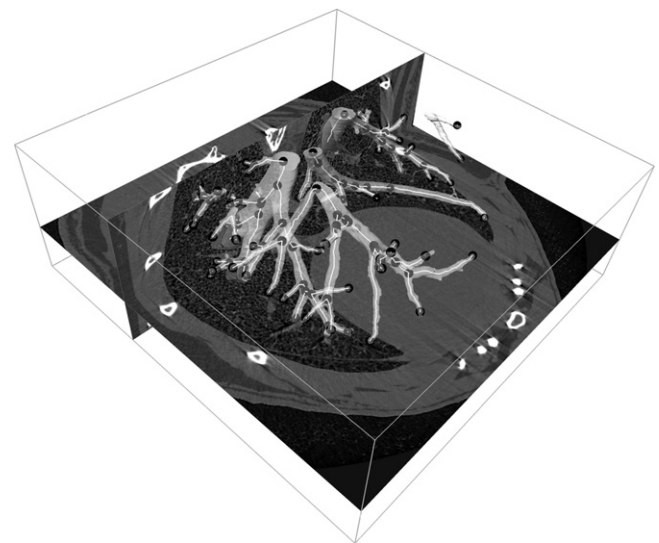


Fig. 3. Airway segmentation and skeleton. The boundary box indicates three-dimensional volume data obtained in one rotation. The airway skeleton (white lines) and branching voxels (black spheres) agreed with the airway surface (transparent).

value of the local diameters stored at skeleton voxels between branching points. The ratios of increases in *Law* and *Daw* (δLaw and δDaw , respectively) were normalised by *Law* and *Daw* at 0 cmH₂O to enable comparison of airways of different sizes. *Vaw* was the sum of the partial volumes for all voxels of a segment, assuming a truncated cone between branching voxels. From the middle-skeleton voxel of the selected airway skeleton, rays fanning out circumferentially were examined, with the exception of the adjacent vessel, and airway wall thickness (*WTaw*) values were calculated using the standard full-width at half-maximum technique (Jong et al., 2005; Hasegawa et al., 2006; Washko et al., 2009). It is well known that there is an overestimation of wall thickness and that this effect is greater for vessels of smaller diameter (Jong et al., 2005; Washko et al., 2009). Thus, to reduce the effect, *WTaw* was measured when lung pressure was 20 cmH₂O. The localised compliance of the airway segment (*sCaw*) was evaluated from 0 to 20 cmH₂O, as follows:

$$sCaw = \frac{(Vaw_{20\text{ cmH}_2\text{O}} - Vaw_{0\text{ cmH}_2\text{O}})}{20\text{ cmH}_2\text{O} \cdot Vaw_{0\text{ cmH}_2\text{O}}}$$

Airway segments were classified into three groups based on the luminal diameter at 0 cmH₂O (Phillips and Kaye, 1995): *Daw* > 1000 μm (large-airway group; group 1), 500–1000 μm (medium airway group; group 2) and < 500 μm (small-airway group; group 3). The rodent airways show more monopodial and asymmetrical branching than did those of humans (Phalen et al., 1978; Phillips and Kaye, 1995), suggesting that the rodent airways should be classified not by generation but by diameter. In our previous paper, we focused on the airway distension of small airways, and then airway segments (< 900 μm) were classified into some groups (Sera et al., 2007). On the other hand, in this study, we focused on the airway distension and compliance of whole airways *in vivo*, and the airway segments (< 2500 μm) were classified to compare airway distension and compliance statistically. Differences in airway changes (δDaw and δLaw) and *sCaw* among airway groups within the healthy and allergic-sensitised mice were analysed by Tukey–Kramer multiple comparison. Analysis of variance with unpaired *t*-test was used to determine the significance of differences in clinical characteristics between the healthy and allergic-sensitised mice. In all analyses, a value of *P* < 0.05 was taken to indicate statistical significance.

Table 1
Clinical characteristics of healthy and allergic-sensitised mice.

	<i>n</i>	Weeks	Weight (g)	<i>V</i> at 20 cmH ₂ O (μL)	<i>sC_{lung}</i> [cmH ₂ O ^{−1}]	IgE (ng/mL)
Healthy mice	5	15	27.5 ± 2.2	1001.7 ± 92.1	0.124 ± 0.011	1.28 ± 0.13
Allergic sensitised mice	5	15	24.9 ± 1.5 [#]	776.0 ± 54.7 [§]	0.107 ± 0.008 [§]	50.5 ± 15.3 [#]

Data are presented as means ± SD.

[#] *P* < 0.001.

[§] *P* < 0.05.

3. Results

3.1. Clinical characteristics of healthy and allergic-sensitised mice

Table 1 shows the clinical characteristics and total serum IgE levels of the healthy and allergic-sensitised mice. We calculated the specific whole lung compliance (*sC_{lung}*) as follows:

$$sC_{lung} = \frac{V_{20\text{cmH}_2\text{O}} - V_{0\text{cmH}_2\text{O}}}{20\text{cmH}_2\text{O} - V_{0\text{cmH}_2\text{O}}}$$

The lung volume (*V*) was measured using whole-body plethysmography and estimated from the CT images (Lundblad et al., 2007). However, as the height of the synchrotron beam was smaller than that of the mouse lung and not all CT images of the mouse lung could be acquired, *V* was not estimated from synchrotron CT images. Therefore, we estimated *V* at 20 cmH₂O approximately from the mechanical ventilator settings. *V* at 0 cmH₂O (functional residual capacity, FRC) for healthy mice was reasonably defined as 400 μL (Schulz et al., 2002). We assumed that FRC was proportional to body weight, and FRC for allergic-sensitised mice was estimated from that of healthy mice. The values of *V* at 20 cmH₂O and *sC_{lung}* for allergic-sensitised mice were significantly smaller than those for healthy mice (*P* < 0.05). IgE levels were significantly higher in allergic-sensitised mice than in healthy controls (*P* < 0.001).

3.2. Synchrotron CT images

This CT system enables the visualisation of airways in mice (diameter > 150 μm) at 0 and 20 cmH₂O *in vivo* (Fig. 4). Fig. 5 shows representative CT images of identical airways in healthy and allergic-sensitised mice at 0 and 20 cmH₂O; the changes in diameter were smaller in allergic-sensitised mice than in healthy controls.

3.3. Airway distension

Fig. 6 shows (A) δD_{aw} and (B) δL_{aw} of the healthy and allergic-sensitised mice, and Table 2 shows the averaged values of δD_{aw} , δL_{aw} , and *WTaw* in each group. In δD_{aw} of the healthy mice, the average value in the small-airway group was significantly larger than in the large-airway group, although there was no significant difference between the large and medium groups. In contrast, in the allergic-sensitised mice, there were no significant differences among the groups. Compared with the healthy mice, δD_{aw} of the allergic-sensitised airway was significantly smaller in group 3 and there were no significant differences among the other groups. The peripheral airways showed complicated branching and δL_{aw} could be affected by branching angle. After inflation, the branching angles varied and δL_{aw} could be less than 1 (Fig. 6(B)). However, there were no significant differences in δL_{aw} among the groups, and the values remained relatively constant in healthy and allergic-sensitised mice (Table 2). In comparison to healthy mice, there were no significant differences in any group. *WTaw* in allergic-sensitised mice increased significantly in all airway groups, but the change was much larger in the small than in the large-airway group.

Fig. 7 shows the average *sCaw* in each group of the healthy and allergic-sensitised mice. *sCaw* is presented as the value normalised to the airway volume at 0 cmH₂O, such as *sC_{lung}*. In both healthy and allergic-sensitised mice, *sCaw* in the small-airway group was found to be greater than *sC_{lung}*. And, *sCaw* (150 < *Daw* < 500 μm) was higher in living mice than euthanised mice (Sera et al., 2007). In healthy mice, the average *sCaw* in the small-airway group was significantly larger than in the large-airway group, although there were no significant differences between the medium and large groups. In the allergic-sensitised mice, there were no significant differences among the groups. In the small-airway group, the average *sCaw* of allergic-sensitised mice was significantly lower than that of healthy mice. These results are consistent with those reported previously (Sera et al., 2007).

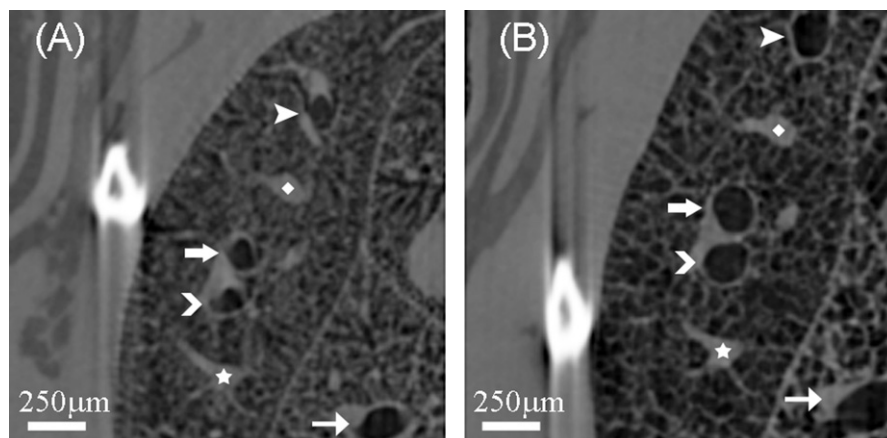


Fig. 4. Enlarged pictures of the airway diameter changes in a normal mouse at 0 cmH₂O (A) and at 20 cmH₂O (B). The same airways at 0 and 20 cmH₂O are indicated by identical arrows, and the same vessels are indicated by stars and diamonds.

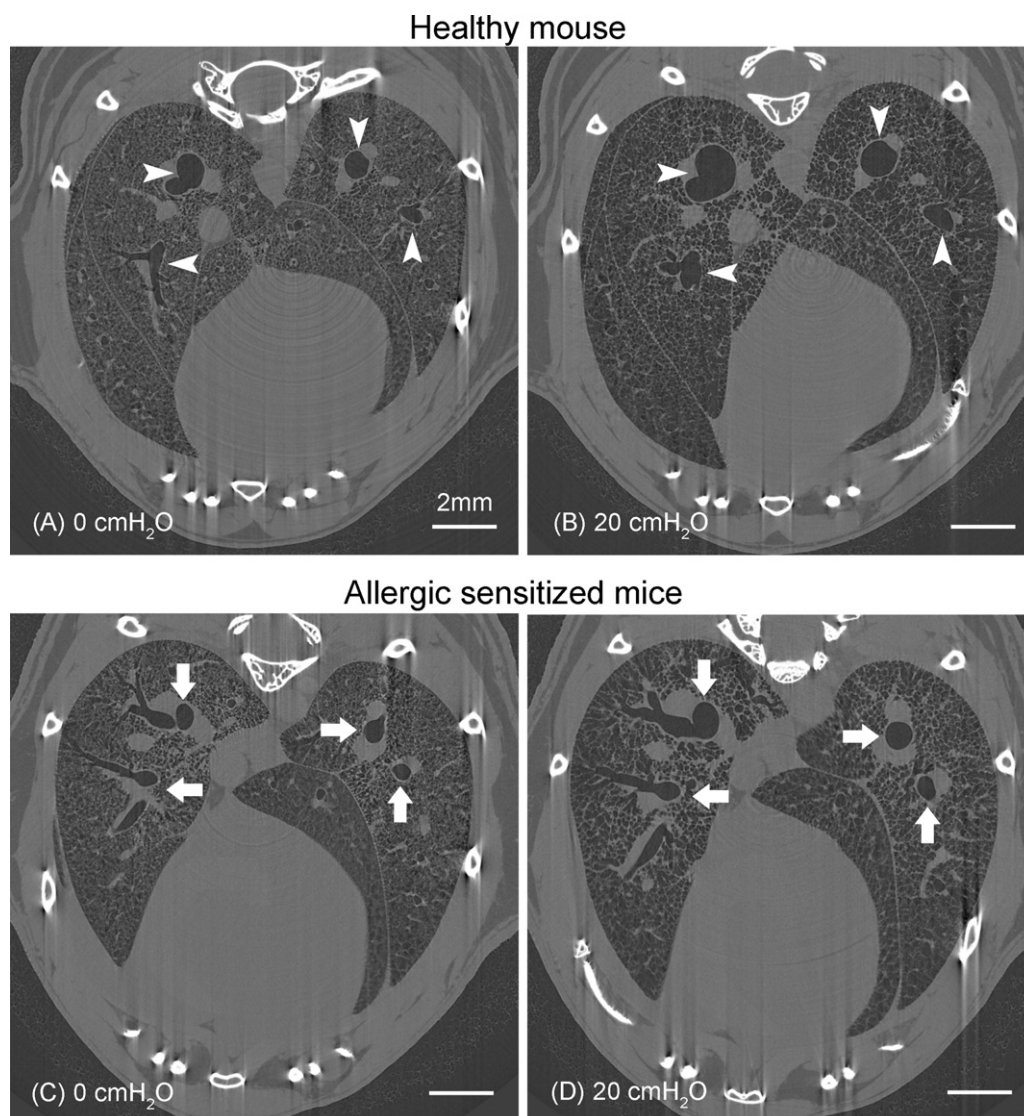


Fig. 5. Representative CT images from a normal mouse (left panel: at 0 cmH₂O (A) and at 20 cmH₂O (B)) and an asthmatic mouse (right panel: 0 cmH₂O (C), and 20 cmH₂O (D)). In healthy and allergic-sensitised mice, arrows in the same direction indicate identical airways at 0 and 20 cmH₂O.

4. Discussion

In this study, we evaluated the airway distension between 0 and 20 cmH₂O lung pressures, in terms of L_{aw} , D_{aw} and V_{aw} , with varying airway size in healthy and allergic-sensitised mice *in vivo*; the evaluation was performed in three-dimensions from synchrotron *in vivo* CT images. In clinical characteristics, total serum IgE levels in asthmatic mice were significantly higher than that in healthy controls (Table 1). Furthermore, the airway wall was thicker,

although the body weights of allergic-sensitised mice were lower than that of healthy controls (Fig. 5 and Table 2). In both healthy and allergic-sensitised mice, sC_{aw} in the small-airway group was found to be greater than sC_{lung} . And, sC_{aw} ($150 < D_{aw} < 500 \mu\text{m}$) was higher in living mice than euthanised mice (Sera et al., 2007). With respect to airway size, δD_{aw} and sC_{aw} were significantly smaller in the small-airway group in healthy mice, but not in allergic-sensitised mice. Compared with the airways of healthy mice, δD_{aw} and sC_{aw} of allergic-sensitised mice were significantly

Table 2
 δD , δL and WT_{aw} of healthy and allergic-sensitised airways.

	Airway group 1 $D_{aw} > 1000 [\mu\text{m}]$		Airway group 2 $1000 > D_{aw} > 500 [\mu\text{m}]$		Airway group 3 $500 > D_{aw} [\mu\text{m}]$	
	Healthy (n = 34)	Allergic sensitised (n = 15)	Healthy (n = 53)	Allergic sensitised (n = 53)	Healthy (n = 94)	Allergic sensitised (n = 82)
δD_{aw}	0.59 ± 0.16	0.53 ± 0.14	0.71 ± 0.25	0.51 ± 0.22	$0.97 \pm 0.36^*$	$0.71 \pm 0.33^\#$
δL_{aw}	0.19 ± 0.21	0.17 ± 0.20	0.13 ± 0.25	0.10 ± 0.24	0.16 ± 0.38	0.16 ± 0.38
$WT_{aw} [\mu\text{m}]$	68.2 ± 16.9	$86.5 \pm 21.5^\S$	51.1 ± 11.3	$83.0 \pm 20.8^\#$	42.6 ± 8.5	$71.8 \pm 14.8^\#$

Data are presented as means $\pm$ SD. N is the number of airway segments in each airway group.

* $P < 0.001$, vs. large-airway group 1 (D_{aw} at 0 cmH₂O) > 1000 .

^\# $P < 0.001$ vs. a same-size group of healthy mice.

^\S $P < 0.05$ vs. a same-size group of healthy mice.

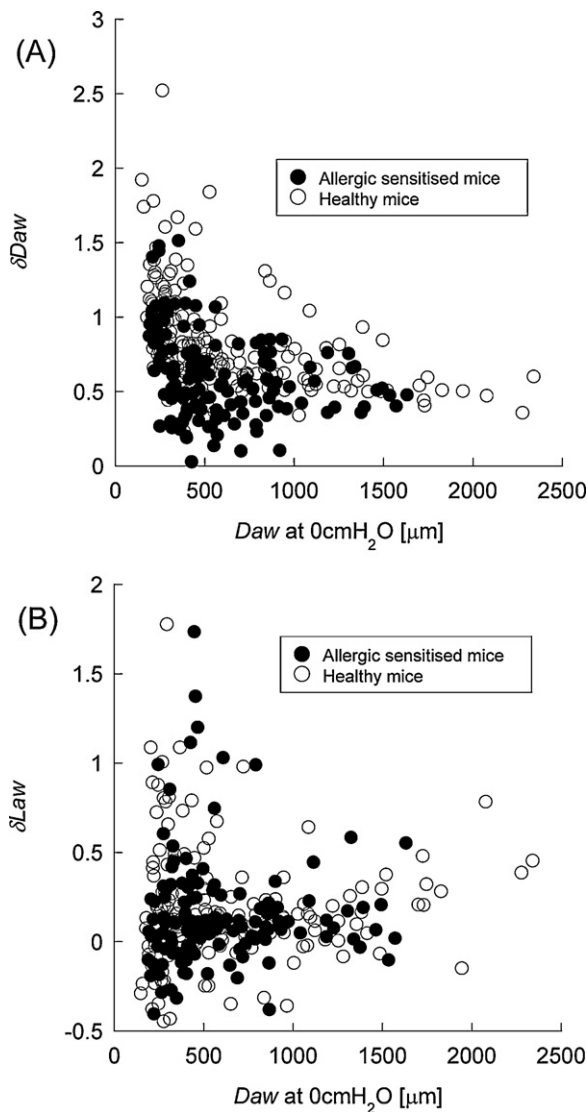


Fig. 6. δD_{aw} (A) and δL_{aw} (B) in healthy and allergic-sensitised airways. Table 2 shows the averaged values of δD_{aw} , δL_{aw} , and WT_{aw} in each airway group. Compared with the healthy mice, δD_{aw} of the allergic-sensitised airway was smaller as the diameter decreased. However, there were no significant differences in δL_{aw} .

smaller in the small-airway group. These results are consistent with those of a previous study using euthanised mice (Sera et al., 2007). WT_{aw} in allergic-sensitised mice was significantly thicker than in healthy mice in all airway groups, but the change was much larger in the small than in the large-airway group. These results are also consistent with those reported previously (Carroll et al., 1993). These observations that $sCaw$ of allergic-sensitised small airways was decreased compared with healthy small airways could account for the significance of small airways in hyperresponsiveness.

Our 4D *in vivo* CT system allows the evaluation of euairway mechanics (i.e., changes in diameter and length) and compliance of varying size in mice (diameter > 150 μm). In this system, we acquired each projection during iso-pressure breath holding. We hypothesised that the geometry would be stable during iso-pressure holding, and that the geometry after the respiratory cycle was identical to that before, and so each projection was obtained in one respiratory cycle in synchrony with airway pressure and heartbeat to reduce motion artefacts (Sera et al., 2008). We also used the X-ray shutter to reduce the radiation dose. In this study, the total radiation dose was 5.6 Gy. Micro-computed tomography

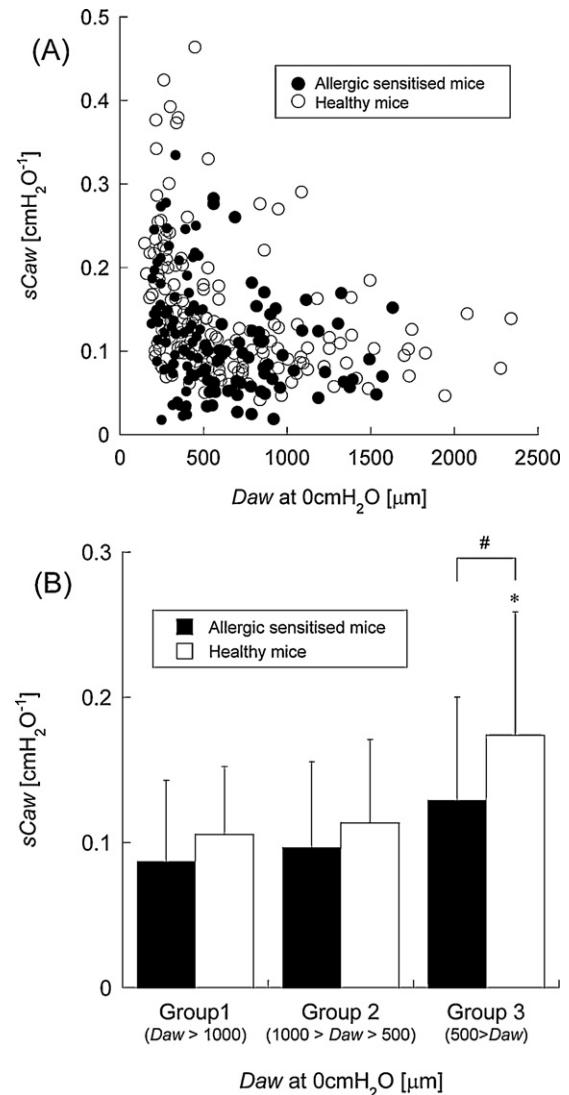


Fig. 7. Specific airway compliance ($sCaw$) of healthy and allergic-sensitised mice. (A) Scatter diagram and (B) histogram of airway groups. Data are presented as means $\pm$ SD. * $P < 0.001$, vs. the large-airway group (D_{aw} at $0 \text{ cmH}_2\text{O}$) > 1000. # $P < 0.001$, vs. a same-size group.

(micro-CT) systems that provide high-resolution, non-invasive CT images of living small animals have been developed (Badea et al., 2004). In addition, prospective (Ford et al., 2005) and retrospective (Ford et al., 2007) respiratory gating micro-CT systems have been proposed for 4D lung visualisation in free-breathing animals. In the former approach, the projections are obtained during the specific respiratory phase, and 4D results always require several scans. In the latter approach, the projections are acquired without any trigger during acquisition and are sorted into a different number of phase bins after complete acquisition. The radiation dose is low in all micro-CT systems; however, the lung microstructure (small airways) cannot be observed *in vivo*. In previous studies, the mice were alive (at least 200 days) after 16.0 Gy thoracic irradiation, despite a reduction in pulmonary compliance (Guerrero et al., 2007). In addition, the whole-body irradiation dose lethal for 50% of the population (LD_{50}) for all types of lung damage in mice ranges from 9 to 12 Gy (Safwat et al., 1996), which is higher than the dose received during the image sequence. After chest CT (dose, 20 Gy), the changes in histology and CT images were detected from week 4 and week 12, respectively. Clinical manifestations (loss of weight, mobility, breathing, increased mortality) occurred from week 14

(Plathow et al., 2004). These previous reports suggest that lung function and histology changes would not have occurred during our investigation (<1 h).

In healthy mice, δD_{aw} was significantly larger in the small-airway group, although there was no significant difference between the large and medium groups. In terms of δL_{aw} , there were no significant differences among the airway groups, and the averaged δL_{aw} values were smaller than those of δD_{aw} in all groups (Table 2). In addition, sC_{aw} was also higher in the small-airway group. These results were consistent with previous observations in euthanised mice (Sera et al., 2005, 2008) and excised rat lung (Sera et al., 2004). In particular, the relationship between airway size and compliance was similar to the results of human studies (Kelly et al., 2010). Our results suggest that airway geometry may not change homogeneously during respiration. This may have been due to the heterogeneities of the mechanical properties of airways. In mice, the mechanical properties of the respiratory system are characterised by the forced oscillation technique, which might be affected by the airway and tissue heterogeneities (Ito et al., 2007). Airways of varying diameter size penetrate into the depth of the parenchyma. Due to the complexity of the arrangement of the airways within the parenchyma, the airway geometry changes markedly and in a complicated manner during respiration. In particular, morphological studies have demonstrated some heterogeneities in alveolar size in normal mice (Soutiere and Mitzner, 2004). In contrast, measurement of the mechanical properties of the airway membrane in mice is difficult because of its small size; therefore, other mammalian lungs are commonly used. The airway wall structure varies along its path, and the fibrous coat contains cartilage only in large airways, gradually becoming thinner in smaller airways (Weibel, 1984). The mechanical properties of muscle strips from canine airways of differing airway generations were analysed, and there was heterogeneity in airway smooth muscle, indicating that the geometry of small and large airways may not change homogeneously (Ma et al., 1997; Noble et al., 2002). A study of the morphological structure of the rabbit tracheal mucosal membrane, performed to determine why the membrane is stiffer in the longitudinal than in the circumferential direction of the airway, indicated that the elastic fibres were oriented mainly in the longitudinal direction (Wang et al., 2000).

In allergic-sensitised mice, there were no significant differences in airway mechanics (δD_{aw} , δL_{aw} or sC_{aw}) in the various airway groups. Compared with healthy controls, δD_{aw} and sC_{aw} in allergic-sensitised mice were significantly smaller in the small-airway group, but not in the large- or medium-airway groups, and there were no significant differences in δL_{aw} . Airway compliance decreased in distal airways, and was significantly lower in patients with asthma, with the differences between asthmatic patients and healthy subjects becoming smaller towards the trachea (Brackel et al., 2000). These results were consistent with those of the present study, and support the suggestion that chronic inflammation and remodelling of the airway wall may result in stiffer dynamic elastic properties of the asthmatic smaller airway. In humans, the changes in airway compliance in asthma after bronchodilator use are greater in smaller airways (Kelly et al., 2011). Elastin in asthmatic patients had either virtually disappeared or was fragmented, and the deeper layer of elastic fibres was abnormal, with the fibres becoming patchy, tangled and thickened (Bousquet et al., 1996). Based on electron microscopy visualisation, elastolysis and fragmentation of elastic fibres occur in asthmatic patients, possibly as a result of repair elicited by chronic inflammation and mechanical stretching induced by breathing and oedema. The mechanical properties of airways depend on the quantity and organisation of the elastic components, particularly collagen and elastic fibres. In particular, elastic fibres with greater distensibility can lead to high strain. Moreover, in this study, WT_{aw} in allergic-sensitised mice

was increased significantly in all airway groups, but the change was much larger in the small-airway group than in the large-airway group. According to the reference histological measurements, the smooth muscle layer in OVA-challenged mice showed progressive thickening, with an increase of 2.25-fold (Shinagawa and Kojima, 2003). Our results were consistent with a previous report that structural changes of the airway wall occur predominantly in the small airways, although they are present in both large and small airways (Carroll et al., 1993). In an animal model of asthma, the volume proportion of smooth muscle-specific actin is augmented in distal airways and alveolar duct walls (Xisto et al., 2005). In humans, the thickness of the smooth muscle layer is increased in asthmatic patients (Huber and Koessler, 1922), and the smaller airways of the asthmatic subjects are also significantly thickened (Okazawa et al., 1996). In the smaller airways of asthmatic mice, dissolution of elastic fibres and thickening of the smooth muscle could decrease airway compliance (Sera et al., 2007).

In this study, sC_{aw} in the small-airway group was larger than sC_{lung} (Sera et al., 2004). sC_{lung} of allergic-sensitised mice was significantly smaller than that of healthy mice (Table 2). On the other hand, sC_{aw} was significantly smaller only in the small-airway group (Fig. 7). To evaluate respiratory function in detail, it is necessary to determine not only the lung compliance but also the more localised compliance based on microscopic regions of parenchyma and airways. In many respiratory diseases, significant compliance abnormalities occur mainly in localised regions of bronchi and bronchioles, and thus in addition to the overall compliance, the localised or microscopic compliance based on small-scale observations must be identified.

5. Conclusion

The airway distension and specific airway compliance of living healthy and allergic-sensitised mice were measured using a 4D *in vivo* synchrotron-based CT system. Our results indicate that the δD_{aw} of healthy mice was significantly larger in the small-airway group than in the large-airway group; however, that of allergic-sensitised mice was not. Compared with healthy mice, δD_{aw} and sC_{aw} of allergic-sensitised mice were significantly smaller in the small-airway group. WT_{aw} in allergic-sensitised mice increased significantly in all airway groups, but the change was much larger in the small than in the large-airway group. Our findings were consistent with the small airways being relatively important in asthma and suggest that assessment of *in vivo* localised compliance of airway segment in small animal models may be useful for evaluating changes in airway mechanics and further assessment of pharmacological experiments.

Conflict of interest

No conflicts of interest, financial or otherwise, are declared by the authors.

Acknowledgments

These experiments were performed with the approval of the SPring-8 Proposal Review Committee (2008B1384). The work was supported by a Grant-in-Aid for Young Scientists (B) (21700478) from the Japan Society for the Promotion of Science.

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