Aging-related changes in respiratory system mechanics and morphometry in mice

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Elliott JE, Mantilla CB, Pabelick CM, Roden AC, Sieck GC. Aging-related changes in respiratory system mechanics and morphometry in mice. Am J Physiol Lung Cell Mol Physiol 311: L167–L176, 2016. First published June 10, 2016; doi:10.1152/ajplung.00232.2016.—Previous work investigating respiratory system mechanics in mice has reported an aging-related increase in compliance and mean linear intercept (Lm). However, these changes were assessed using only a young (2-mo-old) and old (20- and 26-mo-old) group yet were interpreted to reflect a linear evolution across the life span. Therefore, to investigate respiratory system mechanics and lung morphology across a more complete spectrum of ages, we utilized 2 (100% survival, n = 6), 6 (100% survival, n = 12), 18 (90% survival, n = 12), 24 (75% survival, n = 12), and 30 (25% survival, n = 12)-mo-old C57BL/6 mice. We found a nonlinear aging-related decrease in respiratory system resistance and increase in dynamic compliance and hysteresis between 2- and 24-mo-old mice. However, in 30-mo-old mice, respiratory system resistance increased, and dynamic compliance and hysteresis decreased relative to 24-mo-old mice. Respiratory system impedance spectra were measured between 1–20.5 Hz at positive end-expiratory pressures (PEEP) of 1, 3, 5, and 7 cmH2O. Respiratory system resistance and reactance at each level of PEEP were increased and decreased, respectively, only in 2-mo-old animals. No differences in the respiratory system impedance spectra were observed in 6-, 18-, 24-, and 30-mo-old mice. Additionally, lungs were fixed following tracheal instillation of 4% paraformaldehyde at 25 cmH2O and processed for Lm and airway collagen deposition. There was an aging-related increase in Lm consistent with emphysematous-like changes and no evidence of increased airway collagen deposition. Accordingly, we demonstrate nonlinear aging-related changes in lung mechanics and morphometry in C57BL/6 mice.

IN HUMANS, VARIOUS PARAMETERS of respiratory mechanics and dynamic lung function steadily decline after ~30 years of age at rates of ~1% per year (5, 16). For example, there is an aging-related decrease in chest wall compliance and lung elastic recoil and an increase in closing capacity (22). Animal models that share similarities to the aging process in humans allow exploration into underlying mechanisms, and in this respect mice are frequently used to model aging-related changes in lung biology. Two previous studies explored the association between aging-related changes in lung mechanics and morphometry in mice (13, 14). In one study, Huang et al. (14) reported aging-related changes in respiratory system mechanics and morphometry in 2-, 20-, and 26-mo-old C57BL/6 mice. In a second study, Huang et al. (13) compared aging-related changes in respiratory system mechanics and morphometry in C57BL/6 vs. DBA/2 mice at 2 and 20 mo of age. In these studies, an aging-related increase in lung compliance, but no change in elastic recoil, and a decrease in airway resistance were reported (13, 14). Associated with these changes in lung mechanics, the authors also reported an aging-related increase in mean linear intercept (i.e., Lm; an estimation of the volume-to-surface ratio of acinar air spaces). These results were interpreted to suggest an aging-related change in respiratory system mechanics and morphology that occurs in a linear fashion across the life span. However, previous work by Ranga et al. (25), which investigated respiratory system mechanics across a spectrum of ages in BALB/c mice, found that ~60% of the total aging-related increase in static lung compliance occurred between 1 and 5–7 mo of age (i.e., ~10% per year). The remaining ~40% of the total aging-related increase in static lung compliance occurred between 5–7 and 28 mo of age (i.e., ~2% per year). This more rapid increase in static lung compliance in early age, relative to old age, has also been shown in an accelerated senescent-resistant (SAMRI) mouse strain (10). These data suggest, albeit in BALB/c and SAMRI mice, that aging-related changes in respiratory system mechanics may not evolve linearly across the life span. The distinction between a linear or nonlinear aging-related change in respiratory system mechanics is important, as aging research is often carried out using only a young and old age group, rather than a full spectrum of ages. The majority of work studying respiratory system mechanics and physiology in mice is conducted at ~2–3 mo of age. However, in the context of aging-related research, particularly with an experimental design of only a young and old age group, the age range studied is critically important. As described by Miller and Nadon (24), aging research should utilize a young group that is sufficiently mature to eliminate potential contamination from normal systemic maturation known to be ongoing in C57 mice until 5–6 mo of age when body weight stabilizes (2). Similarly, aging research should utilize an old group that is sufficiently young to avoid potential contamination from normal aging-related comorbidities, often defined by the age corresponding to 50% survival (i.e., ~24 mo of age in C57 mice). In Swiss-Webster albino mice, Amy et al. (1) reported that by ~1 mo of age acinar air space geometry and the number of pores of Kohn resembles the adult lung. However, more recent work in C57BL/6J mice at 3 and ~23 mo of age using reconstruction of high-resolution computed tomography images demonstrated that the development of multiple parameters of lung morphology (e.g., lung volume, number of alveoli, acinus volume, acinus surface area, and acinus...
branch diameter and length) is still ongoing beyond 3 mo of age (19, 30).

Accordingly, aging-related respiratory physiology research in C57 mice should utilize a "young control" group that is sufficiently mature (e.g., 6 mo of age) to avoid contamination by early maturational changes. However, an evidence base is lacking for future work to direct the decision to utilize certain age ranges. Thus the purpose of the present study was to examine the time evolution of natural aging-related changes in respiratory system mechanics and morphometry in C57BL/6 mice across a spectrum of ages. We hypothesized that there is a nonlinear aging-related increase in respiratory system compliance and a corresponding increase in alveolar size.

MATERIALS AND METHODS

All experiments were designed according to the guidelines for animal use in gerontological research (24). All protocols were approved by the Institutional Animal Care and Use Committee (no. A67112) at the Mayo Clinic and were in compliance with the American Physiological Society and National Institute of Health Guidelines.

Animals. Mice (C57BL/6 × 129) were bred and naturally aged in a specific pathogen-free colony maintained at the Mayo Clinic. Animals were maintained on an alternating 12-h light-dark cycle and provided with fresh water and food ad libitum. The present study examined male mice at 2 mo of age (100% survival, n = 6), 6 mo of age (100% survival, n = 12), 18 mo of age (90% survival, n = 12), 24 mo of age (75% survival, n = 12), and 30 mo of age (25% survival, n = 12). Survival estimates are based on published data from our colony (6) and others (32). Male mice were chosen to avoid potential confounding effects of aging-related changes in estrogen. Additionally, in a previous study in C57BL/6 × 129 mice, we found no differences across aging-related changes in diaphragm muscle function between males and females (7).

Respiratory system mechanics. Following induction of anesthesia, from an intramuscular (hindlimb) injection of ketamine (90 mg/kg) and xylazine (10 mg/kg), the trachea was cannulated (18-19G; Brico Medical Supplies, Houston, TX). Care was taken to ensure there was an airtight seal between the trachea and cannula using two strands of firmly tied silk suture. Each animal received a new cannula that was attached to a computer-controlled ventilator (SCIREQ Scientific Respiratory Equipment, flexiVent FX, v.7.5.4, FX1, Montreal, ON, Canada). Mice were mechanically ventilated at 10 ml/kg for ~3 min prior to initiating mechanical perturbations, which were always preceded by a deep inflation maneuver and performed in triplicate. The deep inflation maneuver gradually inflates the lungs to a pressure of 30 cmH2O over a period of 3 s, at which point pressure is held for another 3 s, allowing alveolar pressure to equilibrate with the applied pressure. Accordingly, the deep inflation maneuver takes the subject from functional residual capacity to total lung capacity (TLC) and provides a measure of inspiratory capacity. The first mechanical perturbation applied was the "SnapShot 150," which is a brief (1.25 s), single-frequency (2.5 Hz; corresponding to 150 breaths/min) forced-oscillation perturbation performed at a volume equal to 10 ml/kg. During the maneuver the volume and pressure signals are recorded and the flow signal is derived from the volume measurements, which are fit to the single compartment model by linear regression to derive respiratory system resistance and dynamic compliance. Following the SnapShot 150 maneuver, the "Quick Prime 3" perturbation was performed at a volume equal to 10 ml/kg at oscillatory frequencies above and below the subject’s ventilatory frequency (spanning 1 to 20.5 Hz). The volume and pressure signals from the Quick Prime 3 perturbation are recorded and the flow signal is derived from the volume signal at each frequency. Input impedance is then calculated at every oscillatory frequency and fit to the constant-phase model (9), allowing the calculation of Newtonian resistance, tissue damping, tissue elastance, and hysteresivity. Respiratory system impedance parameters were measured at four different levels of positive end-expiratory pressure (1, 3, 5, and 7 cmH2O). Lastly, the acquisition of pressure-volume curves were obtained by applying stepwise increases in pressure, each with a brief no-flow period allowing the lungs to equilibrate, until the pressure reached 30 cmH2O (corresponding to TLC). The deflation arm of the curve is fit with the Salazar-Knowles equation (26) to calculate static compliance at 5 cmH2O.

Tidal volume ventilation and the SnapShot 150 and Quick Prime 3 perturbations were performed as body weight-dependent (10 ml/kg) volume-driven maneuvers, rather than as a fixed volume (i.e., 0.3 ml regardless of animal’s body weight). This approach was deliberately chosen to avoid employing too large of a volume for animals weighing <30 g (e.g., 0.25 vs. 0.3 ml for a 25-g mouse). However, with this approach it was also important to account for the increased adiposity present in older animals to avoid utilizing too large of a volume for animals weighing excessively over 30 g. Beyond ~6 mo of age, the body weight of C57BL/6 mice progressively increases due to an increased percentage of fat mass (2): 6 mo = ~7% fat mass, 18 mo = ~19% fat mass, and 24 mo = ~15% fat mass. Assuming the body weight at 6 mo of age reflects a standard “lean” body composition, if the body weight for mice at 18 (7/12 animals) and 24 (7/12 animals) mo of age was above the mean body weight for 6-mo-old animals (29.8 ± 1.0 g) it was reduced by ~12 and ~8%, respectively. This correction reduced the average body weight from 31.3 ± 1.2 to 29.2 ± 0.6 g in 18-mo-old animals, and from 32.6 ± 1.1 to 30.9 ± 0.8 g in 24-mo-old animals. No 30-mo-old mice demonstrated excessive adiposity (body weight 28.8 ± 0.5 g).

Of note, all mechanical measurements were obtained with the chest wall intact, as well as during open-chest conditions to remove potential mechanical contributions from the chest wall. No significant differences between closed-chest and open-chest conditions were detected, and therefore data herein reflect only closed-chest (i.e., intact respiratory system) conditions.

Lung morphometry. Lungs were fixed using freshly prepared 4% paraformaldehyde instilled via the tracheal cannula at a pressure of 25 cmH2O. Care was taken to ensure each mouse received the same 25 cmH2O pressure gradient and that complete inflation of both the right and left lungs was observed. Lungs were allowed to equilibrate with the pressure gradient for 15 min before the trachea was carefully and securely tied off prior to withdrawing the cannula and removing the lungs. Postfixation lung volumes were measured by volume displacement at 25 cmH2O pressure (27) to determine the reference lung volume. Lungs were then submerged in 4% paraformaldehyde for at least 24 h prior to processing (Leica Biosystems, ASP300s, Buffalo Grove, IL). The tissue processing procedure was the same for all lungs and consisted of an initial period of buffered formalin fixation, followed by graded ethanol dehydration, xylene clearing, and wax infiltration. Lungs were then embedded in paraffin (Leica Biosystems, EG1150C) and sectioned at 5 μm at the level of the hilum (Leica Biosystems, RM2165). Paraffin-embedded lung sections were picked up on slides and dried at 60°C for 2 h. Slides were then stained with both hematoxylin and eosin and Masson trichrome for histological analysis and were imaged on an Olympus BX50W1 light microscope (Olympus, Tokyo, Japan). Digital images (8-bit, 2,048 × 2,048 pixel array) were obtained via a ×10 and a ×20 objective, calibrated at 0.65 and 0.33 mm²/pixel, respectively. Analysis of mean linear intercept (Lm) was calculated, as before (23), according to the formula $L_m = n/\text{L}$, where $\text{L}$ is the length of the line, $n$ is the number of lines counted, and $I$ is the sum of alveolar wall intercepts (31). Of note, $L_m$ characterizes the entire acinar air space complex (acinar air spaces,
Table 1. Body weight and respiratory system parameters in 2-, 6-, 18-, 24-, and 30-mo-old C57BL/6 mice

<table>
<thead>
<tr>
<th>Age (mo)</th>
<th>Body weight, g</th>
<th>Lung volume, ml</th>
<th>IC, ml</th>
<th>Cst, ml/cmH2O</th>
<th>resistance (cm H2O/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>27.3 ± 0.7</td>
<td>0.63 ± 0.10</td>
<td>0.06 ± 0.03*</td>
<td>0.055 ± 0.005*</td>
<td>0.63 ± 0.06*</td>
</tr>
<tr>
<td>6</td>
<td>29.8 ± 1.0</td>
<td>0.94 ± 0.03*</td>
<td>0.83 ± 0.02*</td>
<td>0.075 ± 0.002*</td>
<td>0.80 ± 0.02*</td>
</tr>
<tr>
<td>18</td>
<td>31.3 ± 1.2</td>
<td>1.06 ± 0.06*</td>
<td>0.90 ± 0.01*</td>
<td>0.091 ± 0.003*</td>
<td>0.90 ± 0.03*</td>
</tr>
<tr>
<td>24</td>
<td>32.6 ± 1.1</td>
<td>1.26 ± 0.05*‡</td>
<td>1.06 ± 0.02*‡</td>
<td>0.091 ± 0.003+i</td>
<td>0.90 ± 0.03+i</td>
</tr>
<tr>
<td>30</td>
<td>28.8 ± 0.5</td>
<td>1.20 ± 0.06*‡</td>
<td>1.20 ± 0.06*‡</td>
<td>0.075 ± 0.004§</td>
<td>0.75 ± 0.004§</td>
</tr>
</tbody>
</table>

Values are means ± SE. IC: inspiratory capacity; Cst: static respiratory system compliance; *P < 0.05, 2 mo vs. 6, 18, 24 and 30 mo; †P < 0.05, 6 mo vs. 18, 24 and 30 mo; ‡P < 0.05, 18 mo vs. 24 and 30 mo; §P < 0.05, 24 mo vs. 30 mo. One-way ANOVA with Tukey’s HSD post hoc test.

RESULTS

The body weights of mice at 2, 6, 18, 24, and 30 mo of age are shown in Table 1. Other than a lower body weight in 2-mo-old mice, no other significant aging-related changes in body weight were observed. Postfixation reference lung volumes increased with age (P < 0.05), reaching a maximum in 24-mo-old mice with a subsequent reduction in lung volume in 30-mo-old mice (Table 1). The greatest increase in lung volume (49%) occurred between 2 and 6 mo of age. Inspiratory capacity followed a similar aging-related increase, reaching a maximum in 24-mo-old mice with a subsequent reduction in 30-mo-old mice (Table 1).

Respiratory system mechanics. There was a nonlinear aging-related decrease (P < 0.05) in respiratory system resistance (Fig. 1A) between 2 and 24 mo of age. The greatest decrease in respiratory system resistance (54%) occurred between 2 and 6 mo of age. Respiratory system resistance decreased by 26% between 6 and 18 mo of age and by 20% between 18 and 24 mo of age. Interestingly, respiratory system resistance increased (P < 0.05) in 30-mo-old animals compared with both 18- and 24-mo-old animals (Fig. 1A).

Respiratory system dynamic compliance also displayed a nonlinear aging-related increase (P < 0.05) between 2 and 24 mo of age (Fig. 1B). The greatest increase in respiratory system dynamic compliance (51%) occurred between 2 and 6 mo of age. Respiratory system dynamic compliance increased by 22% between 6 and 18 mo of age, and by 28% between 18 and 24 mo of age. However, respiratory system dynamic compliance decreased (P < 0.05) in 30-mo-old animals compared with 24-mo-old animals (Fig. 1B).

Analysis of the quasi-static pressure-volume curves revealed a main effect for age and pressure (P < 0.05) when expressed as a change in volume (Fig. 2A) as well as when expressed relative to total lung volume (Fig. 2C). However, no significant interaction between age and pressure was observed (Fig. 2A and C). There was an aging-related increase (P < 0.05) in respiratory system hysteresis, which reached a maximum at 24 mo of age and decreased at 30 mo of age (Fig. 2B). The greatest increase in respiratory system hysteresis (69%) occurred...
curred between 2 and 6 mo of age. Respiratory system hyster-
esis increased by 7% between 6 and 18 mo of age and by 24% bet-
between 18 and 24 mo of age. There was also a nonlinear
aging-related increase (P < 0.05) in static respiratory system
compliance calculated at 5 cmH2O on the deflation limb of the
pressure-volume curve, with the greatest increase (56%) oc-
curring between 2 and 6 mo of age. Static respiratory system
compliance reached a maximum at 24 mo of age and decreased
at 30 mo of age (Table 1).

Respiratory system impedance. Respiratory system imped-
ance parameters (Fig. 3) were evaluated across a range of
frequencies (between 1 and 20.5 Hz) and at 1, 3, 5, and 7
cmH2O of positive end-expiratory pressure (PEEP). Respira-
tory system impedance spectra (i.e., resistance and reactance)
differed only in the 2-mo-old mice, which displayed signifi-
cantly increased respiratory system resistance, and decreased
respiratory system reactance, at 1, 1.5, and 2.5 Hz for each
level of PEEP (Fig. 3). There was a main effect of both age and
PEEP (P < 0.05), without a significant interaction, for New-
tonian resistance, tissue damping, and tissue elastance (Table
2). However, there was a significant interaction (P < 0.05)
between age and PEEP for hysteresivity (Table 2). Significant
post hoc comparisons for hysteresivity were only observed at
24 and 30 mo of age.

Lung and airway morphology. Representative hematoxylin
and eosin-stained lung cross sections illustrating alveolar and
parenchymal structure in each age group are presented in Fig. 4.
Consistent with these aging-related changes in respiratory
mechanics, there was an aging-related increase (P < 0.05) in
mean linear intercept, reflecting an increase in the volume-to-
surface ratio of acinar air spaces (Fig. 5). Based on Fig. 5 it
appeared that there was a roughly linear aging-related increase
in mean linear intercept. However, when mean linear intercept
was expressed as a volume (mm3) and plotted against postfix-
ation lung volumes (Fig. 6), the relationship between mean
linear intercept and lung volume was exponential. These data
were fit with a nonlinear exponential growth curve using least
squares and demonstrate a strong correlation (r^2 = 0.74).

Pulmonary fibrosis was assessed and scored according to the
modified Ashcroft score (15). There was no evidence for
pulmonary fibrosis in mice at 2, 6, 18, and 24 mo of age.
However, there was mild to moderate bronchiolitis with some
fibrosis in mice at 30 mo of age. Representative Masson
trichrome-stained small airways (~100–200 μm) in each age
group are presented in Fig. 4. There was no evidence of an
increase in airway collagen deposition. Further histological
analysis of small airways revealed evidence for focal mild to

Fig. 2. Absolute pressure-volume loops (A), overall hysteresis, i.e., the volume of each pressure-volume loop reflecting the work of breathing (B), and pressure-volume loops expressed relative to total lung volume (C) in 2 (•, continuous line), 6 (○, dashed line), 18 (□, dotted line), 24 (●, dashed-dotted line), and 30 (▲, gray continuous line)-mo-old mice. Data were analyzed via a two-way ANOVA (A and C) and a one-way ANOVA (B) with Tukey’s HSD post hoc test. *P < 0.05, 2 mo vs. 6, 18, 24, and 30 mo; †P < 0.05, 6 mo vs. 18, 24, and 30 mo; ‡P < 0.05, 18 mo vs. 24 and 30 mo; §P < 0.05, 24 mo vs. 30 mo.
Fig. 3. Real (i.e., resistance; \( R_{rs} \)) (A, C, E, G) and imaginary (i.e., reactance; \( X_{rs} \)) (B, D, F, H) parts of the respiratory system impedance spectra at 1, 1.5, 2.5, 3.5, 5.5, 6.5, 8.5, 9.5, 11.5, 14.5, 15.5, 18.5, and 20.5 Hz and positive end-expiratory pressures of 1, 3, 5, and 7 cmH\(_2\)O in 2 (●, continuous line), 6 (○, dashed line), 18 (■, dotted line), 24 (□, dashed-dotted line), and 30 (▲, gray continuous line)-mo-old mice. Data were analyzed via a two-way ANOVA with Tukey’s HSD post hoc test. *\( P < 0.05 \), 2 mo vs. 6, 18, 24, and 30 mo; †\( P < 0.05 \), 6 mo vs. 18, 24, and 30 mo; ‡\( P < 0.05 \), 18 mo vs. 24 and 30 mo; §\( P < 0.05 \), 24 mo vs. 30 mo.
moderate bronchiolitis only in 18-, 24-, and 30-mo-old animals.

**DISCUSSION**

The present study demonstrates nonlinear aging-related changes in respiratory system mechanics and lung morphometry across a spectrum of ages in mice, thereby establishing an evidence base to direct future aging-related respiratory physiology research in mice. The greatest changes in respiratory mechanics occurred between 2 and 6 mo of age. Specifically, respiratory system resistance decreased by 54%, dynamic compliance increased by 51%, and hysteresis increased by 69% (i.e., ∼9% per month). However, between 6 and 18 mo of age these parameters followed a more gradual change (i.e., ∼1–2% per month), which tended to accelerate thereafter between 24 and 30 mo of age (i.e., ∼5% per month). With respect to lung morphometry, mean linear intercept was relatively unchanged between 2 and 6 mo of age, but thereafter it increased exponentially in relation to total lung volume.

Aging-related physiological research often relies on two age groups to draw conclusions regarding the effect of the natural aging process on the parameter(s) in question. Clearly, with only two age groups, knowledge of the time evolution for the parameter(s) in question is not attained, but such information is often critical for researchers to determine appropriate ages of their young and old subject groups. To this point, Miller and Nadon (24) established principles for animal use in gerontological research. The basic experimental framework they set forth states that aging research should utilize a young group that is sufficiently mature to eliminate potential contamination from normal systemic maturation known to be ongoing in mice until 5–6 mo of age when body weight stabilizes (2). Similarly, aging research should utilize an old group that is sufficiently young to avoid potential contamination from normal aging-related comorbidities, often defined by the age corresponding to 50% survival (i.e., ∼24 mo of age in C57 mice).

**Respiratory system mechanics.** Previous work by Huang et al. (14) compared aging-related changes in respiratory system mechanics between young (2-mo-old) and older (20- and 26-mo-old) C57BL/6J mice. In this study, Huang et al. reported that 80–84% of the total aging-related increase in lung volumes and respiratory system compliance occurred between 2 and 20 mo of age, with only 16–20% occurring between 20 and 26 mo of age (14). Although the authors acknowledge that their study does not offer insight into the rate of change for these parameters in the intervening months, their interpretation was that lung volumes and respiratory system compliance increased roughly linearly from 2 to 26 mo of age. However, previous work by Ranga et al. (25), which investigated respiratory system mechanics across a spectrum of ages in BALB/c mice, found that ∼60% of the total aging-related increase in static lung compliance occurred between 1 and 5–7 mo of age (i.e., ∼10% per year). The remaining ∼40% of the total aging-related increase in static lung compliance occurred between 5–7 and ∼24 mo of age (i.e., ∼2% per year). This more rapid increase in static lung compliance in early age, relative to old age, has also been shown in SAMR1 mice (10). These data suggest, albeit in BALB/c and SAMR1 mice, that aging-related changes in respiratory system mechanics do not evolve linearly across the life span. The results of the present study support this conclusion, since ∼50–70% of the total aging-related change in respiratory system mechanics occurred between 2 and 6 mo of age (Fig. 1).

The mechanism(s) underlying changes in respiratory system mechanics in mice between 2 and 6 mo of age is unknown but is likely secondary to continued maturational development rather than a consequence of aging per se. Technically, 2-mo-old mice are postpubertal but are inarguably still in the throes of complex maturational development and in this way are the biological equivalent of human teenagers. Although previous work in Swiss-Webster albino mice reported that by ∼1 mo of age acinar air space geometry and the number of pores of Kohn...
resemble the adult lung (1), lung growth and development seems to be ongoing. For example, the simple observation in the present study that the tracheas in 2-mo-old mice could not accommodate an 18G cannula, whereas 6-mo-old mice can, suggests continued growth. More importantly, lung volumes in the present study increased significantly between 2 and 6 mo of age (Table 1). Kawakami et al. (17) have also demonstrated a significant increase in lung volumes in BALB/c mice between
should utilize a young control group at future aging-related respiratory physiology work in C57 mice. It is unknown. However, the present study suggests that time points, the precise aging-related evolution of these parameters is still ongoing beyond 3 mo of age (19, 30). Without knowledge of the intervening time points, the precise aging-related evolution of these parameters is unknown. However, the present study suggests that future aging-related respiratory physiology work in C57 mice should utilize a young control group at ~6 mo of age.

With respect to parameters of respiratory impedance, Huang et al. (14) reported a decrease in airway resistance, tissue damping, and tissue elastance, in mice at 20 and 26 mo of age, compared with mice at 2 mo of age. No changes were observed in mice between 20 and 26 mo of age. The present study did not observe a statistically significant interaction between age and PEEP with respect to airway resistance, tissue damping, and tissue elastance (Table 2). However, the magnitude of these variables within the 2-mo-old age group of the present study (Table 2) and that of Huang et al. (14) are comparable. Importantly, tissue damping and tissue elastance were considerably higher in 2-mo-old mice compared with 6-mo-old mice, which may explain why a significant aging-related change in these parameters was observed by Huang et al. (14). Indeed, previous work by Bozanich et al. (3), has demonstrated that respiratory impedance parameters decrease rapidly during early development (1–8 wk of age) in BALB/c mice. Although by 2 mo of age the rate of change in respiratory impedance parameters appears to be slow, the present study suggests they continue to change beyond 2 mo of age. Additional work examining intervening time points between 2 and 6 mo of age is needed to clarify when these parameters plateau.

The aging-related decrease in respiratory system resistance (between 2 and 24 mo of age) demonstrated in the present study is in agreement with previous reports (10, 14), yet this remains curious considering the aging-related increase in $L_m$ and system compliance suggesting the natural aging process reflects emphysema-like changes in the respiratory system. Indeed, emphysema in humans leads to airway narrowing and a corresponding increase in airway resistance. However, air space enlargement in aged humans is not always correlated with clinically relevant emphysema (16). Furthermore, airway resistance in humans is typically measured via spirometry, which can be difficult for older subjects to perform. Using the forced-oscillation technique that is comparably very simple to perform, recent work by Guo et al. (8) report that airway resistance in elderly subjects (65–100 yr; mean age 83 yr) tends to be slightly lower than previously reported in younger adults. One potential explanation for the aging-related decrease in airway resistance is due to the aging-related increase in lung volume, which is inversely related to airway resistance (4).

Another potential explanation is the possibility that there is an aging-related increase functional residual capacity (i.e., hyperventilation), which would further contribute to airway dilation and decreased airway resistance. Finally, it remains possible that there was an aging-related decrease in airway wall thickness, which may extend to an increased internal lumen diameter and, therefore, decreased resistance. Histological analysis of airways was not sensitive enough to quantitatively address this possibility. However, there was no evidence for an aging-related increase in collagen deposition, which may be expected if respiratory system resistance increased.

Lung and airway morphometry. Previous studies regarding aging-related changes in lung morphometry in mice, reflected primarily by mean linear intercept, have been variable. Most recently, Huang et al. (14) reported a significant increase in mean linear intercept in C57BL/6 mice at 26 mo of age compared with mice at 2 mo of age. Additionally, previous
work in SAMRI mice has reported an aging-related increase in mean linear intercept (21, 28). However, in a comprehensive study by Kawakami et al. (17) no aging-related changes in lung morphometry were observed in BALB/cNNia mice. It has been suggested that BALB/c mice may be resistant to an aging-related increase in mean linear intercept (21). Indeed, potential strain variations in lung morphometry between C57BL/6 and DBA/2 mice appear to exist (13, 18) that may be related to strain differences in alveolar growth vs. multiplication during postnatal lung development (29).

The present study supports and extends these previous findings by demonstrating an aging-related increase in mean linear intercept (Figs. 5 and 6) across a spectrum of ages in C57BL/6 mice. Accurately interpreting the increase in mean linear intercept is critically dependent on knowing postfixation lung volumes (i.e., the reference volume). Indeed, with an increase in compliance, mean linear intercept would be expected to increase solely owing to the corresponding increase in lung volume. However, without normalizing mean linear intercept to lung volume it is impossible to separate whether the increase in mean linear intercept is due to the increase in compliance (i.e., lung volume) or, instead, in part to emphysema-like changes. Although mean linear intercept is a two-dimensional parameter (μm), after extrapolating it to a three-dimensional volume (mm$^3$) and plotting these data against postfixation lung volumes (Fig. 6), an exponential relationship was observed. In this way, mean linear intercept increases more rapidly than the increase in lung volume due to aging-related increases in compliance.

Because of the decrease in respiratory system compliance at 30 mo of age relative to 24 mo of age, we suspected a potential increase in pulmonary fibrosis. Lung sections were analyzed for pulmonary fibrosis and scored according to the modified Ashcroft score. As expected, there was no evidence for pulmonary fibrosis at 2, 6, 18, and 24 mo of age. However, there was mild to moderate bronchiolitis with some fibrosis in mice at 30 mo of age. This mild pulmonary fibrosis is likely insignificant physiologically with respect to explaining the decreased compliance in 30-mo-old mice compared with 24-mo-old mice, but it may explain the increase in airway resistance. Considering that C57BL/6 mice at 30 mo of age have a 25% survival rate, this population of animals likely exhibits a selection bias. In this way, the increase in compliance is less pronounced in 30-mo-old survivors.

**Conclusion.** Although numerous studies have described aging-related changes in respiratory system mechanics and morphometry in humans, relatively few studies have done so in mice. Furthermore, with the exception of previous work by Huang et al. (14), aging-related changes in respiratory system mechanics and morphometry have not been described in C57BL/6 mice. Accordingly, the present study provides important data regarding the nonlinear aging-related changes in respiratory system mechanics and lung morphometry that occur in C57BL/6 mice. Therefore, these data establish an evidence base to direct future work investigating aging-related changes in respiratory physiology in mice. Although aging C57BL/6 do not perfectly emulate human aging, this mouse strain is supported by the USA National Institute on Aging, and, therefore, is commonly used in aging-related research.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**


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