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Restrained whole body plethysmography for measure of strain-specific and allergen-induced airway responsiveness in conscious mice

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Lofgren, Jennifer L. S., Melissa R. Mazan, Edward P. Ingenito, Kara Lascola, Molly Seavey, Ashley Walsh, and Andrew M. Hoffman. Restrained whole body plethysmography for measure of strain-specific and allergen-induced airway responsiveness in conscious mice. *J Appl Physiol* 101: 1495–1505, 2006. First published July 20, 2006; doi:10.1152/jappphysiol.00464.2006.—The mouse is the most extensively studied animal species in respiratory research, yet the technologies available to assess airway function in conscious mice are not universally accepted. We hypothesized that whole body plethysmography employing noninvasive restraint (RWBP) could be used to quantify specific airway resistance (sRaw-RWBP) and airway responsiveness in conscious mice. Methacholine responses were compared using sRaw-RWBP vs. airway resistance by the forced oscillation technique (Raw-FOT) in groups of C57, A/J, and BALB/c mice. sRaw-RWBP was also compared with sRaw derived from double chamber plethysmography (sRaw-DCP) in BALB/c. Finally, airway responsiveness following allergen challenge in BALB/c was measured using RWBP. sRaw-RWBP in C57, A/J, and BALB/c mice was 0.51 ± 0.03 , 0.68 ± 0.03 , and 0.63 ± 0.05 cm/s, respectively. sRaw derived from Raw-FOT and functional residual capacity (Raw*functional residual capacity) was 0.095 cm/s, approximately one-fifth of sRaw-RWBP in C57 mice. The intra- and interanimal coefficients of variations were similar between sRaw-RWBP (6.8 and 20.1%) and Raw-FOT (3.4 and 20.1%, respectively). The order of airway responsiveness employing sRaw-RWBP was AJ > BALB/c > C57 and for Raw-FOT was AJ > BALB/c = C57. There was no difference between the airway responsiveness assessed by RWBP vs. DCP; however, baseline sRaw-RWBP was significantly lower than sRaw-DCP. Allergen challenge caused a progressive decrease in the provocative concentration of methacholine that increased sRaw to 175% postsaline values based on sRaw-RWBP. In conclusion, the technique of RWBP was rapid, reproducible, and easy to perform. Airway responsiveness measured using RWBP, DCP, and FOT was equivalent. Allergen responses could be followed longitudinally, which may provide greater insight into the pathogenesis of chronic airway disease.

murine; specific airway resistance; bronchial reactivity; forced oscillation technique; methacholine

THE USE OF MICE IN RESPIRATORY research is growing, in part, due to the rapid development of new transgenic strains. Precise measurements of airway function may be obtained using invasive technologies that control for the confounding influences of lung volume (i.e., volume history, lung volume during measurement) and respiratory frequency (6). However, an important place remains for the in vivo study of conscious mice, where the influences and risks of anesthesia are absent, and

longitudinal studies are desired. Currently, a problem with the study of conscious mice is the lack of widely accepted techniques for pulmonary function testing. The available methods each have advantages and disadvantages that relate to 1) ease of the procedure, 2) animal tolerance, 3) precision and validation, and 4) their basis in known physical determinants of airway function (i.e., pressure and flow). Recently, concern has been expressed over the widespread use of unrestrained technology to characterize “airway function” per se (5, 6, 28, 41, 50). These opinions were formed after several studies failed to corroborate data derived from unrestrained whole body plethysmography (UWBP) with more rigorous invasive techniques (1, 40, 43, 47). Since UWBP is a commonplace application, this has left many users searching for practical alternatives (33). Several alternative methods have been studied that do provide more direct measures of airway mechanics. Midtidal expiratory flow (EF₅₀) has been evaluated extensively for the ability to characterize bronchoconstriction in conscious mice (21, 22, 24, 55). Outcomes of EF₅₀ have correlated well with simultaneous invasive measures of pulmonary resistance and dynamic compliance during allergen, cholinergic agonist, and hyperoxia challenges (21, 23, 24). While EF₅₀ permits the monitoring of tidal breathing flow limitation, one can only infer airway resistance (Raw) from plethysmographically derived flow. Specific Raw (sRaw), the product of Raw and lung volume, could provide greater insight into airway mechanics (18, 20). This variable can be measured using double-chamber plethysmography (DCP) (42), although there are practical limitations, such as the use of a neck seal and complex restrainer (20). The reproducibility of airway responsiveness derived from DCP has also been challenged (12), and strain-specific responses to methacholine (MCh) were discordant with more invasive methods (16). For these reasons, the current method to measure sRaw using DCP is not widely cited. Transfer impedance (Ztr) is yet another conscious method, which is used to probe central (airway) vs. peripheral (tissue) contributions to bronchoconstriction and permit serial measurements over time (30). One limitation of Ztr is the need for acclimation to obtain acceptable coefficients of variation (CVs), although this can be obtained after 1 day of conditioning the mice to the instrument. In sum, each available system provides a different level of user satisfaction and certainty regarding the status of the airways (6), and therefore the development of novel systems that improve upon this spectrum of technologies should continue.

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We investigated the use of restrained whole body plethysmography (RWBP), an adaptation of the original method of body plethysmography (pressure plethysmography) in humans (15) that was later used in guinea pigs (3). Mice are fast breathers (3–6 Hz), so special considerations concerning plethysmographic design and validation were addressed. The intent of this study was to provide an initial proof of concept for RWBP in conscious mice and thus stimulate future applications and comparisons with alternative systems. We hypothesized that 1) baseline sRaw-RWBP and associated MCh responses would be similarly reproducible to Raw-forced oscillation technique (FOT), 2) values for sRaw measured with RWBP (sRaw-RWBP) would be comparable to sRaw derived from a combination of Raw from the FOT (Raw-FOT) and Boyle's law plethysmography to obtain functional residual capacity (FRC), or DCP to obtain an alternative conscious sRaw (sRaw-DCP), 3) strain-specific airway responsiveness would be accurately characterized by RWBP, and lastly, 4) RWBP could be used to characterize longitudinally the increase in airway responsiveness associated with chronic (10 wk) allergen challenge in a single group of BALB/c mice.

METHODS

The experimental protocol followed National Institutes of Health guidelines and was approved by the Institutional Animal Care and Use Committee at Tufts University Cummings School of Veterinary Medicine (IACUC Protocol G670–04).

Animals. Pathogen-free female C57BL/6 ($n = 18$) (Charles River Laboratories, Wilmington, MA), A/J ($n = 18$), and BALB/c ($n = 59$) (Jackson Laboratories, Bar Harbor, MA), purchased at 8–10 wk of age (19–22 g), were used for this experiment. Each mouse was individually identified; they were housed in cages in groups of four to five in an Association for Assessment and Accreditation of Laboratory Animal Care accredited facility that provided only HEPA filtered air. Food and water free of ovalbumin (OVA) were provided ad libitum. A sentinel program ruled out the presence of any of the following infectious agents in the housing area of the mice during the study: parvoviruses (mouse parvovirus-1, mouse parvovirus-2, microvillus membrane, nonstructural protein-1), Sendai virus, pneumonia virus of mice, mouse hepatitis virus, Theiler's murine encephalitis virus, reovirus, *Mycoplasma pulmonis*, and mouse rotavirus (epizootic diarrhea of infant mice).

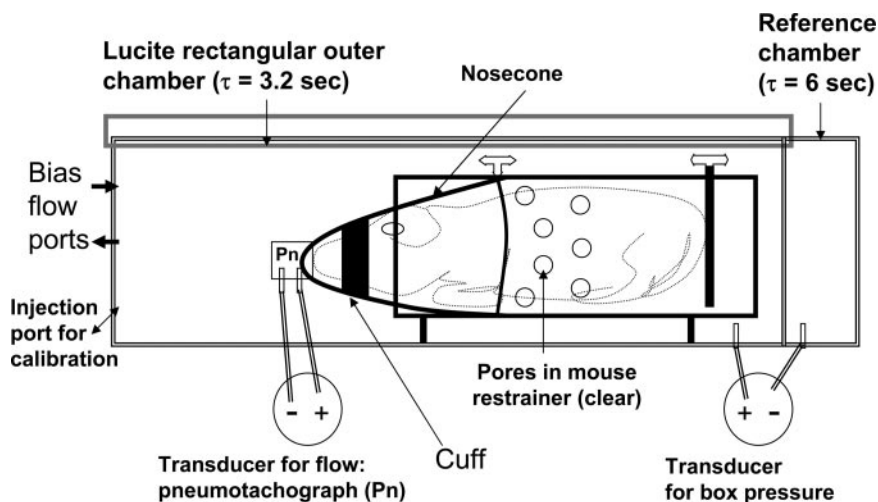
Study design. In A/J, C57, and BALB/c mice, the responses to MCh aerosol were measured using RWBP and the FOT. In BALB/c mice,

we additionally used DCP. Finally, we measured MCh responses in the BALB/c mice after allergen sensitization (intraperitoneal) and aerosol challenges.

Physical properties of the RWBP. The custom-built whole body pressure plethysmograph (RWBP) consisted of clear Lucite outer chamber walls (5.8 cm height $\times$ 7.7 cm width $\times$ 20.2 cm length = 902 ml, thickness 12 mm). The outer chamber held an inner restraint chamber (e.g., nose cone restrainer, Kent Scientific, Torrington, CT) (Fig. 1). The restraint chamber was modified by drilling several holes (2–3 mm) into the wall, overlying the region where the thorax and abdomen of the mice lie.

The dynamic properties of the plethysmograph were studied by varying pressures in the chamber (32). A leak (τ 3.2 s to 36% peak pressure) was created by a Luer connector mounted in the wall of the box, which was attached to a length of tubing, a stopcock, and needle (22 g, 2 cm). The leak resistance was measured to be 5.37 $\text{cmH}_2\text{O} \cdot \text{ml}^{-1} \cdot \text{s}$ and inductance 0.029 s^2/ml . A step pressure created by 1) hammering gently the plunger of a syringe loaded to an expected volume (0.02 ml), and 2) balloon burst both peaked between 19 and 21 ms, with a thermal time constant (described by a single compartment) of 0.95 s. Amplitude of box volume (V_{box}) was examined as a function of input frequency using digitally controlled square and sinusoidal flows delivered with a piston-driven mouse ventilator (flexiVent, Scireq, Montreal, Canada). V_{box} amplitude remained within 3% of the delivered volume (0.1 ml) from 1 to 10 Hz, and within 0.075% between 2 and 5 Hz, the range most relevant to breathing in conscious mice. Therefore, the conditions were adiabatic across these frequencies. Time shifts between flow, delivered via the pneumotachograph within the box, and peak box pressure averaged <3 ms (range 1–6 ms) from 0.5–19.5 Hz generated using a broadband input generator (flexiVent, Scireq). Pressure in the plethysmographic chamber was sampled using a low-range (± 10 cmH_2O) differential pressure transducer (TRD 5700, Buxco Electronics, Wilmington, NC) referenced to a chamber open to atmosphere ($\tau = 6$ s). A pneumotachograph (8431 series, Hans Rudolph, Kansas City, MO) with dead space of 0.3 ml, heated to 38–39°C, was custom fitted via O-ring assembly to the proximal port of the nose cone for measurement of flow and flow-derived parameters. Data were sampled at 2,500 Hz per channel using commercial hardware (Max1420 Buxco Electronics, PCI 6024E, National Instruments) and software (Biosystem XA version 2.7.4, Buxco Electronics). The pneumotachograph was calibrated by integration of the flow, injected as a known volume (0.5 ml). V_{box} derived from box pressure was calibrated by rapid injection of a known volume (0.1 ml) into the chamber, between each mouse experiment, and checked repeatedly using injections of varying volumes and quasi-sinusoidal inputs. Bias flow (0.5 l/min) was employed

Fig. 1. Schematic of restrained whole body plethysmography (RWBP). The RWBP consisted of a clear Lucite restrainer and plethysmographic chambers with assorted ports for bias flow, calibration, and leak testing. Mice entered the rear of the mouse restrainer and were then pushed forward using the adjustable backplate until the nose engaged the inner surface of the cuff (see METHODS for leak testing data). The nose cone was attached to a heated fiber screen pneumotachograph. The lid was closed, and the box periodically vented during the first 30–60 s of data collection to counteract thermal drift, if necessary. Bias flow (0.5 l/min) was introduced between data recordings. The mouse and mouse restrainer were removed only for aerosol challenges.



between recordings, and the box vented fully between MCh challenges.

Measurement of sRaw using RWBP. For measurements using RWBP, mice were loaded into the restrainer from the rear and using the mobile back plate, were pushed forward until their heads were straight and muzzles in contact with the inner wall of a rubber O-ring (i.e., "cuff") that was used to prevent leak.

The amount of leak around the muzzle was investigated in a pilot study involving BALB/c female mice ($n = 11$, 20–26 gm). A steady airflow (30 ml/s) was delivered simultaneously via Y piece to (1) the mouse assembly (i.e., pneumotachograph, nose cone, and mouse), and (2) a parallel "shunt" pathway opened to atmosphere. Flow through the shunt pathway was measured using a separate calibrated pneumotachograph. After total flow was measured through the shunt pathway, the other pathway was attached to the mouse assembly. The proportion of flow lost to the mouse pathway was considered the percent leak.

The mean ($\pm$ SD) reduction in shunt flow was $0.93 \pm 0.97\%$ (range 0 to 2.6%), indicating that the flow resistance through the cuff area was on average >100 times greater than the shunt pathway. Thus the leak in the cuff contributed to $<1\%$ error in the measurement of flow or sRaw.

The Vbox-flow (x - y) plots were constructed post hoc from the primary signals using commercial software (Acknowledge v. 3.7.3, Goleta, CA). Primary waveforms (Vbox, flow) were reviewed and the time-period indicative of peak responses to MCh was identified by repeated sampling. Peak responses to MCh were found in pilot studies to occur between 1.5 and 2.5 min after the initiation of exposure to MCh. The slope (θ) of the Vbox-flow plot was measured manually using a protractor (accurate to 0.5°) on the straightest possible segment between 1 to -1 ml/s (i.e., the left side of the loop). Examples of a complete bronchoprovocation and the derivation of sRaw (sRaw-RWBP) from primary signals are shown in Fig. 2A. The straight segment corresponded to the rapid (10–12 ms) transition from expiration to inspiration where volume shift was negligible. This technique has been shown to minimize the contribution of heating and humidification to the box pressure (3, 4), and sRaw derived from this period was equivalent in panting and nonpanting human subjects (35). Gains were set to produce angles (tangents) between 40 and 75° . Breaths with evidence of laryngeal braking were avoided (56). sRaw-RWBP was computed from the tangents as follows:

$$\text{sRaw-RWBP} = (1/\tan\theta) * (\text{Patm} - \text{PH}_2\text{O}) * \text{Cf} * (\text{Vbox} - \text{bwt mouse})/\text{Vbox} \quad (\text{Ref.2})$$

where Patm is atmospheric pressure, PH_2O is water vapor pressure, Cf is a scaling factor for the x - and y -axes, Vbox is total volume of the outer chamber, and bwt of mouse is body weight (g).

One major concern with the use of an unheated plethysmograph was the potential for errors to arise in the computation of sRaw, from heating and humidification of inspired gas, which has been shown to cause the box pressure amplitude to nearly double (39, 40, 57). We explored this issue by performing a pilot experiment with conscious mice (BALB/c female, $n = 5$, 21.6–27.1 g), whereby sRaw was determined in the heated and humidified (37°C , 90% humidity) vs. unheated (26°C , 66% humidity) box. Calibrations were performed before each measurement, and mice were removed from the restrainers between measurements. In the unheated box, the amplitude of Vbox was only 10% greater, suggesting that there is a significant amount of gas conditioning (heating and/or humidification) while under restraint for RWBP (Table 1). sRaw did not change with heating of the box, although the loops were tighter with heating of the plethysmograph (Fig. 2B). Hence, the measurement of the sRaw based on the tangent as described was repeatable, despite slight differences in gas conditioning between heated and unheated conditions. In contrast to RWBP, we found in a separate pilot study in BALB/c mice ($n = 10$, 18.6–25.3 g) similar to Lundblad et al. (40) that Vbox

doubled when mice were left unrestrained in the plethysmograph (Table 1). Hence, the use of RWBP to obtain a tangent for computation of sRaw appeared to be relatively free from errors associated with gas conditioning, at least to the extent that could be measured at baseline before any provocations.

DCP. The DCP was purchased from a commercial source (PLY3351, Buxco Electronics, Wilmington, NC). The techniques for measurement with this technique have been described previously (12, 20). Briefly, the difference between RWBP and DCP is that RWBP isolates nasal flow and DCP isolates both nasal and thoracic flow (i.e., they are separated). Hence the measurement of DCP relies on the comparison of phase and magnitude of the nasal and thoracic flows, whereby RWBP relies on comparison between nasal flow and box pressure. For DCP, the flow for each chamber (nasal and thoracic) of the DCP was calibrated separately by rapid injection of a known volume (0.5 ml) into the chamber; volume was matched by the integration of flow. The accuracy of calibration was checked volumetrically before the each mouse was placed in the chamber. An AC offset was used to condition each signal (nasal, thoracic) to compensate for the bias flow in the nasal chamber. The phase lag between the chambers in the absence of a mouse was negligible (<0.01 ms up to 10 Hz). For DCP, mice were loaded into the rear of the thoracic chamber and pushed forward until the head protruded through a hole in a latex neck seal provided with the equipment. Four different sized neck seal openings were used (0.6–1.0 mm), with the smallest size that did not diminish peak flow or minute ventilation ($\dot{V}_E$) (20) employed for measurements. Once the mouse was secured within the thoracic chamber with the head protruding through an appropriate neck seal, the nasal chamber was attached, and bias flow (0.5 l/min) initiated. For measurements, the bias flow was turned off temporarily to maximize signal to noise. Computation of sRaw measured with DCP (herein "sRaw-DCP") followed protocols established by Pennock et al. (42) and later applied to mice by Flandre et al. (20), whereby the time lag (dT) between the thoracic and nasal flow at zero crossing (during transition between inspiration and expiration) was utilized as follows:

$$\text{sRaw-DCP} = (\text{T}_i + \text{T}_e)/(2\pi) \times (\text{Patm} - 47) \times 1.362 \times \pi \times dT/(\text{T}_i + \text{T}_e)$$

where T_i and T_e are inspiratory and expiratory time (s), respectively, and Patm is in cmH_2O . The peak dT was identified, and 10 sequential breaths free from movement artifacts were measured for that period.

Measurement of respiratory system impedance using the FOT. Methods for the FOT, including calibration techniques, followed previous publications (25, 54). Briefly, mice were anesthetized with ketamine (50–75 mg/kg) and xylazine (5 mg/kg) (Butler, Dublin, OH) intraperitoneally, and a tracheostomy was performed with a 19-g cannula (Becton Dickinson, Franklin Lakes, NY). Once anesthesia was confirmed by lack of response to toe pinch, mice were paralyzed with pancuronium (1 mg/ml, Baxter Healthcare, Irvine, CA). Supplemental ketamine (25 mg/kg ip) was provided every 0.5 h. Ventilation was set at frequency of 200 breaths/min, tidal volume (V_T) 0.3 ml, positive end-expiratory pressure 3.0 cmH_2O , and inspired oxygen was supplemented at all times. Repeated measurements were performed using a commercial data acquisition system for input impedance between 0.5 and 19.5 Hz (Quick Prime 3 analyzer, FlexiVent System, SCIREQ, Montreal, Quebec). A constant-phase model (25) was employed to compute Raw ("Raw-FOT"), in addition to tissue resistance (G_{ti}) and elastance (H_{ti}) coefficients. Baseline Raw, G_{ti} , and H_{ti} were tabulated, but the MCh responses using G_{ti} and H_{ti} were not used for comparisons with conscious measurements; only Raw was used for this purpose. To derive a value of sRaw (i.e., $\text{Raw} \times \text{FRC}$) from FOT for comparison with conscious sRaw-RWBP, we measured FRC using the Boyle's law method in a separate group of similarly anesthetized-tracheostomized female C57 mice ($n = 30$, 19–22 g) using a commercial plethysmograph (PLY 3111, Buxco Electronics), data acqui-

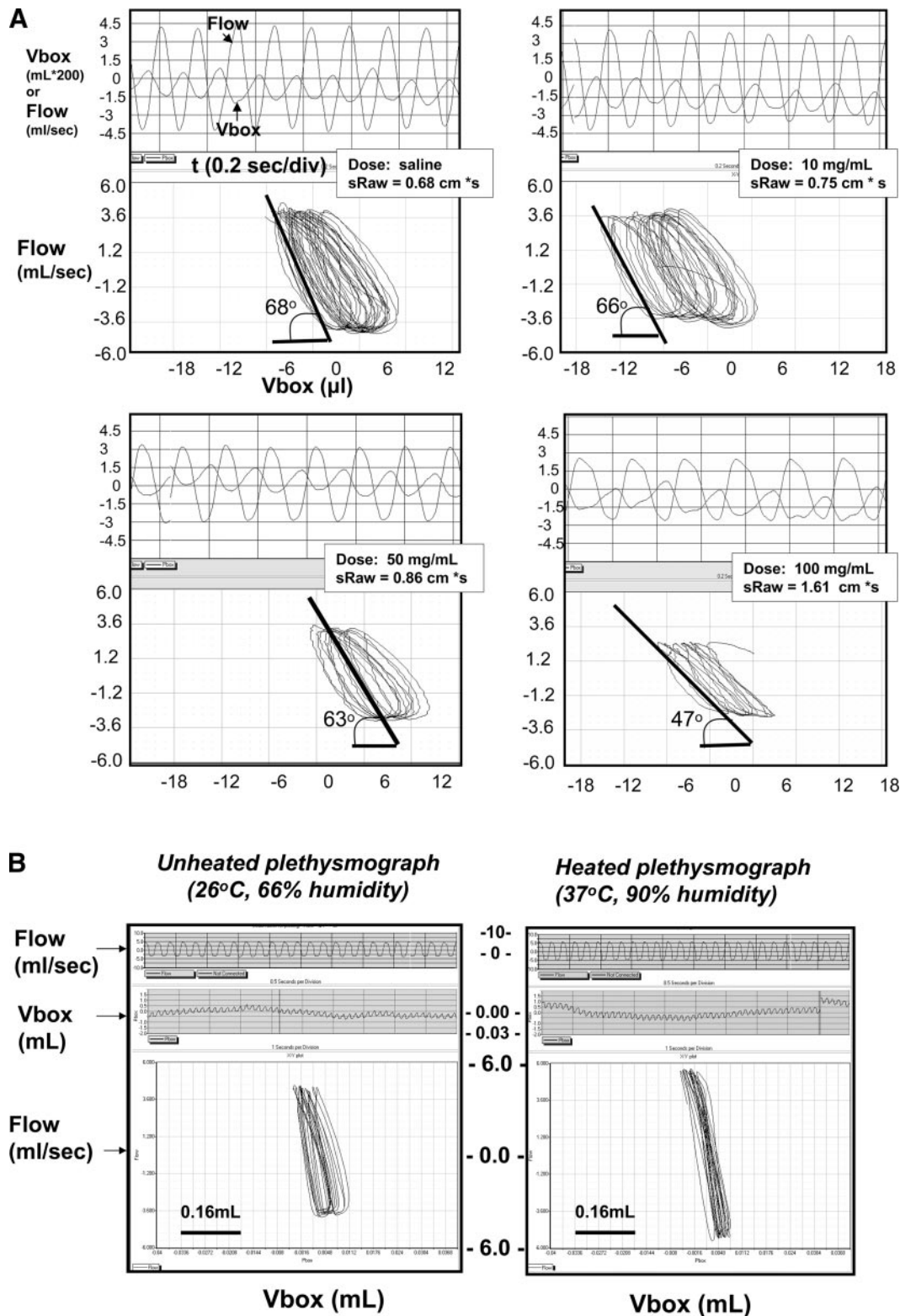


Fig. 2. A: derivation of specific airway resistance (sRaw) from primary data in a C57BL/6J mouse. Shown for each dose of methacholine (MCh) are strip charts for pneumotachograph flow and plethysmographic volume. Below each strip chart are the corresponding plots of flow (y-axis) and box volume (Vbox) shift (x-axis). Units for x- and y-axes are shown in the top left plot ("postsaline"). Also shown are the average slopes obtained for each dose of MCh in this mouse. As described in the text, sRaw was computed as follows: $sRaw = 1/\tan \theta * (P_B - P_{H_2O}) * C_f * (V_{pleth} - bwt/V_{pleth})$, where $\tan \theta$ is tangent of angle shown in radians, P_B is barometric pressure, P_{H_2O} is water vapor pressure, C_f is the scale factor of printout used for measurement of angle (2.0 s), V_{pleth} is total volume of plethysmograph (900 ml), and bwt is body weight of mouse in grams. B: effect of heating and humidification of the plethysmograph (RWBP) on the appearance of xy plots in a BALB/c mouse. Measurements can be found in Table 1. Heating caused significant increases in tidal volume, minute ventilation ($P < 0.05$), and peak inspiratory flow (PIF) ($P = 0.06$). There was less evidence of xy looping when the box was heated, but these changes did not affect the values of sRaw.

Table 1. Effects of heating the plethysmograph to body temperature (37°C) on flow-derived parameters, amplitude of box pressure signal, and sRaw

Variable	Units	Unheated Restrained (n = 5)	Heated Restrained (n = 5)	P Value	Unheated Restrained (n = 10)	Unheated Unrestrained (n = 10)	P Value
f	breaths/min	298 (20.1)	316 (33.4)	0.08	304 (27)	367 (63)	0.001
V _T	ml	0.304 (0.025)	0.352 (0.018)	0.03			
V _E	ml/min	91 (6.7)	109 (10.8)	0.04			
PIF	ml/s	4.03 (0.315)	4.75 (0.53)	0.06			
PEF	ml/s	4.46 (0.23)	4.78 (0.72)	NS			
sRaw	cm/s	0.576 (0.112)	0.592 (0.089)	NS			
ΔV _{box}	ml	0.0098 (0.0019)	0.0089 (0.0012)	NS	0.0135 (0.0007)	0.0278 (0.002)	0.001

Values are means (SD); n, no. of mice. Additionally shown are the effects of removing the mice from the restrainer and placing them in the open plethysmograph on respiratory frequency (f) and box pressure (V_{box}). V_{box} (in ml) and flow (ml/s) were recalibrated before each perturbation. V_T, tidal volume; V_E, minute ventilation; PIF, peak inspiratory flow; PEF, peak expiratory flow; sRaw, specific airway resistance; Δ, change; NS, not significant.

sition system (XA Biosystem 2.7.4, Buxco Electronics), and standard calibration routines.

Reproducibility of baseline (unprovoked) measurements. A subset of C57BL/6 mice (n = 12) used for RWBP and FOT were initially used to examine reproducibility (within animal, within group) of sRaw-RWBP. Several recordings were made over 45 min, on a separate day in the AM and PM (10 min each), and on a third day for 10 min. For each time point, an average of 10 breaths selected at random was used for analysis. The results were expressed as CV and 95% confidence intervals (see *Statistical analyses*). The within-group CV was also compared between strains of mice.

MCh challenges in conscious mice. Challenges were conducted at the same time of day (morning or afternoon) for each mouse. Ambient temperatures in the laboratory ranged 21–23°C and humidity 25–45%. Baseline recordings were obtained after 2 min acclimation to the chamber. For aerosol exposures, the mice within their restraint chambers were detached from the pneumotachograph and attached to an adjacent enclosure setup for nose-only exposures. For RWBP, aerosols were generated with an ultrasonic nebulizer (Aerogen, Aeroneb, Dangan, Galway, UK), and directed through the T piece using a low-flow (470 ml/min) regulator, thus permitting the mice to breathe a standardized aerosol concentration (reported mass median diameter = 3.1 μm). Following each exposure, mice were returned within 20 s to the RWBP, and the recording of data resumed for 5 min. For DCP, aerosols were delivered from the Aerogen nebulizer directed via stopcock into the nasal chamber. Bias flow caused the aerosol to traverse the nasal chamber at the level of the nares, assuring exposure to the aerosol. Doses of MCh were chosen that induced, on average, an increase in sRaw-RWBP to 300% baseline. Specifically, MCh (Provacholine, Methapharm, Brantford, Ontario) was nebulized for 60 s to C57 at concentrations of 0 (i.e., saline), 10, 50, and 100 mg/ml, in A/J mice at 0, 1, 5, and 10 mg/ml, and in BALB/c mice at 0, 6.25, 12.5, and 25 mg/ml (pre- and post-OVA exposure). During the comparison of mouse strains (A/J, C57, and BALB/c) using RWBP, the above doses were delivered without stopping criteria. However, during the comparison of techniques (RWBP, DCP, and FOT) and following allergen challenge in BALB/c mice, we stopped MCh challenge when EF₅₀ to ≤1 ml/s, since these mice were expected to become more responsive over time, and it was therefore deemed unsafe to give all doses of MCh. The provocative concentration that caused sRaw to increase to >175% postsaline value was computed by log-linear interpolation across the final two concentrations of MCh employed for each mouse, according to Sterk et al. (51). The provocative concentration of MCh that increased sRaw to 175% postsaline values (ED₁₇₅) was used as an index of airway responsiveness.

MCh challenges using the FOT. Aerosols were delivered directly into the tracheal cannula during lung inflation during mechanical ventilation (flexiVent, Scireq, Montreal, Quebec). Ten-second nebulization periods were used, followed immediately by a series (8–15) of measurements. The lung was inflated to total lung capacity (30

cmH₂O airway pressure) once after each aerosol delivery. Forced oscillations during apnea (3 s in duration) were applied every 17 s for 5 min. Dosage ranges were predetermined in pilot studies to evoke between 10 and >75% increase in Raw. MCh in C57 mice was delivered at 0 (saline), 4, 8, and 16 mg/ml, and in A/J we used 0, 2, 4, and 8 mg/ml. The concentration of MCh that provoked an increase in Raw-FOT to 175% baseline (ED₁₇₅) was determined by log-linear interpolation as described above.

Allergen sensitization and aerosol exposure of BALB/c mice. A subset of the BALB/c mice (n = 9) were immunized and sensitized to OVA using a procedure modified from past studies (10, 36, 37, 53). Briefly, mice received an intraperitoneal injection of 50-μg OVA (grade V, 98% pure, Sigma Aldrich, St. Louis, MO) precipitated in aluminum hydroxide and magnesium hydroxide (Imject Alum, Pierce, Rockford, IL) 14 and 7 days before inhalational exposure. Mice were then exposed to 2.5% aerosolized OVA for 30 min, three times a week for a total of 10 wk, and on the 5th and 10th wk, MCh challenges were performed 48 h after OVA exposure. Inhalation exposures were performed using a custom-built whole body exposure system during which air was drawn through a 0.4 m³ chamber at a flow rate of 80 l/min. The OVA solution was delivered into the chamber using a Pari LC JET fine-particle nebulizer (Pari, Paris, France), delivering particles with reported mean median diameter of 1.6 μm, and compressor (model NE-CO8, Omron Healthcare, Vernon Hills, IL). The mass concentration of the particles within the breathing zone of the mice was continuously monitored using a laser photometer (SidePak, AM510, TSI, Shoreview, MN). Flow from the compressor was regularly adjusted throughout the exposure period to keep the OVA concentration between 15 and 20 mg/m³.

Statistical analyses. The intra-animal and within-group reproducibility of noninvasive sRaw vs. invasive Raw were expressed as CV and 95% confidence limits, and time effects were tested using repeated-measures ANOVA. The MCh responses were analyzed using repeated-measures ANOVA. The effect of mouse strains on sRaw-RWBP, sRaw-DCP, Raw-FOT, or indexes of airway reactivity (ED₁₇₅) was analyzed using ANOVA. Pairwise comparison between strains was performed using Student's *t*-tests. Paired tests were employed to test the effect of allergen on ED₁₇₅ in BALB/c mice. Significance was attributed to data when *P* < 0.05. All values are expressed as means ± SE, except where indicated.

RESULTS

Reproducibility of sRaw using RWBP. In the group of C57 mice used to study repeatability, there were no significant changes in sRaw-RWBP within the 45-min measurement periods, between AM and PM measurements, or between different experimental days (Table 2). The mean intra-animal CV over the short term (i.e., 3 measurements evenly spaced over

Table 2. Specific airway resistance measured repeatedly in a group of conscious C57 mice ($n = 12$) using restrained whole body plethysmography

Time	0 min	10 min	30 min	45 min	Day 1 AM	Day 1 PM	Day 2	Day 3
Mean sRaw, cmH ₂ O/s	0.573	0.609	0.544	0.564	0.619	0.556	0.602	0.645
SD sRaw, cmH ₂ O/s	0.101	0.100	0.132	0.128	0.095	0.127	0.088	0.052
Coefficient of variation*, %	17.6	16.5	24.3	22.7	15.4	22.8	14.6	8.1
95% CI, cmH ₂ O/s	0.057	0.057	0.075	0.072	0.054	0.072	0.05	0.030

CI, confidence interval. *Within group (within time period).

45 min) was 6.8% for sRaw-RWBP and for Raw-FOT it was 3.4% (for 3 successive measurements). The mean intra-animal CV for Raw-FOT was significantly lower ($P < 0.05$). The mean intra-animal CV in the longer term (across days 1, 2, and 3) was 22.8% for sRaw-RWBP. The inter-animal CV for sRaw-RWBP for this set of C57 mice on days 1, 2, and 3 were 26, 27, and 14%, respectively (mean 22.3%). For the subsequent groups used in MCh challenges, the interanimal group CV for sRaw-RWBP was 26.5% for C57, 17.2% for AJ, and 30.2% in BALB/c mice (pre-OVA). In comparison, interanimal CV for Raw-FOT was 19.5% for C57 and 20.7% for AJ. Therefore, the reproducibility of these techniques was very similar.

Comparison between sRaw-RWBP and sRaw-FOT. The measurement of sRaw by RWBP at baseline in C57 mice was compared with sRaw derived from 1) Raw-FOT and 2) plethysmographically derived FRC also in anesthetized intubated mice. We measured an average FRC of 0.27 ml, which is close to published values in C57 (0.25 ml) (38). Hence, sRaw-FOT was computed as follows: Raw-FOT $\times$ FRC, or $0.353 \text{ cm H}_2\text{O} \cdot \text{ml}^{-1} \cdot \text{s} \times 0.27 \text{ ml} = 0.09531 \text{ cm/s}$. Therefore, sRaw-FOT was approximately one-fifth of sRaw-RWBP.

Strain-specific airway responsiveness. Measurement of sRaw-RWBP took between 3 and 6 min, including loading, and MCh challenges took between 16 and 30 min, depending on airway responsiveness. Baseline sRaw-RWBP was significantly lower ($P < 0.001$) in C57 compared with AJ or BALB/c (Table 3). Baseline Raw-FOT was not different between AJ ($0.35 \pm 0.12 \text{ cm} \cdot \text{ml}^{-1} \cdot \text{s}$), C57 ($0.35 \pm 0.16 \text{ cm} \cdot \text{ml}^{-1} \cdot \text{s}$), and BALB/c ($0.30 \pm 0.06 \text{ cm} \cdot \text{ml}^{-1} \cdot \text{s}$) mice. The mean $\pm$ SE FRC determined in a separate group of C57 mice was $0.265 \pm 0.009 \text{ ml}$. These values were used to construct sRaw-FOT. MCh caused a significant dose-dependent increase in sRaw-RWBP (Fig. 3A). Accompanying the change in sRaw-RWBP was a significant decrease in respiratory frequency but no significant change in V_T for any strain of mice (Fig. 3, B and C). MCh

caused a dose-dependent increase in Raw in all three strains of mice (Fig. 4). Airway responsiveness was significantly different between AJ, C57, and BALB/c mice for sRaw-RWBP, and the descending order of reactivity was $\text{AJ} > \text{BALB/c} > \text{C57}$ (Table 4). Using Raw-FOT, there was no difference between C57 and BALB/c ED₁₇₅, but both strains had a significantly higher ED₁₇₅ than AJ. Therefore, airway reactivity in descending order by FOT was $\text{AJ} > \text{BALB/c} = \text{C57}$. A comparison of MCh responses between methods using a single strain of mouse (BALB/c) and standard doses for MCh is shown in Fig. 5. There was no difference in ED₁₇₅, derived for RWBP, DCP, and FOT. However, the percentage change in sRaw-RWBP or sRaw-DCP was significantly higher than the percent change in Raw-FOT. Baseline sRaw-RWBP ($0.63 \pm 0.05 \text{ cm/s}$) was significantly lower ($P < 0.001$) than mean sRaw-DCP ($1.82 \pm 0.11 \text{ cm/s}$).

Allergen responses. In BALB/c mice exposed to OVA for 5 or 10 wk, there was no significant change ($P > 0.1$) in baseline sRaw (mean $\pm$ SD: pre-OVA: 0.75 ± 0.12 , 5-wk OVA: 0.66 ± 0.16 , 10-wk OVA: $0.79 \pm 0.26 \text{ cm/s}$). There was a significant ($P = 0.001$) decrease in ED₁₇₅ following 5 and 10 wk of OVA challenge in immunized mice (Fig. 6).

DISCUSSION

In this study, we demonstrate for the first time the application of whole body plethysmography (WBP) in conscious mice, as originally described by DuBois et al. (15). WBP akin to the technique performed in humans for measurement of sRaw was first adapted for animals by Agrawal (3), who used conscious guinea pigs (3, 19, 26). More recently, WBP for direct determination of sRaw has been employed in dogs (8) and sheep (7) using a head-out configuration. These studies demonstrate that WBP holds promise for characterization and longitudinal studies of airway mechanics and airway responsiveness in conscious animals. Measurement of sRaw appears

Table 3. Baseline measurements of conscious and invasive physiology by strain of mice

Strain	RWBP								FOT		
	sRaw, cmH ₂ O/s	f, breaths/min	V_T , ml	$\dot{V}_E$, ml/min	PEF, ml/s	PIF, ml/s	T_I , s	T_E , s	Raw, cmH ₂ O $\cdot$ ml ⁻¹ $\cdot$ s	Gti, cmH ₂ O/ml	Hti, cmH ₂ O/ml
C57	0.509	357.7	0.281	100.3	4.90	4.36	0.089	0.082	0.35	5.28	28.84
	± 0.033	± 0.02	± 0.014	± 0.02	± 0.02	± 0.02	± 0.03	± 0.01	± 0.02	± 0.92	± 6.72
A/J	0.676*	277.9*	0.245*	67.7*	3.27*	2.92*	0.109*	0.108*	0.35	4.95	21.94*
	± 0.027	± 0.03	± 0.06	± 0.06	± 0.05	± 0.05	± 0.02	± 0.03	± 0.03	± 0.049	± 1.82
BALB/c	0.633*	327	0.210*	67.1*	3.25*	3.04*	0.0964	0.109*	n/d	n/d	n/d
	± 0.045	± 5.5	± 0.007	± 2.79	± 0.13	± 0.12	± 0.0018	± 0.045			

Values are means $\pm$ SE. Values shown were derived from C57 ($n = 18$), AJ ($n = 18$), and BALB/c ($n = 18$) mice. Methods used were restrained whole body plethysmography (RWBP) and the forced oscillation technique (FOT). T_I , duration of inspiration; T_E , duration of expiration; Raw, resistance of airways; Gti, tissue resistance; Hti, tissue elastance; n/d, not determined. *Significant difference from C57 mice ($P < 0.05$).

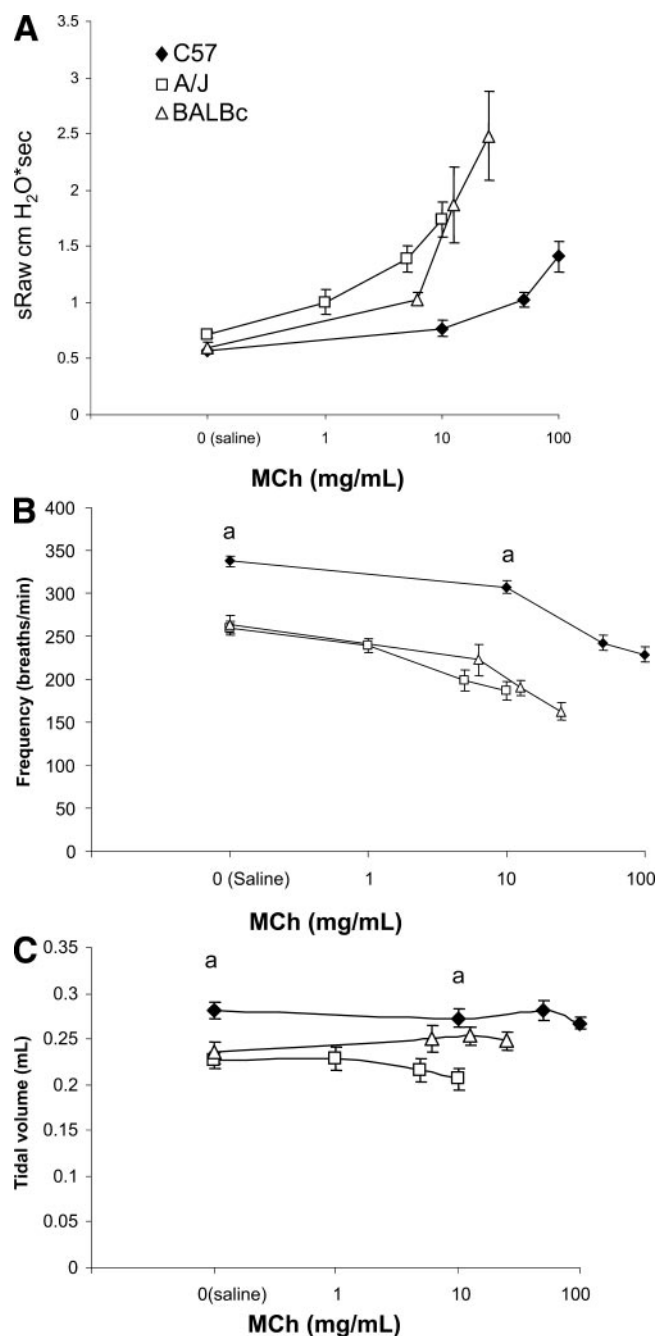


Fig. 3. Log-linear dose response curves in response to MCh in awake C57 ($n = 18$, ◆), A/J ($n = 18$, □), and BALB/c ($n = 18$, △) mice ($P < 0.05$). ^aStatistical significance between C57 and A/J at equivalent doses of MCh. A: sRaw. B: breathing frequency. C: tidal volume.

to be an appropriate end point for MCh responses, since it combines information related to Raw and lung volume, both of which can be modified during tidal breathing after bronchoconstriction.

Advantages of RWBP may include the ease of loading, nose-only exposure, direct nonplethysmographic measurement of flow, and the lack of a neck seal that may constrict the airway or impair loading. For use of RWBP, none of the mice required acclimation to complete several sets of baseline measurements and one or more bronchoprovocations, which may

be an additional advantage. While we did not specifically measure indexes of stress directly, and therefore it is not possible to comment on their physiological responses to RWBP, the mice were active, grooming, and appeared unharmed each time they were removed from the chamber.

Critique of the plethysmographic device (RWBP). The size of the box (902 ml, or 45 ml/g) was relatively large compared with past studies employing pressure plethysmographs in guinea pigs or mice (49, 56). This contributed to consistent adiabatic conditions, an acceptable feature of pressure plethysmography (32, 49). One problem was frequent drift in the baseline of the pressure signal due to warming of the chamber by reloading of the mouse. This could be avoided in future experiments by conducting the entire study without opening the chamber, i.e., aerosolizing agonists to the mice within the chamber using special delivery systems (3, 19). Incorporation of heating elements or warmed water (39, 40, 42) or decreasing the size or thickness of the chamber walls may also serve to stabilize the thermal conditions of the box interior. Alternatively, flow-type plethysmography has been described (49), which would minimize the effects of baseline thermal drift, although pose additional challenges, e.g., maintaining calibration. A significant challenge in the use of any plethysmograph is to understand the confounding influence of heating and humidification on the box pressure signal. Heating and humidification of inspired gas likely created some looping in the XY plots (31) based on our pilot studies. However, this source of looping appeared not to confound the measurement of sRaw (Table 1). Furthermore, heating of the plethysmograph caused a decrease in box pressure amplitude (i.e., V_{box}) by only 10%, suggesting that gas conditioning within the dead space in front of the mouse is significant compared with the absence of conditioned gas as in the unrestrained setting. In sum, there are several improvements that could be made to the current design to improve stability and convenience, but none of these would improve fundamental accuracy of sRaw derived by RWBP.

Reproducibility of sRaw. An understanding of test reproducibility is paramount to the application of any device used to measure pulmonary function. The intra-animal CV for sRaw-RWBP (measurements made every 15 min) in mice (6.6%) was similar to guinea pigs (26) and very similar to Raw-FOT

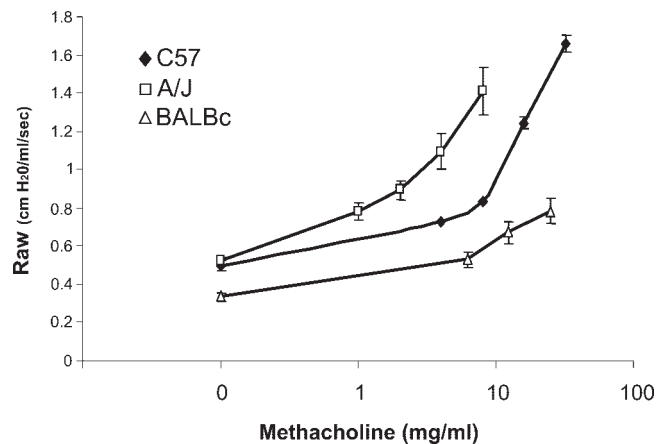


Fig. 4. Forced oscillation technique (FOT) performed in anesthetized, tracheotomized C57 ($n = 18$, ◆), A/J ($n = 18$, □), and BALB/c ($n = 10$, △) mice. Shown are saline (0 mg/ml MCh) followed by MCh concentrations, which depended on strain.

Table 4. Measures of airway responsiveness in three strains of mice

Groups	n	Raw ED ₁₇₅ , mg/ml	sRaw-RWBP ED ₁₇₅ , mg/ml	sRaw-DCP ED ₁₇₅ , mg/ml
C57	18	8.3 (6.5–10.2)	40.5† (26.9–54.2)	
AJ	18	2.3* (1.5–3.1)	4.49*† (3.1–5.9)	
BALB/c (OVA–/OVA–)	18		10.9* (7.8–13.9)	
BALB/c (OVA+/OVA–)	9	9.48 (6.2–12.8)	10.2* (7.3–13.1)	7.1* (4.9–9.3)

n, No. of mice. Airway responsiveness was measured using FOT (Raw), RWBP (sRaw-RWBP), and double-chamber plethysmography (sRaw-DCP). The provocative concentration that caused an increase in Raw or sRaw to 175% baseline (ED₁₇₅) is expressed as the mean and 95% confidence interval (in parentheses). OVA, ovalbumin. OVA–, no sensitization or exposure to OVA; OVA+/OVA–, sensitization intraperitoneally without aerosol exposure. *Significantly different from C57 mice ($P < 0.01$). †Significantly different from ED₁₇₅ for Raw within strain ($P < 0.01$).

(3.4%) as measured. Similarly, the CV for respiratory system resistance in conscious mice using Ztr was 6% (30), although this was obtained after two previous acclimation periods of measurement, each 2 h apart. The within-group CVs were equivalent for sRaw-RWBP and Raw-FOT in the multiple strains of mice in this study. In sum, sRaw-RWBP was no less reproducible at baseline or after MCh than Raw, despite the fact that the measurement was made without control of several important factors such as V_T and PEEP.

Comparison between sRaw-RWBP, sRaw-FOT, and sRaw-DCP. The measurements using FOT and FRC were employed in part to understand the validity of RWBP for measurement of airway mechanics, specifically sRaw. The baseline values for Raw in both C57 and AJ strains were comparable to previously published values (9, 25, 44) and, therefore, served as a reasonable gold standard. The mean value of sRaw-RWBP in C57 (0.51 cm/s) was about five times the value obtained for sRaw-FOT (0.095 cm/s), commensurate with the contribution from the upper airways. In support of this notion, the upper airways contributed to about four-fifths of total Raw in rats (13). The exact contribution of the upper airways to the baseline and postprovocation measurements of sRaw-RWBP were not disclosed by this study but require further study in conscious mice.

An additional approach that was employed to better understand the validity of sRaw-RWBP was the comparison with sRaw-DCP. In BALB/c mice where both techniques were

performed, sRaw-RWBP (0.63 ± 0.05 cm/s) was significantly lower than sRaw-DCP in our study (1.82 ± 0.11 cm/s) and lower than past measures of sRaw-DCP in BALB/c mice, i.e., 1.02 (20), 1.18 ± 0.4 (18), and 1.50 cmH₂O/s (12). Our values for sRaw-RWBP were also lower than sRaw-DCP in other strains of mice, i.e., 1.2 (20) or 1.44 ± 0.5 cmH₂O/s (18) in C57 and 1.68 cmH₂O/s in AJ mice (20). The basis for the large discrepancy between sRaw-RWBP and sRaw-DCP was not disclosed by our study. The higher values across the board for sRaw-DCP compared with sRaw-RWBP may relate to differences in the computation methods and the constrictive effect of the neck seal used for DCP. The computation for DCP is based entirely on nasal vs. thoracoabdominal phase lag, which may be influenced by several instrument and host factors previously reviewed by Pennock et al. (42). Another factor that may have confounded our measurements of sRaw-DCP was the tension on the neck seal. The seal must be tight enough to restrain the mouse while avoiding leak, yet loose enough to avoid constriction (20). As it is difficult to standardize the tightness of neck seals, this may introduce some additional variability in the measurements and heighten baseline values.

The use of the restraint system used for RWBP may also confound measurements of sRaw by depressing lung volumes (FRC, total lung capacity). Although the position of the rear plate was adjusted according to the size of the mouse, lung volumes may be reduced in this position. Further study em-

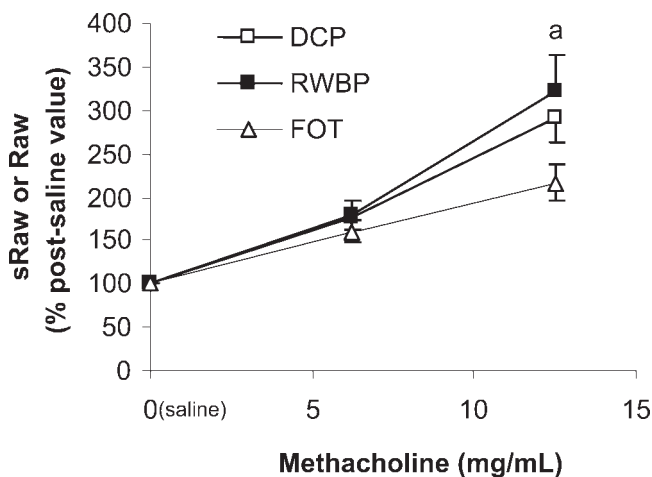


Fig. 5. Comparison of MCh responses between measurement methods. Conscious methods included sRaw-RWBP ($n = 10$) and double chamber plethysmography (sRaw-DCP, $n = 10$). Invasive method was the FOT, which produced Raw-FOT ($n = 9$). *Significant difference ($P < 0.001$) between RWBP or DCP vs. FOT.

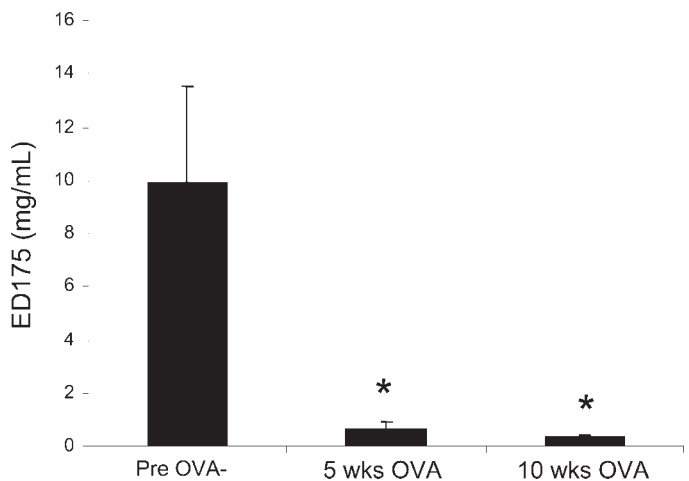


Fig. 6. Longitudinal measurements of airway responsiveness provocative concentration of MCh that increased sRaw to 175% postsaline values (ED₁₇₅) in immunized BALB/c mice ($n = 9$) before exposure to ovalbumin (OVA) aerosol and 5 and 10 wk after OVA aerosol exposures three times per week (OVA+/OVA+). *Compared with baseline, there was a significant ($P < 0.001$) reduction in ED₁₇₅ at 5 and 10 wk.

ploying imaging (e.g., computed tomography) holds promise to explore the effects of tube restraint.

Strain-specific airway responsiveness. The C57 mice exhibited significantly higher breathing frequency, minute volume, and peak flows than A/J or BALB/c mice, and higher $\dot{V}_T$ than A/J mice. The C57 strain was previously found to have higher $\dot{V}_E$ than BALB/c (20), and this was thought to reflect their greater basal metabolism, body temperature, and lower hematocrit. Breathing frequency, $\dot{V}_T$, and $\dot{V}_E$ were slightly higher in this study than several past studies employing DCP (18, 20) than head-out plethysmography (17, 21, 55) or UWBP (48), although Delorme and Moss (12) using DCP and Hamelmann and coworkers (27) using unrestrained plethysmography found similar values for frequency. We speculate that the higher ventilatory rates in this study relate to the presence of excessive dead space (0.3 ml), which cause the mice to compensate appropriately. This did not appear to complicate the process of data collection or cause harm to the mice. However, the presence of hyperpnea may have contributed to differences in MCh delivery or response to MCh due to altered lung volumes.

Airway responsiveness in the three strains of mice differed significantly (Table 3). Concerning our gold standard (FOT), MCh responsiveness paralleled past data on this subject using BALB/c mice (54). The descending order of airway responsiveness differed between RWBP (A/J > BALB/c > C57) and FOT (A/J > BALB/c = C57) largely due to relatively lower responsiveness of conscious C57 mice. The relative airway responsiveness found in this study using RWBP (A/J > BALB/c > C57) mirrored past studies that have employed invasive technologies in two or three of the same strains (11, 16, 52), as well as published data on airway reactivity (using UWBP) from the vendor for C57 and A/J mice (46). One study that compared double-chamber (sRaw) to single-chamber (enhanced pause) measures of airway responsiveness disclosed rank orders BALB/c > A/J > C57 for DCP and A/J > BALB/c > C57 as in our study for UWBP (12). The inability to characterize A/J mice as hyperresponsive, and the poor reproducibility of airway responsiveness indexes (e.g., provocative concentration of MCh that doubled resistance) were cited as major weaknesses of DCP by that study. In another study using UWBP (58), the order was AJ > BALB/c = C57 as seen in our invasive measurements. Hence there are inconsistencies in the literature with regard to the airway responsiveness of BALB/c vs. C57 mice, but most studies show that A/J mice are considerably more responsive to MCh than other strains before any sensitization or exposure to allergen. We do not have an explanation for the differences between conscious and invasive measures of airway responsiveness in our study. It is possible that conscious measurements of airway responsiveness in C57 mice, which are typically hyporesponsive, were influenced by their uniquely exaggerated $\dot{V}_E$, or another conscious factor that altered drug delivery.

To further compare RWBP to DCP and FOT for measurement of airway responsiveness, we employed all three techniques in a single strain (BALB/c) of mice. Despite different modes of delivery and concentrations of MCh employed to construct a dose-response curve, the three methods produced equivalent ED₁₇₅. Rather than imply that these methods are equally sensitive, the data would suggest that MCh delivery methods and doses have been adjusted over time by investigators to obtain a dose-response curve in nonsensitized BALB/c

mice between 0 and 30 mg/ml. If we had employed the same method of MCh delivery (e.g., intravenous route) for the same time, we might have obtained different results. For example, it would be expected that the delivery of MCh for 60 s into the lower respiratory tract of intubated mice would likely have produced greater responses, and therefore our conclusion concerning airway responsiveness as a function of technique would have been different (i.e., FOT would have detected greater airway responsiveness). Because intravenous responses to MCh are poorer than aerosolized responses, we chose the aerosol route.

Furthermore, it is impossible to determine the contribution of the nose or glottis vs. lower airways in the response to MCh. This could be a significant limitation to these methods during pharmacological testing. The higher percentage responses in the conscious animals (RWBP, DCP) vs. anesthetized intubated mice (FOT) (Fig. 5) may have resulted from upper airway constriction, either by direct stimulation of cholinergic receptors in the nasal mucosa (34, 45) or cholinergic reflexes involving the glottis, nasal cavities, or other structures following lower airway deposition of MCh (13, 14), as observed previously in rats. In one study in rats (14), frequent glottic closure was noted, and this explained the observed marked increase in expiratory Raw with lower airway deposition of MCh. We too noted frequency glottic closures (periods of zero flow at the initiation of expiration), but breaths with glottic closures, while excluded for analysis in this study, could be analyzed on the basis of the tangent immediately after glottic opening. Thus it is tempting to speculate that RWBP permitted a qualitative analysis of the "source" of expiratory resistance and, therefore, exclusion of resistance caused purely by glottic closure. Further studies are warranted to disclose the contribution of the upper airways to the responses measured using RWBP, particularly in mice where there is a paucity of such data.

Allergen responses. The effect of OVA exposure by aerosol in sensitized mice did not evoke a change in baseline sRaw (pre-OVA: 0.711 ± 0.038 , 5 wk: 0.66 ± 0.02 ; 10 wk: 0.785 ± 0.084 cm/s). Similarly, no effect was seen in baseline airway or tissue mechanics in a past study using a similar protocol comparing OVA-/OVA- to OVA+/OVA+ BALB/c mice (54). Using RWBP, there was a significant decrease in ED₁₇₅ at 5 and 10 wk, demonstrating that it was feasible to track significant changes in airway responsiveness longitudinally. The site of antigen effects was not established in this study. While OVA challenge in sensitized mice is thought to induce airway hyperresponsiveness primarily due to lower airway inflammation and remodeling, similar responses to antigen have been found in the nasal passages of mice (14, 29). Further studies are warranted to relate structural to functional responses to allergen challenge. However, it is evident from our data that RWBP offers an opportunity to perform longitudinal studies of chronic airway disease.

In conclusion, this study demonstrates for the first time the measurement of sRaw using RWBP in conscious mice. The noninvasive method of RWBP permitted highly reproducible measurements of sRaw, without acclimation to the instrument. Values for sRaw were very similar to those derived from the invasive FOT. Strain-specific and allergen-induced effects on airway reactivity were demonstrated using RWBP. The tech-

nique of RWBP holds promise for acute or longitudinal studies of airway disease in conscious mice.

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REFERENCES

- Adler A, Cieslewicz G, and Irvin CG. Unrestrained plethysmography is an unreliable measure of airway responsiveness in BALB/c and C57BL/6 mice. *J Appl Physiol* 97: 286–292, 2004.
- Agrawal A and Agrawal KP. Body plethysmographic measurement of thoracic gas volume without panting against a shutter. *J Appl Physiol* 81: 1007–1011, 1996.
- Agrawal KP. Specific airways conductance in guinea pigs: normal values and histamine induced fall. *Respir Physiol* 43: 23–30, 1981.
- Agrawal KP and Kumar A. Fall in specific airway conductance at residual lung volume in small airway obstruction. *Respir Physiol* 40: 65–78, 1980.
- Bates J, Irvin C, Brusasco V, Drazen J, Fredberg J, Loring S, Eidelman D, Ludwig M, Macklem P, Martin J, Milic-Emili J, Hantos Z, Hyatt R, Lai-Fook S, Leff A, Solway J, Lutchen K, Suki B, Mitzner W, Pare P, Pride N, and Sly P. The use and misuse of Penh in animal models of lung disease. *Am J Respir Cell Mol Biol* 31: 373–374, 2004.
- Bates JH and Irvin CG. Measuring lung function in mice: the phenotyping uncertainty principle. *J Appl Physiol* 94: 1297–1306, 2003.
- Bedenice D, Bar-Yishay E, Ingenito EP, Tsai L, Mazan MR, and Hoffman AM. Evaluation of head-out constant volume body plethysmography for measurement of specific airway resistance in conscious, sedated sheep. *Am J Vet Res* 65: 1259–1264, 2004.
- Bedenice D, Rozanski E, Bach J, Lofgren J, and Hoffman AM. Canine awake head-out plethysmography (HOP): characterization of external resistive loading and spontaneous laryngeal paralysis. *Respir Physiol Neurobiol* 151: 61–73, 2006.
- Bozanich EM, Collins RA, Thamrin C, Hantos Z, Sly PD, and Turner DJ. Developmental changes in airway and tissue mechanics in mice. *J Appl Physiol* 99: 108–113, 2005.
- Chang YS, Kim YK, Bahn JW, Kim SH, Park HW, Kim TB, Cho SH, Min KU, and Kim YY. Comparison of asthma phenotypes using different sensitizing protocols in mice. *Korean J Intern Med* 20: 152–158, 2005.
- De Sanctis GT, Singer JB, Jiao A, Yandava CN, Lee YH, Haynes TC, Lander ES, Beier DR, and Drazen JM. Quantitative trait locus mapping of airway responsiveness to chromosomes 6 and 7 in inbred mice. *Am J Physiol Lung Cell Mol Physiol* 277: L1118–L1123, 1999.
- DeLorme MP and Moss OR. Pulmonary function assessment by whole-body plethysmography in restrained vs. unrestrained mice. *J Pharmacol Toxicol Methods* 47: 1–10, 2002.
- DiMaria GU, Wang CG, Bates JH, Guttmann R, and Martin JG. Partitioning of airway responses to inhaled methacholine in the rat. *J Appl Physiol* 62: 1317–1323, 1987.
- DiMaria GU, Bellofiore S, Sapienza S, Martin JG, and Mistretta A. The site of airway responses to methacholine or antigen inhalation challenge. *Eur Respir J Suppl* 6: 523S–526S, 1989.
- DuBois A, Botelho S, and Comroe JH Jr. A new method for measuring airway resistance in man using a body plethysmograph: values in normal subjects and in patients with respiratory disease. *J Clin Invest* 35: 327–335, 1956.
- Duguet A, Biyah K, Minshall E, Gomes R, Wang CG, Taoudi-Benchekroun M, Bates JH, and Eidelman DH. Bronchial responsiveness among inbred mouse strains. Role of airway smooth-muscle shortening velocity. *Am J Respir Crit Care Med* 161: 839–848, 2000.
- Fairchild GA. Measurement of respiratory volume for virus retention studies in mice. *Appl Microbiol* 24: 812–818, 1972.
- Faisca P, Anh DB, and Desmecht DJ. Sendai virus-induced alterations in lung structure/function correlate with viral loads and reveal a wide resistance/susceptibility spectrum among mouse strains. *Am J Physiol Lung Cell Mol Physiol* 289: L777–L787, 2005.
- Finney MJ and Forsberg KL. Quantification of nasal involvement in a guinea pig plethysmograph. *J Appl Physiol* 76: 1432–1438, 1994.
- Flandre TD, Leroy PL, and Desmecht DJ. Effect of somatic growth, strain, and sex on double-chamber plethysmographic respiratory function values in healthy mice. *J Appl Physiol* 94: 1129–1136, 2003.
- Glaab T, Daser A, Braun A, Neuhaus-Steinmetz U, Fabel H, Alarie Y, and Renz H. Tidal midexpiratory flow as a measure of airway hyperresponsiveness in allergic mice. *Am J Physiol Lung Cell Mol Physiol* 280: L565–L573, 2001.
- Glaab T, Hoymann HG, Hohlfeld JM, Korolewicz R, Hecht M, Alarie Y, Tschernig T, Braun A, Krug N, and Fabel H. Noninvasive measurement of midexpiratory flow indicates bronchoconstriction in allergic rats. *J Appl Physiol* 93: 1208–1214, 2002.
- Glaab T, Mitzner W, Braun A, Ernst H, Korolewicz R, Hohlfeld JM, Krug N, and Hoymann HG. Repetitive measurements of pulmonary mechanics to inhaled cholinergic challenge in spontaneously breathing mice. *J Appl Physiol* 97: 1104–1111, 2004.
- Glaab T, Ziegler M, Baelder R, Korolewicz R, Braun A, Hohlfeld JM, Mitzner W, Krug N, and Hoymann HG. Invasive vs. noninvasive measurement of allergic and cholinergic airway responsiveness in mice. *Respir Res* 6: 139, 2005.
- Gomes RF, Shen X, Ramchandani R, Tepper RS, and Bates JH. Comparative respiratory system mechanics in rodents. *J Appl Physiol* 89: 908–916, 2000.
- Griffiths-Johnson DA, Nicholls PJ, and McDermott M. Measurement of specific airway conductance in guinea pigs. A noninvasive method. *J Pharmacol Methods* 19: 233–242, 1988.
- Hamelmann E, Schwarze J, Takeda K, Oshiba A, Larsen GL, Irvin CG, and Gelfand EW. Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. *Am J Respir Crit Care Med* 156: 766–775, 1997.
- Hantos Z and Brusasco V. Assessment of respiratory mechanics in small animals: the simpler the better? *J Appl Physiol* 93: 1196–1197, 2002.
- Hellings PW, Hessel EM, Van Den Oord JJ, Kasran A, Van Hecke P, and Ceuppens JL. Eosinophilic rhinitis accompanies the development of lower airway inflammation and hyper-reactivity in sensitized mice exposed to aerosolized allergen. *Clin Exp Allergy* 31: 782–790, 2001.
- Hessel EM, Zwart A, Oostveen E, Van Oosterhout AJ, Blyth DI, and Nijkamp FP. Repeated measurement of respiratory function and bronchoconstriction in unanesthetized mice. *J Appl Physiol* 79: 1711–1716, 1995.
- Jaeger MJ and Bouhuys A. Loop formation in pressure vs. flow diagrams obtained by body plethysmographic techniques. *Prog Respir Res* 4: 116–130, 1969.
- John J, Drefeldt B, Taskar V, Mansson C, and Jonson B. Dynamic properties of body plethysmographs and effects on physiological parameters. *J Appl Physiol* 77: 152–159, 1994.
- Kips JC, Anderson GP, Fredberg JJ, Herz U, Inman MD, Jordana M, Kemeny DM, Lotvall J, Pauwels RA, Plopper CG, Schmidt D, Sterk PJ, Van Oosterhout AJ, Vargaftig BB, and Chung KF. Murine models of asthma. *Eur Respir J* 22: 374–382, 2003.
- Klaassen AB, Kuijpers W, Scheres HM, Rodrigues de Miranda JF, and Beld AJ. Cholinergic receptors in the upper respiratory system of the rat. *Arch Otolaryngol Head Neck Surg* 112: 428–431, 1986.
- Krell WS, Agrawal KP, and Hyatt RE. Quiet-breathing vs. panting methods for determination of specific airway conductance. *J Appl Physiol* 57: 1917–1922, 1984.
- Kumar RK, Herbert C, and Kasper M. Reversibility of airway inflammation and remodelling following cessation of antigenic challenge in a model of chronic asthma. *Clin Exp Allergy* 34: 1796–1802, 2004.
- Kumar RK, Herbert C, Webb DC, Li L, and Foster PS. Effects of anticytokine therapy in a mouse model of chronic asthma. *Am J Respir Crit Care Med* 170: 1043–1048, 2004.
- Lai YL and Chou H. Respiratory mechanics and maximal expiratory flow in the anesthetized mouse. *J Appl Physiol* 88: 939–943, 2000.
- Lai-Fook SJ and Lai YL. Airway resistance due to alveolar gas compression measured by barometric plethysmography in mice. *J Appl Physiol* 98: 2204–2218, 2005.
- Lundblad LK, Irvin CG, Adler A, and Bates JH. A reevaluation of the validity of unrestrained plethysmography in mice. *J Appl Physiol* 93: 1198–1207, 2002.

41. Mitzner W and Tankersley C. Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. *Am J Respir Crit Care Med* 158: 340–341, 1998.
42. Pennock BE, Cox CP, Rogers RM, Cain WA, and Wells JH. A noninvasive technique for measurement of changes in specific airway resistance. *J Appl Physiol* 46: 399–406, 1979.
43. Petak F, Habre W, Donati YR, Hantos Z, and Barazzzone-Argiroffo C. Hyperoxia-induced changes in mouse lung mechanics: forced oscillations vs. barometric plethysmography. *J Appl Physiol* 90: 2221–2230, 2001.
44. Pillow JJ, Korfhagen TR, Ikegami M, and Sly PD. Overexpression of TGF- α increases lung tissue hysteresivity in transgenic mice. *J Appl Physiol* 91: 2730–2734, 2001.
45. Rodrigues de Miranda JF, Scheres HM, Salden HJ, Beld AJ, Klaassen AB, and Kuijpers W. Muscarinic receptors in rat nasal mucosa are predominantly of the low affinity agonist type. *Eur J Pharmacol* 113: 441–445, 1985.
46. Schwartz D. The Mouse Phenome Project. <http://phenome.jax.org/pub/cgi/phenome/mpd.cgi?rtn=projects/details&id=98>.
47. Schwarze J, Hamelmann E, Gelfand EW, Adler A, Cieslewicz G, and Irvin CG. Barometric whole body plethysmography in mice. *J Appl Physiol* 98: 1955–1957, 2005.
48. Shore SA, Johnston RA, Schwartzman IN, Chism D, and Krishna Murthy GG. Ozone-induced airway hyperresponsiveness is reduced in immature mice. *J Appl Physiol* 92: 1019–1028, 2002.
49. Sinnett EE, Jackson AC, Leith DE, and Butler JP. Fast integrated flow plethysmograph for small mammals. *J Appl Physiol* 50: 1104–1110, 1981.
50. Sly PD, Turner DJ, Collins RA, and Hantos Z. Penh is not a validated technique for measuring airway function in mice. *Am J Respir Crit Care Med* 172: 256, 2005.
51. Sterk PJ, Fabbri LM, Quanjer PH, Cockcroft DW, O'Byrne PM, Anderson SD, Juniper EF, and Malo JL. Airway responsiveness. Standardized challenge testing with pharmacological, physical and sensitizing stimuli in adults. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal Official Statement of the European Respiratory Society. *Eur Respir J Suppl* 16: 53–83, 1993.
52. Takeda K, Haczku A, Lee JJ, Irvin CG, and Gelfand EW. Strain dependence of airway hyperresponsiveness reflects differences in eosinophil localization in the lung. *Am J Physiol Lung Cell Mol Physiol* 281: L394–L402, 2001.
53. Temelkovski J, Hogan SP, Shepherd DP, Foster PS, and Kumar RK. An improved murine model of asthma: selective airway inflammation, epithelial lesions and increased methacholine responsiveness following chronic exposure to aerosolised allergen. *Thorax* 53: 849–856, 1998.
54. Tomioka S, Bates JH, and Irvin CG. Airway and tissue mechanics in a murine model of asthma: alveolar capsule vs. forced oscillations. *J Appl Physiol* 93: 263–270, 2002.
55. Vijayaraghavan R, Schaper M, Thompson R, Stock MF, and Alarie Y. Characteristic modifications of the breathing pattern of mice to evaluate the effects of airborne chemicals on the respiratory tract. *Arch Toxicol* 67: 478–490, 1993.
56. Vinegar A, Sinnett EE, and Leith DE. Dynamic mechanisms determine functional residual capacity in mice, *Mus musculus*. *J Appl Physiol* 46: 867–871, 1979.
57. Wagers S, Lundblad L, Moriya HT, Bates JH, and Irvin CG. Nonlinearity of respiratory mechanics during bronchoconstriction in mice with airway inflammation. *J Appl Physiol* 92: 1802–1807, 2002.
58. Whitehead GS, Walker JK, Berman KG, Foster WM, and Schwartz DA. Allergen-induced airway disease is mouse strain dependent. *Am J Physiol Lung Cell Mol Physiol* 285: L32–L42, 2003.

