



Effect of somatic growth, strain, and sex on double-chamber plethysmographic respiratory function values in healthy mice

Thierry D. Flandre, Pascal L. Leroy and Daniel J.-M. Desmecht

Journal of Applied Physiology 94:1129-1136, 2003. First published Nov 27, 2002;
doi:10.1152/jappphysiol.00561.2002

You might find this additional information useful...

This article cites 46 articles, 21 of which you can access free at:

<http://jap.physiology.org/cgi/content/full/94/3/1129#BIBL>

This article has been cited by 3 other HighWire hosted articles:

Role of interleukin-6 in murine airway responses to ozone

R. A. Johnston, I. N. Schwartzman, L. Flynt and S. A. Shore

Am J Physiol Lung Cell Mol Physiol, February 1, 2005; 288 (2): L390-L397.

[\[Abstract\]](#) [\[Full Text\]](#) [\[PDF\]](#)

CXCR2 is essential for maximal neutrophil recruitment and methacholine responsiveness after ozone exposure

R. A. Johnston, J. P. Mizgerd and S. A. Shore

Am J Physiol Lung Cell Mol Physiol, January 1, 2005; 288 (1): L61-L67.

[\[Abstract\]](#) [\[Full Text\]](#) [\[PDF\]](#)

The Use and Misuse of Penh in Animal Models of Lung Disease

J. Bates, C. Irvin, V. Brusasco, J. Drazen, J. Fredberg, S. Loring, D. Eidelman, M. Ludwig, P. Macklem, J. Martin, J. Milic-Emili, Z. Hantos, R. Hyatt, S. Lai-Fook, A. Leff, J. Solway, K. Lutchen, B. Suki, W. Mitzner, P. Pare, N. Pride and P. Sly

Am. J. Respir. Cell Mol. Biol., September 1, 2004; 31 (3): 373-374.

[\[Full Text\]](#) [\[PDF\]](#)

Updated information and services including high-resolution figures, can be found at:

<http://jap.physiology.org/cgi/content/full/94/3/1129>

Additional material and information about *Journal of Applied Physiology* can be found at:

<http://www.the-aps.org/publications/jappl>

This information is current as of July 22, 2005 .



Effect of somatic growth, strain, and sex on double-chamber plethysmographic respiratory function values in healthy mice

THIERRY D. FLANDRE,¹ PASCAL L. LEROY,² AND DANIEL J.-M. DESMECHT¹

Departments of ¹Pathology and ²Biostatistics, University of Liège, B-4000 Liège, Belgium

Submitted 26 June 2002; accepted in final form 16 November 2002

Flandre, Thierry D., Pascal L. Leroy, and Daniel J.-M. Desmecht. Effect of somatic growth, strain, and sex on double-chamber plethysmographic respiratory function values in healthy mice. *J Appl Physiol* 94: 1129–1136, 2003. First published November 27, 2002; 10.1152/jappphysiol.00561.2002.—Double-chamber plethysmography has been recognized since 1979 as a reference technique to measure pulmonary function values in guinea pigs, but it has not gained attention for use in mice. Theoretically, however, this technique combines the advantages of single-chamber plethysmography with a quantitative assessment of flow and/or volume and a calculated resistance, the interpretation of which in terms of bronchoconstriction is not disputed. Here we show that, when appropriately preconditioned, mice are able to gradually grow accustomed to the apparatus and display extremely stable nasal and thoracoabdominal flow tracings. Overall, strain, sex, and somatic growth had a significant effect on pulmonary function values. The changes in specific airway resistance (sRaw) and enhanced pause (Penh) values were never in the same direction, indicating that they measure different things. The respiratory frequency was far higher in C57BL/6 compared with BALB/c mice. Peak flows, minute volume, specific tidal and minute volumes, and sRaw were also higher, but Penh was smaller. Males breathed at a higher frequency than females, leading to a higher minute volume. Nevertheless, the specific volumes were considerably higher among females. Penh was lower in males, whereas sRaw was identical in both sexes. Changes associated with somatic growth were rapid and important between 5 and 9 wk, then slowed down between 9 and 12–13 wk and became almost imperceptible after.

airway resistance; BALB/c; C57BL/6

MICE HAVE BECOME A PERMANENT part of biomedical research laboratories (25). Briefly, they owe this success to the low costs incurred, the existence of thousands of inbred strains, and the fact that the mouse is the species with the greatest density of known genetic markers, which facilitates the tracing of the alleles partly or wholly responsible for complex phenotypes (26). In this respect, numerous recent examples in both pulmonary toxicology (22) and physiology (46) or in the field of asthma (9) clearly demonstrate that in the future there will be a constantly increasing need for practical and fast respiratory function investigative

techniques yielding accurate results independent of external factors.

In this area, there is the classical physiological approach on the basis of the analysis of transpulmonary pressure and nasal flow. This gold standard provides values relating to ventilatory mechanics (compliance and resistance) that are invaluable in terms of understanding and quantifying the phenomena involved. Unfortunately, the use of this approach implies an anesthetic, intubation, pleural catheterization, and artificial ventilation, all of which are procedures that generate significant artifacts (5, 17). Moreover, it is clearly not possible to consider screening a large number of animals in this way, although this would be indispensable to identify any quantitative trait loci. Finally, this approach provides single-point measurements that do not allow follow-up studies.

A second option is to use the low-frequency forced oscillation technique (LFOT), which has been recently validated in mice (18, 41). LFOT provides direct, separate, and high-grade assessment of airway and tissue mechanics but, unfortunately, also implies the use of anesthetized, intubated, and artificially ventilated mice.

The third option is to use single-chamber barometric plethysmography, whereby the respiratory function is assessed on the basis of the characteristics of the pressure wave generated by respiration in a chamber in which the animal can move around (30). This approach to investigation of the respiratory function in mice owes its success to its decisive advantages in terms of practical implementation (quick and easy) and its specific features (no artifacts linked to anesthetic and invasive manipulations). In addition, it enables prolonged and/or repeated measurements over time and provides a bronchoconstriction index known as “enhanced pause,” or Penh (19). However, this technique has a number of defects: the variability between respiratory cycles is high (19), the current volumes measured cannot be quantitatively compared (10), and the interpretation of Penh is the subject of intensive debate (16, 29).

In this context, double-chamber plethysmography, validated from 1979 onward (39) and recognized since then as a reference technique for guinea pigs, has not

Address for reprint requests and other correspondence: D. Desmecht, Dept. of Pathology, Univ. of Liège, B43 Sart-Tilman, B-4000 Liège, Belgium (E-mail: daniel.desmecht@ulg.ac.be).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

had any success in mice. Theoretically, however, this technique combines the advantages of single-chamber plethysmography with quantitative flow and/or volume measurements and a calculated resistance the interpretation of which in terms of bronchoconstriction is not disputed. The purpose of this work was to assess the feasibility of this technique in mice, in particular by defining conditions under which the psychological constraints linked to stress can be kept to a minimum. Differences linked to somatic growth, strain, and sex on the double-chamber plethysmographic pulmonary function values (PFVs) were then examined, yielding reference values in growing male and female conscious and healthy BALB/c and C57BL/6 mice.

MATERIALS AND METHODS

Animals. Eighty pathogen-free BALB/cByJico and C57BL/6Jico mice, 4–14 wk of age, were used in all experiments ($n = 40$ for each strain, sex ratio 1:1). The animals were kept under standard housing conditions (22°C, 12:12-h light-dark cycle), fed a commercial diet, and given water ad libitum. To enable them to become gradually accustomed to the experimental environment, the mice were placed in the equipment described below for 15 min every day from their fourth week. After this, between 5 and 14 wk of age, they were placed in the plethysmograph once a week, at a set time (between 9:00 and 11:30 AM), for ~10 min. After the final measurements had been taken, the mice were euthanized by an intraperitoneal overdose of pentobarbital sodium. All the serological analyses carried out in the postmortem proved negative for the most common murine pathogenic agents. All the procedures used complied with National Institutes of Health guidelines, and the experimental protocol was approved by the Ethics Committee of the University.

Measurement of pulmonary function. The pulmonary function was measured by using the two-chambered, whole body plethysmograph devised by Buxco (model no. PLY-3351). Briefly, this equipment consists of two plastic cylinders that can be attached to one another (Fig. 1). The first cylinder, which is used as the thoracoabdominal compartment, takes the form of a large syringe (30 mm ID) with a rigid orifice at the end, through which the head is passed. The mouse is placed in this cylinder carefully, head first, from the opposite side. The piston is then connected, and its gradual movement causes the mouse to put its head in the orifice. The diameter of this orifice is adapted to the animal, so that its head and

neck can pass through but not its shoulders. When the second cylinder, which acts as the nasal compartment, is connected to the first, a latex film is interposed so as to guarantee that the system is airtight. A hole is made in the center of this latex film through which the head can pass, and the diameter of this hole is adjusted to each animal so that the collar thus formed is airtight but does not compress the upper respiratory tract. Preliminary experiments consisted of comparing minute volumes (MV) from the same mouse measured by the equipment for a series of sheets with a hole of decreasing diameter (per notch of 1 mm). A gradual increase in MV was observed (owing to the reduction in leaks), followed by a plateau. The ideal diameter was defined as the largest diameter at which the MV plateau could be attained. A table of ideal diameters depending on the live weight was therefore prepared in advance for mice weighing between 15 and 30 g.

Each plethysmographic chamber was equipped with the same wire screen pneumotachograph (1 mesh stainless steel cloth screen) and the same differential pressure transducer (EMKA Technologies, model DP-T). Before the experiments were started, the phase compatibility of all the equipment had been established by demonstrating the absence of any phase lag between the signals coming from the two transducer chambers when a pressure sine wave (15 Hz) was imposed on the system by connecting a loudspeaker to it. Once the mouse was in position, all the equipment was placed in a casing containing 2,000 liters of air kept permanently at a temperature of 22°C and a relative humidity of 70%. A hole was formed in the nasal chamber to allow the continuous extraction of the stale air at a constant flow rate (500 ml/min). This air was therefore constantly replaced by fresh air from the casing.

For each mouse, the protocol consisted of breathing in the plethysmograph for 10 min, 14 times. The first four manipulations were designed to familiarize the animals with the experimenter's hand, the positioning in the equipment, and the environment. The following ten procedures were used to collect the nasal and thoracoabdominal flows generated in the plethysmographic chambers by calm breathing. The flows from the two chambers were systematically calibrated before and after the experiment. If a difference of >5% was observed, the data collected were eliminated. In four mice, inhalations of saline and methacholine were administered. Aerosols were generated by an ultrasonic nebulizer (LS-2000, SystAM, Villeneuve sur Lot, France), which produces particles with a mean aerodynamic diameter of 3.5 μm . Aerosols were delivered into the nasal chamber for 30 s in a dose-response manner: 0 (saline), 10, and 100 mg of methacholine per milliliter.

Analysis of individual patterns. The raw flow curves were acquired by sampling the signals at 2 kHz. The regularity of the breathing pattern was first qualitatively assessed by monitoring the constancy of peak flows. On the basis of this criterion, some cough or movements excepted, the vast majority of mice breathed regularly between the second and the tenth minute spent in the plethysmograph. On the basis of this criterion too, a 3-min window of regular breathing was chosen for quantitative analysis, corresponding to ~900 successive cycles. A specially designed software program (IOX, version 1533, EMKA Technologies, Paris, France) was used to process the two flow curves directly during the experiment. A series of parameters was measured directly on the basis of the thoracoabdominal flow curve (Fig. 2): duration of inspiration (T_I), duration of expiration (T_E), peak inspiratory flow (PIF), peak expiratory flow (PEF), and tidal volume (TV). This last parameter was systematically measured twice per cycle, once on the inspiratory portion (ITV) and once on the

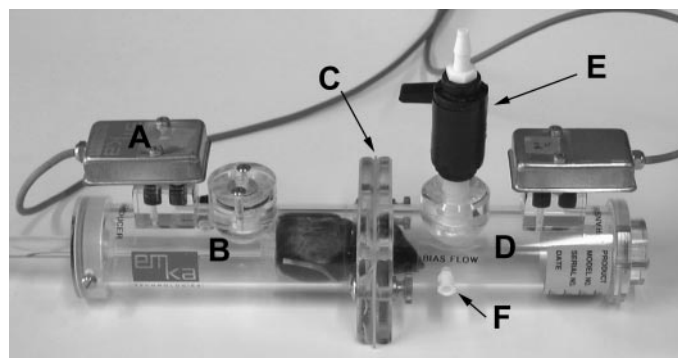


Fig. 1. Double-chamber plethysmograph. A: differential pressure transducer. B: thoracoabdominal compartment. C: sealing latex film. D: nasal compartment. E: aerosol inlet. F: port for constant extraction of stale air.

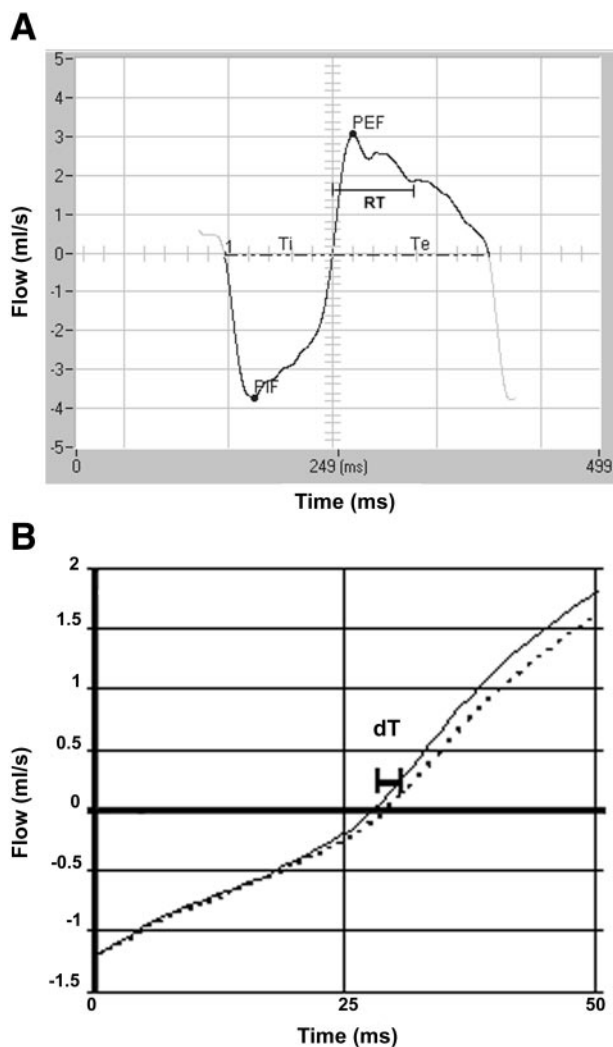


Fig. 2. A: characteristic thoracoabdominal flow tracing. T_i , inspiratory time; T_e , expiratory time; PIF, peak inspiratory flow; PEF, peak expiratory flow. Inspiratory tidal volume and expiratory tidal volume correspond to the area enveloped by the flow tracing below and above the zero-flow line, respectively. RT, time taken to exhale the first 64% of the tidal volume. B: characteristic nasal and thoracoabdominal flows at the time of end-inspiratory zero flow. dT , time lag used to calculate specific resistance of the airways.

expiratory portion of the breathing cycle (ETV). Two other parameters were calculated: the time needed to exhale the first 64% of the TV, known as relaxation time (RT), and the bronchoconstriction index (Penh) proposed by Hamelmann and colleagues (19): $Penh = [(T_e/RT) - 1] \times (PEF/PIF)$. Furthermore, the delay observed between nasal and thoracoabdominal flows (dT) was measured and used to calculate the specific resistance of the airways (sRaw) by using the approach developed by Pennock et al. (39): $sRaw = [(T_i + T_e)/(2 \times \pi) \times (P_{atm} - 47) \times 1.36 \times 2 \times \pi \times dT/(T_i + T_e)]$, where P_{atm} is atmospheric pressure. Finally, on the basis of the parameters measured above and body weight (BW), the respiratory rate [$RR = 60/(T_i + T_e)$], the MV (= $RR \times TV$), specific TV (sTV = TV/BW), specific MV (sMV = MV/BW), and duty cycle [$\%T_i = T_i/(T_i + T_e)$] were also calculated. Among the 900 quantitative values yielded, the median value was systematically calculated, and the closest 300 individual values (150 up/150 down) were used for calculation of a mean value representative of that mouse and day.

Analysis of pulmonary function values. By the end of the experiments, 80 mice had provided 10 sets of data measured at 1-wk intervals when the mice were between 5 and 14 wk old. All values are reported as means \pm SE. Three-way ANOVA corrected for repeated measurements was used to establish the statistical significance of differences in terms of age, sex, and strain groups. If significant differences among groups were obtained by using the ANOVA, Student's *t*-tests on least square means were used to differentiate the differences between groups. Linear, curvilinear, and allometric equations against age or body weight as independent variables were computed for each dependent variable within each sex and strain. The statistically significant equations matching the data most closely were selected. *P* values <0.05 were considered significant. All statistical analyses were performed with SAS-STAT (SAS Institute, 1989).

RESULTS

By repeating all the stages of the investigative procedure, the mice were able to gradually grow accustomed to the conditions of the experiment. The number and intensity of the excitation phases fell very sharply from the second session onward. The same was true for the attempts to move in the plethysmograph. At the same time, the regularity of the respiratory pattern gradually improved. From the fourth session onward, all signs of discomfort and anxiety had disappeared. The between-breath variability of PFVs, as judged from the variation coefficient of Penh values derived from 20 successive cycles, was consistently $\pm 15\%$. A linear regression of ITV against ETV values produced a straight line, the slope of which did not differ significantly from unity.

Generally speaking, the statistical analyses demonstrated that somatic growth, sex, and strain had a significant effect on all PFVs, with three exceptions: the TV was not affected by sex or strain, the sRaw was not affected by sex and growth, and the PEF and $\%T_i$ were not affected by growth. The least square means characteristic of the two strains, the two sexes, and 5 of the 10 age groups are given in Tables 1 and 2. The significant regression equations that match the data most closely are available on the Web, as is a series of tables giving the reference PFVs depending on age, sex, and strain (13). The PFVs can be divided into three categories, depending on their course during somatic growth: RR, sTV, sMV, and Penh gradually declined; T_i , T_e , TV, MV, and PIF gradually increased; and $\%T_i$, PEF, and sRaw remained stable. A strong evolution was observed between 5 and 9 wk. This evolution then slowed down between 9 and 12–13 wk and disappeared thereafter. Male mice breathed at a higher frequency than females, with shorter T_i and T_e and higher peak flows (PIF and PEF). This led to a higher MV in males. Nevertheless, the specific volumes (sTV and sMV) were considerably higher among females. From a mechanical point of view, the Penh was weaker in males, whereas the sRaw was identical in both sexes. With the exception of the TV, all the PFVs differed between the two strains studied here. The respiratory frequency was far higher in C57BL/6, which necessarily meant shorter T_i and T_e , but also a different strategy, because

Table 1. *Effect of strain and sex on pulmonary function values*

	Strain		Sex	
	BALB/c	C57BL/6	Male	Female
<i>n</i>	517	381	396	502
Body weight, g	21.41 ± 0.22 [#]	20.70 ± 0.24	23.14 ± 0.24 [*]	18.98 ± 0.23
Ti, ms	97.59 ± 0.51 [*]	80.09 ± 0.56	85.88 ± 0.53 [*]	91.18 ± 0.49
TE, ms	125.65 ± 0.66 [*]	121.39 ± 0.74	121.06 ± 0.72 [*]	125.98 ± 0.67
RR, breaths/min	273.1 ± 1.2 [*]	302.3 ± 1.3	293.8 ± 1.3 [*]	281.6 ± 1.2
%Ti	43.59 ± 0.15 [*]	39.73 ± 0.16	41.43 ± 0.16 [#]	41.89 ± 0.15
PIF, ml/s	3.14 ± 0.03 [*]	3.95 ± 0.04	3.67 ± 0.04 [*]	3.42 ± 0.03
PEF, ml/s	2.80 ± 0.04 [*]	3.09 ± 0.05	3.06 ± 0.04 [*]	2.83 ± 0.04
ITV, ml	0.219 ± 0.002	0.224 ± 0.003	0.224 ± 0.002	0.219 ± 0.002
MV, ml/min	59.73 ± 0.69 [*]	67.83 ± 0.77	65.92 ± 0.75 [*]	61.63 ± 0.71
sTV, μl/g	10.46 ± 0.08 [*]	11.07 ± 0.09	9.84 ± 0.09 [*]	11.69 ± 0.09
sMV, ml·min ⁻¹ ·g ⁻¹	2.85 ± 0.02 [*]	3.36 ± 0.03	2.91 ± 0.03 [*]	3.30 ± 0.02
Penh	0.732 ± 0.013 [*]	0.540 ± 0.015	0.620 ± 0.014 [*]	0.653 ± 0.013
sRaw, cmH ₂ O·s	1.021 ± 0.014 [*]	1.195 ± 0.016	1.097 ± 0.015	1.119 ± 0.014

Values are least square means ± SE; *n*, no. of mice. Ti, duration of inspiration; TE, duration of expiration; RR, respiratory rate; PIF, peak inspiratory flow; PEF, peak expiratory flow; ITV, inspiratory tidal volume; MV, minute volume; sTV, specific TV; sMV, specific MV; Penh, enhanced pause; sRaw, specific resistance of airways. * and #Significantly different at $P < 0.01$ and $P < 0.05$, respectively.

the relative amount of time devoted to inhaling (%Ti) was higher in BALB/c. The peak flows (PIF and PEF), the MV, the sTV, the sMV, and the sRaw were higher in C57BL/6. However, the Penh was higher in BALB/c. The methacholine concentration-response curve for sRaw is reported in Table 3.

DISCUSSION

Artifacts of biological origin. To eliminate the impact of the circadian cycle (43, 45), the temperature (31), and the relative humidity (20) of the air inhaled when breathing, recordings were made between 9:00 and 11:30 AM, while the nasal chamber was constantly ventilated with air at 22°C and 70% relative humidity, in accordance with the legal recommendations on the well-being of animals used for experimental purposes. Moreover, judging from the calm and indifference of the animals on the one hand and the stability of the nasal and thoracoabdominal patterns obtained on the other hand, we may conclude that the standardized protocol for the acclimatization of the mice made it

possible to keep the impact of the psychological constraints linked to the manual handling and the discovery of a new environment to a minimum.

Artifacts of technical origin. The thoracoabdominal flow was obtained by taking pneumotachographic measurements of the movements of incoming and outgoing ambient air in the thoracoabdominal chamber. By definition, these measurement conditions are quasi-isothermic. At the very most, given the presence of the mouse, a gradual heating of the air in the chamber might be expected, which could invalidate the initial calibration of the pneumotachograph. This possible artifact was overcome by demonstrating that the average TVs calculated in the first and the fifth minute were equal. The nasal pattern was obtained by pneumotachography of the incoming and outgoing air movements in the nasal chamber. In a system like this, the outgoing air is cyclically reheated and humidified because it is partially enriched by the air exhaled, which could also invalidate the calibration. The extent of the phenomenon was minimized from the outset by the

Table 2. *Effect of somatic growth on pulmonary function values*

	Age, wk				
	5	7	9	12	14
<i>n</i>	79	79	96	94	89
Body weight, g	16.12 ± 0.17 ^a	19.12 ± 0.17 ^b	21.43 ± 0.17 ^c	23.07 ± 0.17 ^d	24.31 ± 0.17 ^e
Ti, ms	81.76 ± 1.02 ^a	86.77 ± 1.02 ^b	87.76 ± 0.90 ^b	89.66 ± 0.90 ^c	93.42 ± 0.94 ^d
TE, ms	114.85 ± 1.38 ^a	120.75 ± 1.38 ^b	122.05 ± 1.22 ^b	125.25 ± 1.23 ^c	126.59 ± 1.28 ^c
RR, breaths/min	308.6 ± 2.6 ^a	294.2 ± 2.6 ^b	290.6 ± 2.3 ^b	283.4 ± 2.3 ^c	277.8 ± 2.4 ^d
%Ti	41.54 ± 0.29	41.66 ± 0.29	41.81 ± 0.25	41.66 ± 0.25	42.34 ± 0.27
PIF, ml/s	3.25 ± 0.07 ^a	3.43 ± 0.07 ^b	3.72 ± 0.06 ^c	3.76 ± 0.06 ^c	3.61 ± 0.06 ^c
PEF, ml/s	2.98 ± 0.07	2.92 ± 0.07	3.02 ± 0.06	3.05 ± 0.06	3.03 ± 0.06
ITV, ml	0.191 ± 0.004 ^a	0.210 ± 0.004 ^b	0.230 ± 0.003 ^c	0.238 ± 0.003 ^c	0.236 ± 0.004 ^c
MV, ml/min	58.85 ± 1.23 ^a	61.77 ± 1.23 ^b	67.31 ± 1.09 ^c	67.71 ± 1.10 ^c	65.76 ± 1.14 ^c
sTV, μl/g	12.08 ± 0.18 ^a	11.19 ± 0.18 ^b	10.88 ± 0.16 ^b	10.46 ± 0.16 ^c	9.94 ± 0.17 ^d
sMV, ml·min ⁻¹ ·g ⁻¹	3.73 ± 0.06 ^a	3.29 ± 0.06 ^b	3.17 ± 0.05 ^b	2.97 ± 0.05 ^c	2.75 ± 0.05
Penh	0.812 ± 0.022 ^a	0.703 ± 0.022 ^b	0.606 ± 0.020 ^c	0.556 ± 0.020 ^c	0.596 ± 0.021 ^c
sRaw, cmH ₂ O·s	1.042 ± 0.031	1.144 ± 0.031	1.156 ± 0.027	1.066 ± 0.028	1.090 ± 0.029

Values are least square means ± SE. Least square means with different letters are significantly different at $P < 0.05$.



Table 3. Effect of inhaled methacholine on resistance values

	Saline	Methacholine, 10 mg/ml	Methacholine, 100 mg/ml
sRaw, from the present study, cmH ₂ O·s	0.89 ± 0.23	1.91 ± 0.46	7.17 ± 1.33
RL, from the present study,* cmH ₂ O·s·ml ⁻¹	1.79 ± 0.46 (100%)	3.81 ± 1.02 (213%)	14.35 ± 3.16 (802%)
RL, from Nagase et al.,† cmH ₂ O·s·ml ⁻¹	0.45 (100%)	0.75 (167%)	1.40 (311%)

Values are means ± SE. RL, lung resistance. *Calculated from sRaw, assuming an end-inspiratory thoracic volume of 0.5 ml; †extrapolated from Fig. 3 in the paper of Nagase et al. (36).

imposition of a constant flow of fresh air into the nasal chamber. Under these conditions, it was possible to check that the average TVs calculated on the inspiratory or the expiratory branch of the curve were equal. Finally, it was necessary to demonstrate that the intrabreath reheating and humidification of the air inhaled did not cause sufficient dilatation of the TV for the delay between the thoracoabdominal and the nasal curves to be influenced by this. This artifact in turn was overcome by demonstrating that the slope of the regression line of ITV against ETV (calculated on the thoracoabdominal pattern) did not differ significantly from the unit, which implies that the intrabreath dilatation and any impact it may have had are negligible.

The interposition of a latex collar designed to ensure airtightness between the two chambers is also a source of possible error, either due to a fault if the collar is too loose or due to excess if the collar is too tight. In the first case, the PIF, PEF, TV, MV, and sRaw values may be expected to be underestimated, with the latter value tending toward 0. The second case may be expected to result in an overestimated sRaw, defense movements, and a modification in the breathing pattern. These artifacts were eliminated by standardizing the procedure for choosing the collar as described above. Another possible issue leading to artifacts in flow-volume measurements is the presence of a back-and-forth mouse-collar movement synchronous with respiration. If this occurs, then the calculated TV would be the sum or difference of the true TV and the volume cyclically displaced from one compartment into the other. Although such collar movement probably occurs with respiration, the amplitude of the volume displaced is probably negligible because the body is gently but surely wedged between the rigid orifice (shoulders) and the piston (back), thus damping out possible oscillations. Also, a leakage associated with mouse-collar movement would result in unequal ITV and ETV, which was not the case. In practice, this artifact was ruled out on-line during the experiment by monitoring the superimposition of successive thoracic vs. mouth volume Lissajous loops. Furthermore, it can be anticipated that any error introduced by air leakage between the two chambers would be magnified as the resistance

of the lungs and airways increases. Inhaled methacholine was used to demonstrate that the apparatus gives reliable resistance data in such conditions too. From the comparison of resistance data derived from sRaw measurements and from intubated mice inhaling similar methacholine concentrations (Table 3), it can be seen that the magnitude of the changes recorded by double-chamber plethysmography is more important, suggesting that the leakage-related underestimation, if any, is not significant.

Some comparison of the relative magnitude of the sRaw with other literature measurements is presented in Table 4. To our knowledge, there are no published sRaw values in mice available, but by assuming an end-inspiratory lung volume at ~0.5 ml, one can calculate a total lung resistance from sRaw. From Table 4, it is obvious that resistance data derived from intubated mice yielded significantly lower values than those reported here. This can be explained by the fact that sRaw measures the sum of lung and upper airway or nasal resistance, not only that of the compartment distal to the trachea. In turn, total lung resistance data obtained from intact mice (upper airways included) reveals a similar order of magnitude: 2.08 (present study) vs. 1.27 (Ref. 17) or 2.32 (Ref. 48) cmH₂O·s·ml⁻¹. Also, according to the intermammalian allometric equation calculated by Bennett and Tenney (4), the expected value of total lung resistance in a 20-g mouse amounts to 1.92 cmH₂O·s·ml⁻¹, which is very close to the value calculated here from sRaw measured in the 20-g BALB/c mouse (2.08 cmH₂O·s·ml⁻¹).

Effect of sex. As regards the PFVs, to our knowledge no significant differences between sexes have ever been reported for mice. The respiratory frequency was higher in male mice, which is comparable to observations made in rats. The androgens (47) and a sexual dimorphism of the arcuate nucleus (42) have been put forward to explain this difference in rats. The MV of males is also greater, but this is due to the fact that they were heavier at the same age. However, the sMV was considerably higher in females. This difference, which is also found in rats, may be due to a higher basal metabolism in females (40), which has been reported to be accompanied by a higher central temperature and level of activity (34). As with rats, female mice attain this higher sMV by means of a higher sTV rather than by increasing RR.

As regards the parameters used to measure resistance, a surprising contrast was revealed between sRaw and Penh, in that the sRaw was identical for both sexes, whereas the Penh is significantly higher in females. The first conclusion to be drawn from this is that these two parameters do not "quantify" the same thing, as has already been suggested by other works in which, as in this case, an increase in Penh without any increase in resistance is reported in the context of interstitial pneumonia (41). The fact that under our experimental conditions the Penh was measured on the basis of a thoracoabdominal flow pattern rather than on the basis of a typical pressure pattern from single-chamber plethysmography makes the values

Table 4. Resting lung resistance values reported in mice

Mouse strain	Age, wk	BW, g	Sex	n	Anesthetic	Special Procedure	Ventilation	Calculation	Resting RL cmH ₂ O· s·ml ⁻¹	Reference
<i>Upper airways excluded (tracheal cannulation)</i>										
Not Given	Not given	32	Not given	4	Pentobarbital		Forced	IV50	0.48	7
C57BL/6	4–12	20–30	M	52	Pentobarbital	Chest open	Forced	IV50	0.63	27
BALB/c	5–8	20–25	Not given	5–7	Pentobarbital	Esophageal cannula	Spontaneous	IV50	0.56	15
AJ	6–8	Not given	M	3–6	Halothane + Ketamine	Vena cava catheter	Forced	EIO	1.30†	12
C3H/HeJ	6–8	Not given	M	3–6	Halothane + Ketamine	Vena cava catheter	Forced	EIO	0.96†	12
ICR	Not given	35–42	M	5	Pentobarbital + ketamine	Chest open	Forced	IV50	0.58*	36
ICR	8–11	Not given	M	8	Pentobarbital + ketamine	Chest open	Forced	IV50	0.38	35
BALB/c	6–8	18–22	F	16	Pentobarbital	None	Forced	IV50	0.21	37
<i>Upper airways included</i>										
Swiss Webster	Not given	23–25	F	Not given	None	Pleural catheter	Spontaneous	IV50	2.32	48
BALB/c	6–8	18–22	F	18	None	Pleural catheter	Spontaneous	IV50	1.27	17
BALB/c	7	19–20	Both	43	None	None	Spontaneous	TMPS	2.08‡	Present study 4
									1.92	

EIO, end-inspiratory occlusion method (12), IV50, isovolume 50 method (2); TMPS, thorax-to-mouth phase shift method (39); BW, body weight; M, male; F, female. According to the allometric equation among mammals: $RL = 0.078 (BW \text{ in kg})^{-0.819}$ and assuming a BW of 20 g. *Extrapolated from Nagase et al. (36) Fig. 2; †calculated assuming a 35% contribution of chest wall resistance to respiratory system resistance reported; ‡calculated from sRaw assuming an end-inspiratory thoracic gas volume of 0.5 ml.

collected here independent from thermal effects, TV, or functional residual capacity, which is not the case of the Penh derived from single-chamber plethysmography, as recently demonstrated (24). Being calculated on the basis of the thoracoabdominal flow pattern, the Penh from double-chamber plethysmography basically reflects the shape of the waveform, the latter resulting from the integration of the mechanical properties of the respiratory system (including resistance), of the formulation of the central nervous drive, and of the muscular strategy that sets the system in motion. However, it is important to stress that the difference observed here is exactly the opposite of what is reported with rats, where males have the highest Penh (32). There is a temptation to attribute this to a difference in size, because, at the same age, female mice are lighter but female rats are heavier. By combining all the data together, one may therefore observe Penh to be higher in the lighter sex, hence the hypothesis that weight and size influence the value of the Penh owing to their impact on factors whose integration determines the form of the thoracoabdominal flow-volume pattern.

Effect of strain. The two strains tested here differ very clearly from one another owing to their breathing pattern. Strain C57BL/6 breathes more quickly (302 vs. 273 breaths/min) and devotes proportionally less time to inhaling (40 vs. 44%) than strain BALB/c. This accelerated inspiration in C57BL/6 results above all in a far higher PIF, which significantly influences the calculation of the Penh value. The sMV is also higher in C57BL/6, because of an increase in sTV and RR. This higher sMV suggests that C57BL/6 may have a higher basal metabolism than BALB/c, at least at an

ambient temperature of 22°C. There are a number of arguments to back up this explanation: with the same age and sex, C57BL/6 is smaller and lighter, the rectal temperature of C57BL/6 is higher than that of BALB/c (44), the level of activity of C57BL/6 is higher than that of BALB/c (8, 38), the thyroid secretion rate is higher in C57BL/6 (3), and the basal oxygen consumption is higher in C57BL/6 (33). Also, a higher sMV in C57BL/6 like this could be due to differences in the oxygen transport chain. For example, the fact that the hematocrit level, the number of circulating erythrocytes, and the hemoglobin content of the blood are reported to be lower in C57BL/6 could explain at least a part of the difference (14).

As regards the parameters that are supposed to measure resistance, a difference between strains was highlighted for sRaw and Penh, in that the sRaw was higher and the Penh was lower in C57BL/6. Therefore, as between the sexes, a lack of synchronization may be observed between sRaw and Penh, which again suggests that these two parameters do not measure the same thing. Because the C57BL/6 are smaller and lighter at the same age, it is tempting to link their higher sRaw to a lower pulmonary volume, which is generally associated with narrower airways and therefore higher resistance. However, because the sRaw, by definition, normalizes for lung volume, a higher sRaw in C57BL/6 rather suggests some structural differences. On the other hand, it is probable that the lower Penh of C57BL/6 comes from the specific ventilation strategy whereby the accelerated inspiration generates a far smaller PEF/PIF quotient than in BALB/c (0.79 vs. 0.90). This illustrates the existence of a preferred



link between the Penh value and the breathing pattern.

Effect of somatic growth. Generally speaking, the follow-up of the respiratory function parameters undertaken here shows that there are three successive periods. The first of these stretches from 5 to 9 wk of age and is characterized by a high daily weight gain (~190 mg) and rapid evolution of PFVs, with the exception of the %Ti, PEF, and sRaw values, which remain constant. The second period, from 9 to 13 wk of age, is characterized by a smaller daily weight gain (~107 mg); slow evolution of sMV, sTV, RR, Ti, and Te, and stable values for %Ti, PIF, PEF, TV, MV, Penh, and sRaw. Finally, after 13 wk, a third period begins, when the daily weight gain is small (~100 mg) and the evolution of PFVs imperceptible. This course of functional data in three phases corresponds to what is known about the pulmonary morphological modifications occurring during growth in mice, which also initially display a rapid development between 0 and 8 wk, which then slows down or even becomes imperceptible (23). As with other mammals, it may be noted that the rapid development phase ends with the age of sexual maturity (6), whereas the slow development phase tends to end on the entry into adulthood.

More specifically, a gradual reduction in sMV may be observed, corresponding to the expected gradual reduction in the basal metabolism during somatic growth (11, 21). This reduction in the consumption of air per unit of live weight, which can also be detected via the early stabilization of the MV despite persistent weight gain, is achieved by means of a slowdown in rhythm (RR) and the stabilization of TV. The constant sRaw value may a priori seem surprising, because as a general rule resistance decreases as the diameter of the respiratory tract increases, which is supposed to be the case during somatic growth. Nevertheless, recent volumetric data back up this result. In fact, Mitzner et al. (28) showed that with C3H and A/J, somatic growth affects the functional residual capacity only up to the age of 6 wk, which suggests that the increase in pulmonary volumes brought about by growth ceases at 6 wk despite an increase in size and weight that continues well beyond this point. If one supposes that these data can be extrapolated to BALB/c and C57BL/6, it may be suggested that tissular resistance and the resistance due to intrapulmonary airways no longer alter after the age of 6 wk. As regards the upper respiratory tracts, no morphological data are available on the development of their size, but it may reasonably be considered that their development is closely synchronized with that of the more distal tracts (1). In this case, it may indeed be expected that no significant change of the sRaw will be detected in the age group being studied here. However, the Penh declines significantly until 9 wk before stabilizing. This once again indicates that it would be worth undertaking an in-depth study of the biological factors that influence this parameter.

In conclusion, the results presented here lead us to think that, when applied to mice, double-chamber

plethysmography yields stable and reliable pulmonary function values. Compared with pleural catheterization and LFOT, artifacts attributable to anesthesia and tracheal intubation are avoided and the actions required are quick and easy, which makes it possible to examine a large number of animals at the same time or the same animal at several consecutive times. Also, compared with single-chamber plethysmography, double-chamber plethysmography yields quantitative rather than qualitative measurements of the flows, volumes, and resistance, which significantly strengthens interpretation of results. Finally, the results reported unambiguously show that sRaw and Penh measure different things, which suggests that the utility of the latter in assessing airway function is limited.

The research was supported by the Belgian Ministry of Agriculture, Grant S-5929.

REFERENCES

1. **Affolter M and Shilo BZ.** Genetic control of branching morphogenesis during *Drosophila* tracheal development. *Curr Opin Cell Biol* 12: 731–735, 2000.
2. **Amdur LO and Mead J.** Mechanics of respiration in unanesthetized guinea pigs. *Am J Physiol* 192: 364–368, 1958.
3. **Amin A, Chai CK, and Reineke EP.** Differences in thyroid activity of several strains of mice and F₁ hybrids. *Am J Physiol* 191: 34–36, 1957.
4. **Bennett FM and Tenney SM.** Comparative mechanics of mammalian respiratory system. *Respir Physiol* 49: 131–140, 1982.
5. **Chong BTY, Agrawal DK, Romero FA, and Townley RG.** Measurement of bronchoconstriction using whole-body plethysmograph: comparison of freely moving versus restrained guinea pigs. *J Pharmacol Toxicol Methods* 39: 163–168, 1998.
6. **Crispen CG.** *Handbook on the Laboratory Mouse*. Springfield, IL: Thomas, 1975.
7. **Crosfill ML and Widdicombe JG.** Physical characteristics of the chest and lungs and the work of breathing in different mammalian species. *J Physiol* 158: 1–14, 1961.
8. **Davis WM and King WT.** Pharmacogenetic factor in the convulsive responses of mice to flurothyl. *Experientia* 23: 214–215, 1967.
9. **De Sanctis GT, Jiao A, 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.
10. **Enhorning G, van Schaik S, Lundgren C, and Vargas I.** Whole-body plethysmography, does it measure tidal volume of small animals? *Can J Physiol Pharmacol* 76: 945–951, 1998.
11. **Even PC, Rolland V, Roseau S, Bouthegourd JC, and Tome D.** Prediction of basal metabolism from organ size in the rat: relationship to strain, feeding, age, and obesity. *Am J Physiol Regul Integr Comp Physiol* 280: R1887–R1896, 2001.
12. **Ewart S, Levitt R, and Mitzner W.** Respiratory system mechanics in mice measured by end-inflation occlusion. *J Appl Physiol* 79: 560–566, 1995.
13. **Flandre TD, Leroy PL, and Desmecht DJM.** Double chamber plethysmography in healthy BALB/c and C57BL/6 mice: effect of somatic growth. [Online]. Faculty of Veterinary Medicine, University of Liège. <http://www.ulg.ac.be/fmv/anapath/plethysmo/plethdoubch.htm> [3 Jan. 2002].
14. **Frith CH, Suber RL, and Umholtz R.** Hematologic and clinical chemistry findings in control BALB/c and C57BL/6 mice. *Lab Anim Sci* 30: 835–840, 1980.
15. **Garssen J, Van Loveren H, Van Der Vliet H, Bot H, and Nijkamp FP.** T cell-mediated induction of airway hyperresponsiveness and altered lung functions in mice are independent of increased vascular permeability and mononuclear cell infiltration. *Am Rev Respir Dis* 147: 307–313, 1993.



16. **Gelfand EW and Irvin CG.** Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography: correspondence. *Am J Respir Crit Care Med* 158: 340–341, 1998.
17. **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.
18. **Gomes RFM, Shen X, Ramchandani R, Tepper RS, and Bates JHT.** Comparative respiratory system mechanics in rodents. *J Appl Physiol* 89: 908–916, 2000.
19. **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.
20. **Kasa W and Thwaites CJ.** The effects of elevated temperature and humidity on rectal temperature and respiration rate in the New Zealand white rabbit. *Int J Biometeorol* 34: 157–160, 1990.
21. **Kiang-Ulrich M and Horvath SM.** Age-related metabolic modifications in male F344 rats. *Exp Aging Res* 10: 89–93, 2000.
22. **Kleeberger SR, Holroyd KJ, Levitt RC, Zhang L, Longphre M, Harkema J, Eleff SM, and DiSilvestre DA.** Linkage analysis of susceptibility to ozone-induced lung inflammation in inbred mice. *Nat Genet* 17: 475–478, 1997.
23. **Kurozumi M, Matsushita T, Hosokawa M, and Takeda T.** Age-related changes in lung structure and function in the senescence-accelerated mouse (SAM): SAM-P/1 as a new murine model of senile hyperinflation of lung. *Am J Respir Crit Care Med* 149: 776–782, 1994.
24. **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.
25. **Malakoff D.** The rise of the mouse, biomedicine's model mammal. *Science* 288: 248–253, 2000.
26. **Malo D and Skamene E.** Genetic control of host resistance to infection. *Trends Genet* 10: 365–371, 1994.
27. **Martin TR, Gerard NP, Galli SJ, and Drazen JM.** Pulmonary responses to bronchoconstrictor agonists in the mouse. *J Appl Physiol* 64: 2318–2323, 1988.
28. **Mitzner WA, Brown R, and Lee W.** In vivo measurement of lung volumes in mice. *Physiol Genomics* 4: 215–221, 2001.
29. **Mitzner WA and Tankersley CG.** Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography: correspondence. *Am J Respir Crit Care Med* 158: 340–341, 1998.
30. **Mortola JP and Frappell PB.** On the barometric method for measurements of ventilation and its use in small animals. *Can J Physiol Pharmacol* 76: 937–944, 1998.
31. **Mortola JP and Frappell PB.** Ventilatory responses to changes in temperature in mammals and other vertebrates. *Annu Rev Physiol* 62: 847–874, 2000.
32. **Mortola JP and Saiki C.** Ventilatory response to hypoxia in rats: gender differences. *Respir Physiol* 106: 21–34, 1996.
33. **Moshkin MP, Potapov MA, Frolova OF, and Evsikov VI.** Changes in aggressive behavior, thermoregulation, and endocrine responses in BALB/cLac and C57BL/6J mice under cold exposure. *Physiol Behav* 53: 535–538, 1993.
34. **Mousel MR, Stroup WW, and Nielsen MK.** Locomotor activity, core body temperature, and circadian rhythms in mice selected for high or low heat loss. *J Anim Sci* 79: 861–868, 2001.
35. **Nagase T, Kurihara H, Kurihara Y, Aoki T, Fukuchi Y, Yasaki Y, and Ouchi Y.** Airway hyperresponsiveness to methacholine in mutant mice deficient in endothelin-1. *Am J Respir Crit Care Med* 157: 560–564, 1998.
36. **Nagase T, Matsui H, Aoki T, Ouchi Y, and Fukuchi Y.** Lung tissue behavior in the mouse during constriction induced by methacholine and endothelin-1. *J Appl Physiol* 81: 2373–2378, 1996.
37. **Neuhaus-Steinmetz U, Glaab T, Daser A, Braun A, Lommatzsch M, Herz U, Kips J, Alarie Y, and Renz H.** Sequential development of airway hyperresponsiveness and acute airway obstruction in a mouse model of allergic inflammation. *Int Arch Allergy Immunol* 121: 57–67, 2000.
38. **Nikulina EM, Skrinckaya JA, and Popova NK.** Role of genotype and dopamine receptors, in behavior of inbred mice in a forced swimming test. *Psychopharmacology (Berl)* 105: 525–529, 1991.
39. **Pennock BE, Cox CP, Rogers RM, Cain A, and Wells J.** A noninvasive technique for measurement of changes in specific airway resistance. *J Appl Physiol* 46: 399–406, 1979.
40. **Perez VJ, Eatwell JC, and Samorajski T.** A metabolism chamber for measuring oxygen consumption in the laboratory rat and mouse. *Physiol Behav* 24: 1185–1189, 1980.
41. **Petak F, Habre W, Donati YR, Hantos Z, and Barazzone-Argiroffo C.** Hyperoxia-induced changes in mouse lung mechanics: forced oscillations vs. barometric plethysmography. *J Appl Physiol* 90: 2221–2230, 2001.
42. **Schlenker EH.** Gender-specific effects of CNQX administered into the arcuate nucleus on ventilatory patterns in rats. *Respir Physiol* 116: 133–143, 1999.
43. **Seifert EL and Mortola JP.** The circadian pattern of breathing in conscious adult rats. *Respir Physiol* 129: 297–305, 2002.
44. **Shepard CC and Habas JA.** Relation of infection to tissue temperature in mice infected with *Mycobacterium marinum* and *Mycobacterium leprae*. *J Bacteriol* 93: 790–796, 1967.
45. **Sommer B, Montaña LM, Chavez J, Gustin P, and Vargas MH.** Guinea pig lung resistance shows circadian rhythmicity not influenced by ozone. *Respir Physiol* 113: 223–229, 1998.
46. **Tankersley CG, DiSilvestre DA, Jedlicka AE, Wilkins HM, and Zhang L.** Differential inspiratory timing is genetically linked to mouse chromosome 3. *J Appl Physiol* 85: 360–365, 1998.
47. **Tsunoda K, Lee XP, Watanabe S, Doge K, Akiya Y, and Watanabe T.** Sex differences in respiratory and cardiovascular effects of beta-endorphin. *Nippon Hoigaku Zasshi* 47: 193–201, 1993.
48. **Vijayaraghavan R, Schaper M, Thomson 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.