

Aeroallergen-induced dyspnea in freely moving guinea pigs: quantitative measurement by bias flow ventilated whole body plethysmography

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Guinea pigs were sensitized and boosted with i.p. injections of ovalbumin (OA) 10 µg + Al(OH)₃ 100 mg. Thirteen days after the last injection animals (800-1100 g) were placed in bias flow ventilated whole body plethysmographs and allowed to stabilize for 2 h. Lung function was recorded for up to 2 h before and 5 h after aeroallergen challenge (OA 20 mg/ml, 60 s, 20 psi) by a noninvasive pulmonary analyzer for unrestrained rodents. Aeroallergen challenge produced immediate dyspnea and gasping (peaking between 8 and 17 min). Gasping was associated with an increase in amplitude in the box pressure fluctuations (93%), and in the slope of the fluctuations (391%). Respiratory rate increased (103 to 175 breaths/min, 78%), the product of breathing rate times box pressure amplitude increased (161 to 432, 180%). Relaxation time (the time it takes the box pressure signal to drop from its peak to 1/3 of its peak value) declined (0.16 to 0.05 s, 72%). All of these lung dysfunction changes were highly significant ($p < 0.001$). Lung dysfunction persisted for 60 to 120 min after challenge. One of 8 animals tested died within 10 min. None of the animals exhibited late asthmatic responses during the 5 h post-challenge period. Based on these data we conclude that this technique allows quantitative analysis of dyspnea, gasping, and an abnormal pattern (waveform) of breathing. The increase in the slope of the fluctuations, especially at the start of expiration, the concomitant decline in relaxation time, and increased pause time between respiratory cycles appear to be reliable and reproducible markers of early asthmatic responses in the airways of freely moving guinea pigs.

Key words: lung function; dyspnea; immediate allergic response; ovalbumin; freely moving guinea pig; bias flow ventilated whole body plethysmography.

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Whole body plethysmographic techniques have been extensively used in anesthetized, unanesthetized, restrained and unrestrained conscious guinea pigs to evaluate lung dysfunction and bronchial hyperreactivity to mediators and/or pharmacologic stimuli (1, 2, 4, 5, 8, 9, 12). However, anesthesia and restraining (neck collar) have been shown to interfere with repeated or long-term measurements of lung function in rodents (2). In this study a simple non-invasive bias flow ventilated whole body plethysmographic technique (2) and non-invasive pulmonary analyzer were used to measure aeroallergen-induced dyspnea in freely moving guinea pigs.

Material and methods

Ovalbumin, OA (grade VI, Sigma Chemical, St. Louis, MO), was the antigen used in the present investigation. Al(OH)₃ obtained as a dry powder (Fisher Scientific, Fair Lawn, NJ) was used as an adjuvant. Antifoam B Emulsion (Sigma Chemical, St. Louis, MO) was added to prevent the foaming of the aeroallergen.

Male Hartley strain guinea pigs (150-200 g) were obtained from Charles River Breeding Laboratories, N. Wilmington, MA.

Sensitization/booster procedure

Guinea pigs (150–200 g) were sensitized to ovalbumin (OA) by an i.p. injection of OA 10 μ g + Al(OH)₃ 100 mg in 0.5 ml of 0.9% saline. The sensitization procedure was repeated on days 11, 21, and 45 or 52. Guinea pigs were ready for lung function evaluation and aeroallergen challenge 13 days after the last injection.

Evaluation of lung function (Buxco Non-invasive Pulmonary Analyzer)

Each guinea pig (800–1100 g) was placed in a bias flow ventilated whole body plethysmograph (Buxco Electronics, Inc., Sharon, CT, USA, Fig. 1). The changes in box pressure (ΔP) represent the difference between the thoracic expansion/contraction and the tidal volume (air removed from/added to the box during inspiration/expiration). The box pressure fluctuations due to respiration are very small, of the order of 1 mm H₂O. Atmospheric fluctuations and ambient disturbances can far exceed that. The box pressure is measured using a differential pressure transducer (Validyne Engineering, model DP45-14) connected to a Buxco electronics preamplifier. One port of the transducer (+) was attached directly to the whole body plethysmograph while the other port (–) was connected to a 1 liter Nalgene bottle, which was connected to the plethysmograph via a long (125 cm) narrow tubing. The resistance of the tubing and the volume of the reservoir created a time constant of several seconds. Thus both ports of the transducer are connected to the box; one port directly, and the other through a large physical time constant which will not respond to the respiratory

fluctuations. This configuration allowed immunity from ambient atmospheric fluctuations, or slow box pressure variations, and provided a stable, self-correcting baseline for respiration signals. According to the principles described by Drorbaugh & Fenn (6) and Bargeton & Gauge (3), the box pressure amplitude for each respiratory cycle is a function of tidal volume and box temperature. Box pressure increases during inspiration and decreases during expiration. The increase during inspiration is due to the warming of the inspired air and the addition of water vapor. Tidal volume may be computed from ΔP using the formula described by Epstein et al. (7). This computation is valid for unlabored respiration. Under conditions of dyspnea the thoracic movement may become greater, even though the tidal volume remains the same or even diminishes. Furthermore, the phase between the thoracic movement and the tidal volume may change. The difference, which is the box pressure, can rise dramatically due to this phase shift. The box pressure during dyspnea becomes an indicator of effort of breathing, rather than of tidal volume. To maintain the O₂ level and to keep the CO₂ level from rising within the whole body plethysmograph during long measurement periods, a bias air flow is required. Ports on opposite sides of the box allow air intake and exhaust to the atmosphere. Long narrow tubing connects the inlet port to the pressure source, and similar tubing connects the outlet port to the atmosphere. The time constant of the resistance of the tubing times the free volume of the plethysmograph is an order of magnitude greater than the period of the respiratory cycle. Hence the bias flow mechanism does not interfere with the respiratory signal. A bias flow of approximately 10 ml/s was used in the experiment. The box pressure is differentiated to give a pseudo flow signal. This flow signal is proportional to the difference between the rate of change of the thoracic volume and the nasal flow. It is proportional to the nasal flow in the control situation. But like the box pressure signal from which it is derived, it loses correlation to the nasal flow waveform under conditions of dyspnea, thus indicating the effort of breathing, rather than nasal flow. The box pressure and the pseudoflow signal were monitored and recorded using a Compaq Deskpro 286 microcomputer equipped with scrolling monitor hardware and software (Buxco Electronics, Inc.). The respiratory derivations were digitized by a Data Logger (DA16, Buxco Electronics, Inc.), and entered into the microcomputer, and presented alongside the waveforms. Derivations included box pressure amplitude (ml), rate of change of box pressure (ml/s), respiratory rate (breaths/min) and relaxation time (s), i.e. the time it takes the peak box pressure signal to drop from its peak to 1/3 of its peak value.

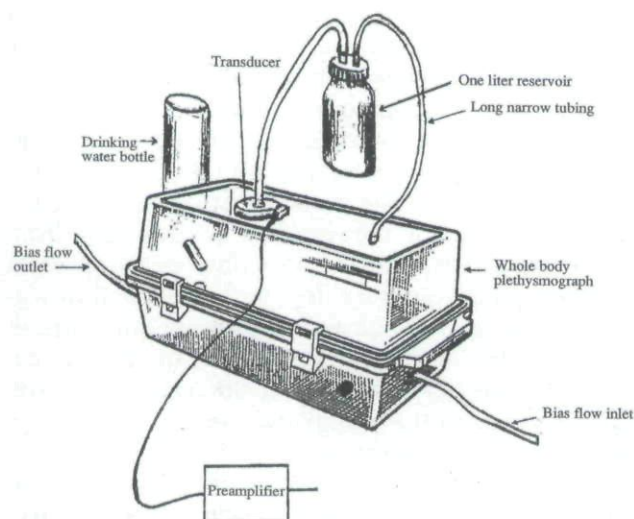


Fig. 1. Whole body plethysmograph configuration for the unrestrained guinea pig.

Acute aeroallergen challenge in unrestrained guinea pigs

Each guinea pig was allowed to stabilize for 2 h. Lung function parameters were recorded for up to 2 h before and 5 h after aeroallergen challenge. Baseline values for each animal were established by averaging each lung function parameter for 5 min prior to challenge. The aeroallergen challenge (OA 20 mg/ml in 0.9% saline containing 2% antifoam emulsion) was produced by forcing compressed air at a pressure of 20 pounds per square inch through a Lovelace nebulizer (In-Tox products, Inc.) connected to the whole body plethysmograph by a 2-way stopcock. The aeroallergen challenge was performed for a period of 60 s. Aeroallergen-induced lung dysfunction was obtained as peak values (minimum or maximum) following aeroallergen challenge over a period of 5 h.

Statistical analysis

The means $\pm$ SD of the aeroallergen-induced pulmonary parameters obtained as peak values following aeroallergen challenge were compared with the means $\pm$ SD of the baseline values using the Student's *t* test for independent observations. Statistical significance as implied in this report indicates a *p* value of ≤ 0.005 .

Results

The box pressure signal is a composite, derived from the difference between the thoracic volume changes and the tidal volume. The pseudoflow signal, generated by differentiating the box pressure signal, is a composite, derived from the difference between the thoracic movement flow and the nasal flow.

We examined thoracic flow and nasal flow in the guinea pig in a double chamber plethysmograph (9) during normal breathing and during the response to a challenge. During normal breathing, both flows appeared identical in shape and were almost identical in phase. The thoracic flow was slightly larger in amplitude than the nasal flow (approximately 10%). The difference between the two produced a waveform, similar in form to each of the components, and approximately 10% of the amplitude of each of the components (Fig. 2A).

During response to challenge, the nasal flow is delayed relative to the thoracic flow. This time delay, according to Pennock et al. (9), is proportional to specific airway resistance. Otherwise the shape of the nasal flow is still similar to the shape of the thoracic flow. The amplitude difference between the thoracic and nasal flows increased (Fig. 2B). The thoracic flow actually increased slightly in amplitude

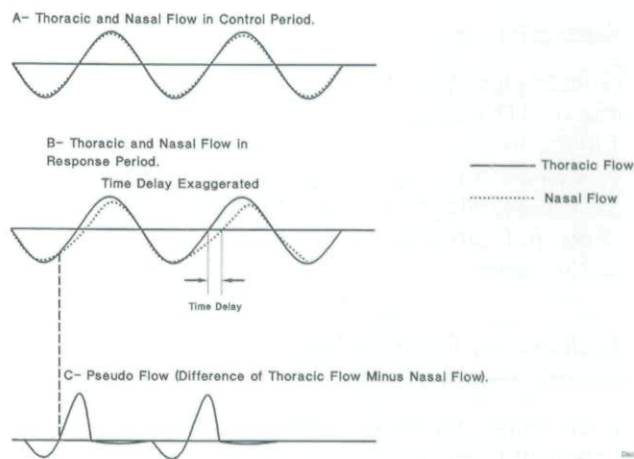


Fig. 2. A: Relationship of thoracic and nasal flow before aeroallergen challenge (Pennock Box Method); B: Shift in the relationship after aeroallergen challenge (Pennock Box; OA 20 mg/ml, 60 breaths, 20 psi, Lovelace nebulizer); C: Corresponding pseudoflow of a freely moving guinea pig in a bias flow ventilated whole body plethysmograph after challenge.

during the response, while the nasal flow decreased slightly.

During response, the expiratory flow decreases from its peak value to zero flow, more gradually than during the control period. The transition from inspiration to expiration is typically abrupt in both the control and the response periods. It is during this transition from inspiration to expiration that the time delay between the thoracic and the nasal flows is most apparent (Fig. 2C). It is also during this transition that the difference between the thoracic and nasal flows is largest. This creates the large amplitude of the pseudoflow waveform at the start of expiration. The peak of the nasal flow in expiration occurs when the thoracic flow is already on the way down. Hence, in the region of end expiration, the difference between the two flows is small, or even negative, giving the appearance in the pseudoflow waveform of a pause, or apnea, between breaths, even though the component flows are not zero.

The following observations may be made from studying the pseudoflow signal in Fig. 2 where the peak amplitude of the pseudoflow signal during the response occurs in the region of the transition from inspiration to expiration in the component signals. It appears as a expiratory flow in the pseudoflow signal. While the ratio of peak expiratory flow to peak inspiratory flow is less than unity in the control period, it is greater than unity in the response period. The peak expiratory amplitude of the pseudoflow signal is related to the time delay between the thoracic and the nasal flow signals, which is related to the specific airway resistance. The pseudoflow waveform underestimates the true inspiratory time of its components. It also indicates an apnea, when in fact

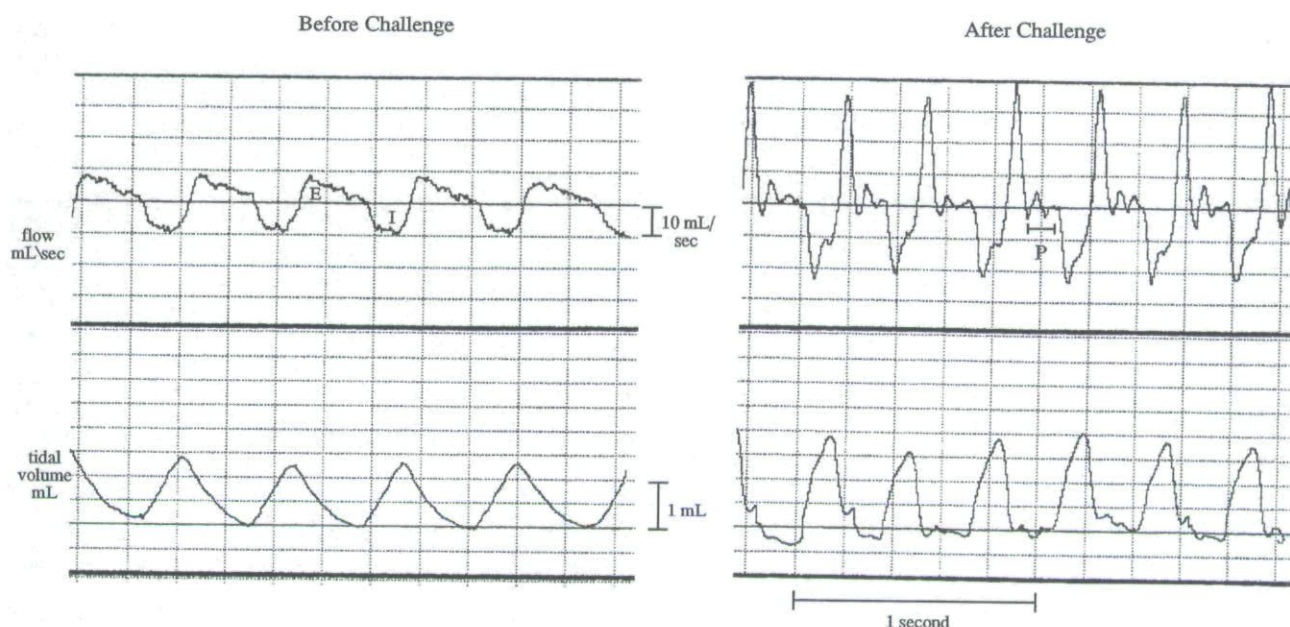


Fig. 3. Typical tracings of the breathing patterns (wave forms) before and after aeroallergen challenge (OA 20 mg/ml, 60 breaths, 20 psi, Lovelace nebulizer) of a freely moving guinea pig in a bias flow ventilated whole body plethysmograph. Note the marked increase in expiratory efforts and pauses between respiratory cycles, which is a characteristic of dyspnea and gasping induced by aeroallergen challenge.

the component flows are not zero. It concentrates the expiratory flow in a narrow interval, giving the appearance of a peak followed by pause. This results in a shortened relaxation time measurement.

Effect of aeroallergen on lung function

The typical tracings of lung function alterations during immediate allergic responses in an unrestrained

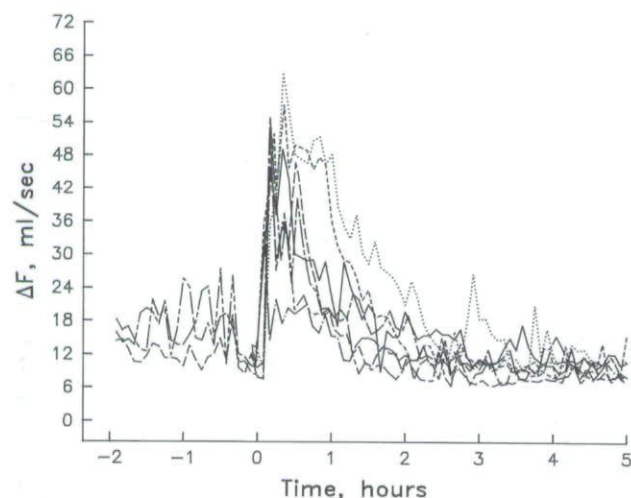


Fig. 4. Differential flow (ΔF = pseudoflow signal derived from ΔP , box pressure) before and after aeroallergen challenge (OA 20 mg/ml, 60 breaths, 20 psi, Lovelace nebulizer) of unrestrained guinea pigs in a bias flow ventilated whole body plethysmograph. In 3 animals prechallenge recordings were made for 2 h and in another 4 animals measurements were taken for 10 min before challenge following a 2-h equilibration period.

guinea pig have been depicted in Fig. 3. The pattern (wave form) of breathing is changed. These alterations included: 1) a marked increase in the box pressure amplitude; 2) a similarly large increase in the pseudoflow amplitude (Fig. 4), especially in the expiratory phase; 3) a decreased relaxation time, as evidenced by a sharp fall-off of the box pressure signal in expiration; and 4) increased incidence in apparent pauses or apneas at the end of expiration. The word "apparent" is used because the pseudoflow differs in form from the nasal flow during dyspnea. Thus there may in fact be air flow during the apparent pause period. It is not visible from the pseudoflow signal. The baseline values for the respiratory parameters, peak alterations in lung function, and time of peak responses have been summarized in Table 1. Aeroallergen challenge (ovalbumin 20 mg/ml, 60 s, 20 psi, Lovelace nebulizer) produced dyspnea and gasping which was accompanied with highly significant ($p \leq 0.001$) abnormalities in lung function, i.e. an increase in box pressure amplitude, slope of box pressure waveform, respiratory rate, product of respiratory rate and box pressure, and a concomitant decrease in relaxation time.

Further, the box pressure waveform of a single guinea pig was analyzed. The box pressure signal was designated as a volume, and its derivative, a flow. A flow-volume loop of a typical breath before challenge (thin inner loop) and after challenge (thick outer loop) has been depicted in Fig. 5. The characteristic shape of the loops allowed quantitative measurements of the respiratory parameters, reflect-

Table 1. Aeroallergen (OA 20 mg/ml, 60 s, 20 psi)-induced lung dysfunction in actively sensitized, boosted, freely moving guinea pigs (bias flow ventilation whole body plethysmography)

Respiratory parameters	Baseline values	Lung function norms		
		After aeroallergen challenge		
		Peak values	% Change	Peak time (min)
ΔF (ml/s)	10.9 $\pm$ 2.2	51.2 $\pm$ 9.2*	391 $\pm$ 134	15.2 $\pm$ 9.2
ΔP (ml)	1.6 $\pm$ 0.3	3.1 $\pm$ 0.7*	93 $\pm$ 35	11.0 $\pm$ 5.6
Respiratory rate (breaths/min)	103.1 $\pm$ 27.0	175.4 $\pm$ 10.6*	78 $\pm$ 37	8.3 $\pm$ 6.4
Respiratory rate $\times\Delta P$	161.1 $\pm$ 26.8	431.7 $\pm$ 101.3*	180 $\pm$ 100	17.1 $\pm$ 6.6
Relaxation time (s)	0.16 $\pm$ 0.03	0.05 $\pm$ 0.01*	-72 $\pm$ 3	17.3 $\pm$ 7.1

Values are means $\pm$ SD. (N=7); OA=Ovalbumin. ΔF (Pseudoflow signal derived from ΔP , box pressure)=the difference between the rate of change of the thoracic volume and the nasal flow. ΔP (Box pressure)=the difference between the thoracic expansion/contraction and the tidal volume. Relaxation time (s)=time it takes the peak box pressure signal to drop from its peak to 1/3 of its peak value. * $p\leq 0.001$ as compared to baseline values (normal lung parameters).

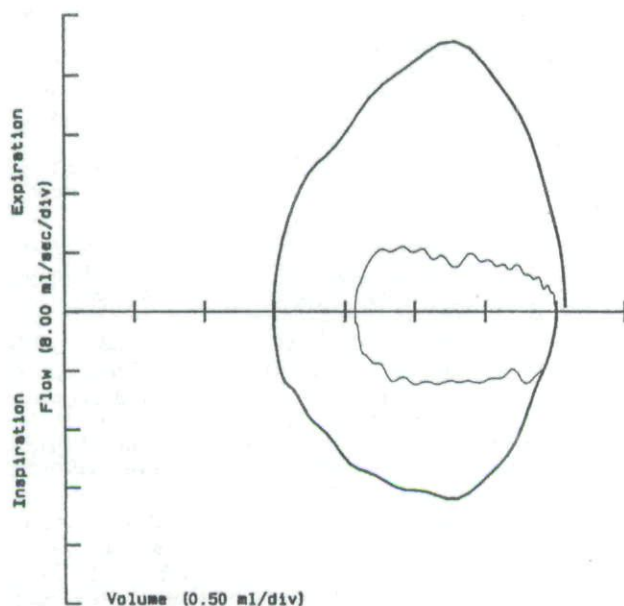


Fig. 5. Flow-volume loop comparing a typical individual breath taken before (thin inner loop) and after (thick outer loop) aeroallergen challenge (OA 20 mg/ml, 60 breaths, 20 psi, Lovelace nebulizer) of an unrestrained guinea pig in a bias flow ventilated whole body plethysmograph.

ing the changes induced by aeroallergen challenge (Table 2).

Discussion

The bias flow ventilated whole body plethysmographic technique and non-invasive analyzer allowed the characterization of lung dysfunction (dyspnea) during early asthmatic responses in freely moving guinea pigs. During visible dyspnea/gasping, these alterations included altered pattern of breathing and waveforms. These consisted of a 2-fold increase in the effort of breathing (box pressure amplitude), a 4-fold increase in the maximum slope of the box pressure waveform during expiration (the pseudo-

Table 2. Aeroallergen (OA 20 mg/ml, 60 s, 20 psi)-induced lung dysfunction analyzed by flow-volume loop in actively sensitized, boosted, freely moving guinea pigs (bias flow ventilated whole body plethysmography)

Respiratory parameters	Lung function norms		
	Before challenge values	After challenge values	% Change
V (ml)	1.42 $\pm$ 0.13	2.90 $\pm$ 1.09*	103 $\pm$ 74
TE (ms)	429 $\pm$ 100	189 $\pm$ 65*	-52 $\pm$ 25
TI (ms)	250 $\pm$ 36	154 $\pm$ 39*	-38 $\pm$ 14
Rate (breaths/min)	88.9 $\pm$ 22.4	182.1 $\pm$ 55.2*	117 $\pm$ 88
PIF (ml/s)	9.0 $\pm$ 1.1	32.3 $\pm$ 4.6*	260 $\pm$ 62
PEF (ml/s)	7.9 $\pm$ 1.0	39.9 $\pm$ 10.3*	403 $\pm$ 104
PEF/PIF	0.89 $\pm$ 0.17	1.23 $\pm$ 0.20*	40 $\pm$ 20
Area In	9.6 $\pm$ 2.1	72.7 $\pm$ 34.2*	685 $\pm$ 467
Area Ex	7.6 $\pm$ 1.5	89.9 $\pm$ 52.6*	1054 $\pm$ 614

Values are means $\pm$ SD. (N=7).

* $p\leq 0.005$ as compared to before challenge values.

V: Volume, i.e. box pressure pulse level. It is proportional to the tidal volume in the control period, is not proportional to tidal volume during the allergic response period, and is representative of respiratory effort during the response period.

TE: Expiration time. Respiratory period minus inspiratory time.

TI: Inspiratory time. From start of inspiration to end of inspiration.

Rate: Respiratory rate. PIF: Peak inspiratory flow. For this and all succeeding parameters, we selected breaths where V rose and fell the same amount during the respiratory cycle; i.e. $V_{\text{inspiration}} = V_{\text{expiration}}$. The "flow" signal is generated by differentiating V. Hence it is representative of actual nasal flow during control period, but not during response period. During the response period it represents respiratory effort, which seems to be proportional to nasal flow during inspiration, but does not look anything like nasal flow during the expiration. While it appears to be proportional to the inspiratory flow during the response, the proportionality factor is much greater than the proportionality factor during the control period.

PEF: Peak expiratory flow. Area In: Area within the flow volume loop, defined by the inspiratory phase relative to the volume axis. Area Ex: Area within the flow volume loop, defined by the expiratory phase relative to the volume axis. The flow-volume loops provides a simple and sensitive indicator of dyspnea, and its degree of severity.

flow), and increase in apparent pauses between respiratory cycles (a characteristic of bronchospastic state), and a concomitant decline in relaxation time in all 7 guinea pigs challenged.

During quiet breathing box pressure changes represent tidal volume, and the rate of change of box

pressure (represented as a pseudoflow) is closely related to air flow. A tremendous increase in box pressure and rate of change of box pressure occurs during allergic bronchoconstriction. Furthermore, box pressure and rate of change of box pressure no longer represent tidal volume and air flow but represent the effort of breathing, i.e. differences in the chest movement and air flow, where little or no air movement is taking place. In other words, dyspnea is characterized by marked chest movement (increase in ΔP and ΔF) without concomitant airflow, and sharper or pulsatile expiratory movements, i.e. 72% reduction in relaxation time (the rate at which the box pressure wave descends during expiration).

The increase in box pressure relative to tidal volume during a response has been noted by Silbaugh & Mauderly (10). They measured dynamic compliance at the same time as box pressure, in a restrained animal. They observed that the ratio of tidal volume to box pressure seemed to relate directly to dynamic compliance. Thus when box pressure increased relative to tidal volume, compliance decreased.

Our observation agrees that box pressure increases during the response. We also observe that the pseudoflow amplitude increases even more than the box pressure amplitude, and the expiratory amplitude increases more than the inspiratory amplitude. Thus the amplitude is a function of the time delay between the thoracic and nasal flow components that make up the pseudoflow signal. We suggest that the amplitude of the expiratory phase of the pseudoflow signal may be related to specific airway resistance.

Thorne & Karol (12) used pyrilamine-treated, restrained inbred guinea pigs (strain 2) and demonstrated a significant increase in respiratory rate (IAR) in 6/7 animals, 4.5 ± 1.4 min, 66% increase; and LAR [late asthmatic response] in 4/7 animals, peak 4.5 ± 2.4 h; 83% increase) to extremely high doses of aeroallergen challenge. In the present study, Charles River unrestrained, outbred guinea pigs exhibited dyspnea and gasping immediately after challenge, peaking around 10 min which was associated with abnormal lung function changes. Factors, such as strain (heredity, genetics), state of health, season, sensitization procedure (aerosol/i.p.), pretreatment with antihistamines, dose and duration of antigen challenge, restraining, recording system, etc. may be important determinants of the type of lung abnormalities (onset, peak, duration, etc.) observed during allergic reactions in animals.

In conclusion, non-invasive measurement of lung function by bias flow ventilated whole body plethysmography in unrestrained guinea pigs may be used to characterize IAR, delayed IAR or LAR, and other lung function abnormalities associated with bronchial inflammation and airway hyperreactivity. The alterations in lung function measurements due to handling, stress, increases in CO_2 /decreases in O_2 (closed unventilated system), restraining (Pennock method), and compromising the animal with anesthesia, surgery, etc. are avoided in the technique utilized in this study.

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