

special communication

Forced expiration: a test for airflow obstruction in horses

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Couëttil, Laurent L., Frank S. Rosenthal, and Chris M. Simpson. Forced expiration: a test for airflow obstruction in horses. *J Appl Physiol* 88: 1870–1879, 2000.—The purpose of this study was to assess whether our method of inducing forced expiration detects small airway obstruction in horses. Parameters derived from forced expiratory flow-volume (FEFV) curves were compared with lung mechanics data obtained during spontaneous breathing in nine healthy horses, in three after histamine challenge, and in two with chronic obstructive pulmonary disease (COPD) pre- and posttherapy with prednisone. Parameters measured in the healthy horses included forced vital capacity (FVC = 41.6 ± 5.8 liters; means \pm SD) and forced expiratory flow (FEF) at various percentages of FVC (range of 20.4–29.7 l/s). Histamine challenge induced a dose-dependent decrease in FVC and FEF at low lung volume. After therapy, lung function of the two COPD horses improved to a point where one horse had normal lung mechanics during tidal breathing; however, FEF at 95% of FVC (4.9 l/s) was still decreased. We concluded that FEFV curve analysis allowed the detection of induced or naturally occurring airway obstruction.

respiratory function; bronchial challenge

MEASUREMENT OF LUNG MECHANICS during tidal breathing is one of the most commonly used tests of respiratory function in the horse, particularly in the study of chronic obstructive pulmonary disease (COPD) or heaves. However, the method appears to lack sensitivity and may not detect differences in the lung function of normal horses, horses with COPD during clinical remission, early cases of COPD, and horses with milder forms of chronic lung disease such as inflammatory airway disease (6, 23). Therefore, there is a need for a sensitive test of peripheral airway obstruction that provides quantitative data on structural changes in the lung.

Forced expiration (FE) is one of the most useful and commonly used lung function tests for the early detec-

tion of small airway disease in humans (26). Maneuvers can be performed in animals, but they demand general anesthesia to avoid any interference of conscious respiratory movements during lung emptying. One method induces FE by connecting the subject's airways to a vacuum reservoir while the body is enclosed in a plethysmograph. Forced expiratory flow (FEF) is measured indirectly by a pneumotachograph that serves as the only opening to room air of the plethysmograph (15, 22).

In previous studies, FE maneuvers have been performed in the anesthetized horse using the vacuum method (9, 15). The horse was suspended upright in a body plethysmograph, and its airways were connected to a vacuum reservoir via a tracheostomy tube. The purpose of the present study was to evaluate the suitability of FE maneuvers in sedated, but not anesthetized, horses using a minimally invasive and non-plethysmographic method. With the use of this method, FE was induced after lungs were inflated to total lung capacity (TLC) by exposing the horses' airways to a vacuum reservoir via a nasotracheal tube. Flow was measured indirectly by computing the instantaneous changes in pressure in the vacuum reservoir.

To evaluate this method of generating FE data, we collected forced expiratory flow-volume (FEFV) curves in normal horses and those with COPD. The following features of this method were assessed: 1) practical implementation in awake, sedated horses, 2) intrasubject reproducibility, 3) the effect of driving pressure (i.e., "effort" dependence) on the results, 4) the ability of the method to detect histamine-induced airflow obstruction, and 5) the ability of the method to distinguish lung function in COPD vs. normal horses.

MATERIALS AND METHODS

Horses

Nine healthy horses [7 Standardbreds, 1 Appaloosa, and 1 Quarter Horse; weight = 480.3 ± 42.6 (SD) kg; age = 8.3 ± 4.3 yr] were studied. All horses were free of respiratory disease for at least 6 mo before the study, and none had a history of chronic or recurrent lung disease.

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Lung function tests were also performed in two horses diagnosed with COPD before and after therapy with oral prednisone. The diagnosis of COPD was based on a history of chronic (>2 yr) coughing, mucopurulent nasal discharge, and recurrent episodes of increased respiratory rate and effort. At the time of the study, both horses exhibited clinical signs consistent with acute COPD crisis. On the basis of clinical examination and lung mechanics' data, one horse was mildly affected (*horse A*) and the other was severely affected (*horse B*).

General Protocol

All tests were performed in a temperature-controlled room (20°C). Each horse was restrained in stocks and fitted with an esophageal balloon catheter, facemask, and flowmeter. Recording of data started when the horse was standing quietly and lasted at least 2 min. After collection of lung mechanics data during spontaneous breathing, the horse was sedated. Five minutes later, a nasotracheal tube was fitted into place and mechanical ventilation was initiated. On average, FE maneuvers were started 15 min after sedative administration and were repeated until at least three reproducible FEFV curves were obtained. Mechanical ventilation was then discontinued, and the horse was allowed to breathe spontaneously. The average session included four FE and lasted 25 min from the time of sedative administration to the completion of the last FE. Bronchoprovocation testing consisted of a series of two FE maneuvers per histamine dilution and lasted between 40 and 50 min. An institutional animal care and use committee approved all procedures.

Lung Mechanics During Spontaneous Breathing

Methods used to measure pulmonary mechanics in the horses were similar to previous descriptions by other investigators (7). Esophageal pressure was measured by means of a condom sealing the distal end of a Teflon catheter (4.8 mm ID, 6.4 mm OD, 240 cm long). An airtight mask was fitted around the horse's nose. The proximal end of the esophageal catheter was connected to a pressure transducer (DP/45-30, Valydine Engineering, Northridge, CA). The other side of the pressure transducer was connected to the facemask via a similar catheter. Transpulmonary pressure (PL) was defined as the pressure difference between mask and esophageal pressure. The port of the mask held the pneumotachometer (no. 4 Fleisch, EMKA Technologies, Paris, France) coupled with a differential pressure transducer (DP/45-14, Valydine Engineering) by two identical Teflon catheters. The output signals from the pressure transducers were recorded simultaneously by a computer (Pulmonary Mechanics Analyzer, XA version, Buxco Electronics, Sharon, CT). Measurements were collected for a minimum of 2 min to obtain at least 10 representative breaths that were selected for data analysis. Pulmonary resistance (RL) and dynamic compliance (Cdyn) were computed using the method of Amdur and Mead (1). Signals from pressure and flow transducers were phase matched, and calibrations were performed according to standard techniques.

FE Apparatus

The experimental apparatus used to induce FE maneuvers is illustrated in Fig. 1. The negative pressure reservoir was composed of three steel tanks (Skolnik Industries, Chicago, IL) connected by a manifold constructed of polyvinyl chloride (PVC) pipe (5.1 cm ID). The tanks, together with the manifold, provided 1,432.6 liters for the vacuum reservoir. A standard gas cylinder (55 liters) was connected by PVC pipe (1.9 cm ID) to the manifold and was used as a vacuum

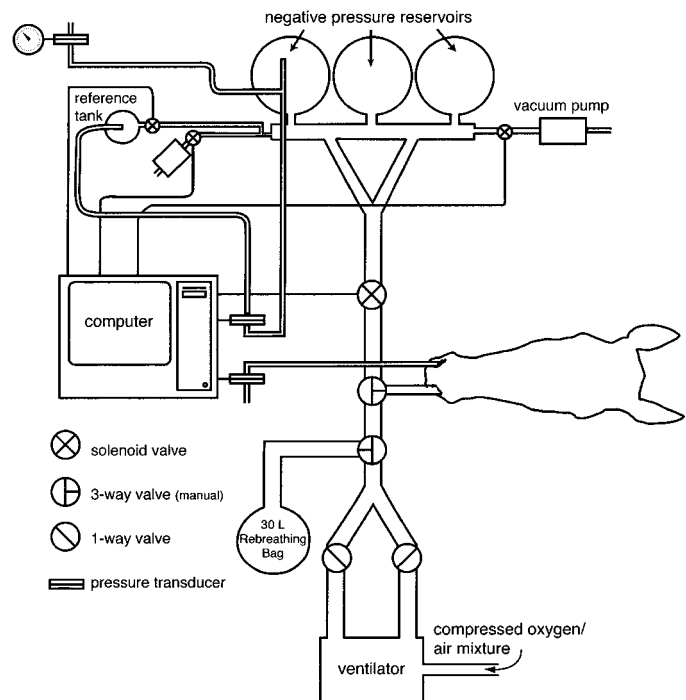


Fig. 1. Diagram of experimental setup to perform forced expiratory maneuvers in the horse.

reference tank against which pressure changes were measured. A solenoid valve (Asco Red Hat II, 0.6 cm ID, Automatic Switch, Florham Park, NJ) was used to isolate the reference tank from the manifold once the entire apparatus (vacuum reservoir and reference tank) had been pumped down to the appropriate subatmospheric pressure. Two additional solenoid valves were used to close off the manifold from the pumps used to evacuate air from the system (vacuum pump, 92 kPa, 91 l/min, Fischer Scientific, Pittsburgh, PA). Each vacuum pump and its corresponding solenoid valve were operated as a unit on individual electrical circuits. The manifold ended at the FE valve, which was a large, high-flow solenoid valve (Asco Red Hat, 3.8 cm ID, Automatic Switch). The FE valve and the reference tank isolation valve were computer controlled.

The vacuum pumps and solenoid valves were controlled by a pressure control device (Photohelic, Dwyer Instruments, Michigan City, IN). The device measured the vacuum pressure and, when enabled, pumped the vacuum chamber down to the selected pressure. Solid state relays were used to accept the digital output from the analog-to-digital conversion board in the computer. These solid state relays, together with electromechanical relays, were used to provide computer control of the isolation valve and the FE valve. Manual control of these valves was provided by toggle switches.

A pressure transducer (Celesco, Transducer Products, Canoga, CA) was used to measure the differential pressure between the reference tank and the vacuum reservoir. The output from the transducer was conveyed to the analog-to-digital conversion board.

FE Maneuver

Protocol. The facemask was removed from the horse after collection of pulmonary mechanics data, but care was taken to leave the esophageal catheter in place. The horse was then sedated with a combination of detomidine hydrochloride (0.03 mg/kg iv) and butorphanol tartrate (0.02 mg/kg iv). Five

minutes later, a tube (20 mm ID, 26.5 mm OD, 90 cm long; Cook Veterinary Products, Bloomington, IN) was introduced transnasally down to the proximal third of the trachea. The head was kept elevated and the neck was extended in a standardized fashion throughout FE maneuvers. The proximal end of the nasotracheal tube was connected to a three-way valve (22 mm ID, model 2100B, Hans Rudolph, Kansas City, MO) with ports leading either to a ventilator (NELAC, North American Dragger, Telford, PA) or to the FE valve of the vacuum reservoir ($-220 \text{ cmH}_2\text{O}$). Mechanical ventilation was initiated at a tidal volume of 6 liters and frequency of 8 breaths/min, using an oxygen-air gas mixture (inspired O_2 fraction of $>70\%$). Ventilatory parameters were designed to maintain end-tidal $\text{CO}_2 < 35 \text{ Torr}$. The FE was induced by an adaptation of a method previously used in dogs (22). Initially, the FE valve was in the closed position. The horse was hyperventilated for at least 10 min to decrease its respiratory drive. The lungs were then inflated to TLC by manually compressing a 30-liter latex bag (large rebreathing bag, Rusch, Duluth, GA). TLC was defined as the lung volume at $30 \text{ cmH}_2\text{O PL}$. The selection of peak inflation pressure of $30 \text{ cmH}_2\text{O}$ was based on the observation that volume-pressure curves generated during passive lung inflation reached a plateau around that value (15). The three-way valve was then turned in the direction of the pressure reservoir. Airways were suddenly exposed to the negative pressure reservoir by opening the FE valve, inducing FE.

The average time between sedative administration and first FE was $\sim 15 \text{ min}$. Between 6 and 8 s were needed to inflate the lungs to TLC, turn the three-way valve, and open the FE valve. Typical sessions included four FE maneuvers and lasted $\sim 22\text{--}25 \text{ min}$ from the time of sedative administration to completion of the last maneuver.

FEFV curve. Calculation of expired volume from tank pressure measurement is detailed in the APPENDIX. Instantaneous flow rate was determined as follows: instantaneous flow rate = $\Delta V/\Delta t$, where ΔV is the difference in volume of air leaving the lung between the beginning and end of one sampling interval in the digital data acquisition system and Δt is the duration of sampling interval. FEFV curves were produced by combining the flow vs. time and volume vs. time data, on a point-by-point basis.

Analysis of the FEFV curve yielded forced vital capacity (FVC); forced expiratory volume in 1 s (FEV_1); forced expiratory flow at 75, 80, 85, 90, and 95% of exhaled vital capacity ($\text{FEF}_{75\%}$, $\text{FEF}_{80\%}$, and so forth); and peak expiratory flow (PEF). The maneuver was repeated until at least three acceptable and repeatable FEFV curves were obtained for each horse. Curves were acceptable if they were free of artifacts (e.g., cough, inspiratory effort) and if a plateau of at least 1 s was observed in the volume-time curve. After three acceptable curves were obtained, they were considered reproducible if the two largest FVC were within 5% of each other and if the two largest FEV_1 were within 5% of each other. Forced expiratory parameters were measured from the curve, which gave the largest sum of FVC plus FEV_1 . Lung volumes were expressed at BTPS according to the standard equation (26). All parameters of the FEFV curves were compared with height and weight measurements in each horse.

Calibration. To verify Eq. A8 (see APPENDIX) and to evaluate the effect of the assumptions made in its derivation on its accuracy, the reservoir system was used together with Eq. A8 to evaluate known volumes of air "exhaled" into the reservoir. Latex bags were filled with known volumes of air using a 3-liter calibrated syringe and a three-way valve. The change in pressure in the reservoir due to the exhalation was measured as described in FEFV curve and APPENDIX. The

change in reservoir temperature due to the exhalation was measured by placing a light-weight thermocouple device (type IT-23, nominal time constant in liquid = 0.01 s; Physitemp, Clifton, NJ) in the pressure reservoir at a position close to the inlet for the pressure transducer.

The temperature and pressure of the reservoir (T_r and P_r , respectively) were recorded immediately before FE of the bag into the reservoir and at 3 s after the start of exhalation, when the bag was fully collapsed and the pressure and temperature had reached a plateau. The volume predicted by Eq. A8 was compared with the known volume of air in the bag at the start of the experiment (Table 1). A linear regression of predicted volume vs. bag volume found that predicted volume was equal to $0.98 (\text{bag volume}) + 3.6$ ($r^2 = 1.00$, $P < 0.00001$). The intercept in this equation was attributed to the volume of air in the tubing connecting the bag to the reservoir.

The calibration data also found that temperature rise was highly correlated with the bag volume ($r = 0.99$, $P < 0.00001$). The proportionality of temperature (T) rise with volume is expected, since the temperature rise is due to the heat dissipated by the work done on the gas as the air enters the reservoir, which in turn is proportional to the volume of air entering the reservoir. If we assume, therefore, that $dT = k \cdot dV$, where k is a constant, then by substituting in Eq. A8 we have

$$dV_L = (V_r)(P_r/P_L)(T_L/T_r)(dP_r/P_r - kdV_L/T_r) \quad (1)$$

$$dV_L[1 + (k/T)(V_r)(P_r/P_L)(T_L/T_r)] = (V_r)(P_r/P_L)(T_L/T_r)(dP_r/P_r) \quad (2)$$

$$dV_L = \frac{(V_r)(P_r/P_L)(T_L/T_r)}{[1 + (k/T_r)(V_r)(P_r/P_L)(T_L/T_r)]} \times (dP_r/P_r) \quad (3)$$

Equation 3, together with the assumptions that the changes in P_r , P_L , T_r , and temperature in the lungs (T_L) are small compared with the initial values, shows that the volume of air leaving the lungs is approximately proportional to the fractional change in P_r . A regression of bag volume vs. dP_r/P_r in the calibration data ($r^2 = 0.995$, $P < 0.0001$) found that

$$dV_L (\text{liters}) = 845.3(dP_r/P_r) - 2.4 \quad (4)$$

A deviation from strict proportionality is indicated by the intercept in Eq. 4. This was attributed to the effect of air at room pressure in the tubing between the bag and the reser-

Table 1. Bag volume predicted by Eq. A8, using reservoir temperature and pressure measurement, compared with known volume of air in the bag before a forced exhalation

Bag Volume, liters	ΔT , $^\circ\text{C}$	Predicted Volume, liters	Predicted Volume - Bag Volume, liters
10	0.8	13.5	3.5
10	0.8	13.2	2.2
15	1.3	18.0	3.0
15	1.2	18.2	3.2
20	1.8	22.6	2.6
20	1.8	24.4	4.4
25	2.2	27.8	2.8
25	2.1	28.0	3.0
30	2.6	33.1	3.1
30	2.6	32.8	2.8

ΔT , increase in reservoir temperature recorded between the start of exhalation and 3 s after.

voir, which was not taken into account in the derivation of Eqs. 3, A8, and A9. It was assumed that the effect of the air in the tube on Pr occurred early in the FE and after a relatively brief initial period did not affect the relationship of expired volume to further changes in Pr. Thus, after this initial period, changes in lung volume are approximated by

$$dV_L \text{ (liters)} = 845.3(dPr/Pr) \quad (5)$$

Equation 5 was used in conjunction with the instantaneous measurement of Pr to provide an estimate of the volume of air leaving the lung (i.e., the forced expired volume) as a function of time.

Effect of driving pressure and tube size. In four horses, lungs were inflated to TLC and FE maneuvers were performed at progressively lower Pr (-60 cmH₂O to -320 cmH₂O) to simulate an increased expiratory effort. FEFV curves that had different driving (i.e., reservoir) pressure were then compared. The influence of tubing diameter on maximal flow rates was examined. FE maneuvers were performed at a Pr of -220 cmH₂O on a horse fitted with nasotracheal tubes of 18, 20, and 22-mm ID successively. The resulting FEFV curves were then compared.

Bronchial challenge. Three horses were studied with histamine bronchial challenge. After two acceptable FE maneuvers, histamine aerosol was delivered to the horse using a jet nebulizer (Pulmo-Aide, DeVilbiss, Somerset, PA) that produced fine particles with mass median aerodynamic diameters of <5 μ m. We measured the output of the nebulizer to be ~ 0.015 ml per 6-liter breath. The order of challenges was normal saline (control) followed by histamine disphosphate in saline solution at 8, 32, 64, and 128 mg/ml. The nebulizer was switched on while the horse was mechanically ventilated, and sixteen 6-liter breaths containing aerosol were delivered for each dilution (0.24 ml over 2 min). FE maneuvers were performed 30 and 120 s after aerosol delivery. The next aerosol challenge was started 6 min after the end of the previous challenge. Histamine challenge was stopped when FEV₁ fell by at least 20% or after administration of the highest concentration (128 mg/ml). The percent fall in FEV₁ for a given histamine concentration was calculated as the difference between the mean baseline FEV₁ and the lowest FEV₁ postchallenge expressed as a percentage of mean baseline FEV₁. We plotted dose-response curves of FEV₁ as a function of histamine dose. The provocative concentration of histamine required to decrease FEV₁ by 20% (PC₂₀) was determined by interpolation between points on the dose-response curve. In addition, histamine dose-response curves were plotted for different FEV_x with $x = 1.3, 1.5, 1.7, 2.0, 2.5,$ and 3.0 s. The provocative concentration of histamine required to decrease FEV_x by 20% (PC_{20,x}) was calculated.

COPD horses. Lung mechanics during spontaneous breathing and FE data were collected in two COPD horses before and after 14 days of treatment with prednisone (1 mg/kg po; every 12 h).

RESULTS

Lung Mechanics During Spontaneous Breathing

Results of the lung function tests obtained before FE maneuvers are summarized in Table 2. Data collected, such as maximum change in PL ($\Delta P_{L_{max}}$), tidal volume, RL, and C_{dyn}, were within the range of previously published measurements of normal horses (23, 27). Between- and within-horse data variability was large. During testing, horses breathed at frequencies ranging from 9 to 16 breaths/min.

Table 2. Summary of pulmonary function tests performed in 9 healthy horses compared with published data for forced expiratory parameters (plethysmographic method) and predicted measurements of vital capacity

Parameters	Mean \pm SD	Range
<i>Lung mechanics during spontaneous breathing</i>		
$\Delta P_{L_{max}}$, cmH ₂ O	5.7 \pm 1.5	3.9–8.0
V _T , liters	6.4 \pm 1.5	4.9–8.5
RL, cmH ₂ O \cdot l ⁻¹ \cdot s	0.56 \pm 0.31	0.29–1.14
C _{dyn} , l/cmH ₂ O	2.28 \pm 0.65	1.01–3.08
<i>Forced expiratory parameters</i>		
FVC, liters	41.6 \pm 5.8	33.1–48.2
FEC ₁ , liters	31.9 \pm 0.8	30.6–32.8
FEV ₁ /FVC	0.78 \pm 0.10	0.67–0.93
PEF, l/s	37.0 \pm 0.3	36.6–37.5
FEF _{75%} , l/s	28.4 \pm 0.8	27.3–29.7
FEF _{80%} , l/s	27.8 \pm 0.9	26.2–29.0
FEF _{85%} , l/s	27.4 \pm 1.3	25.7–29.3
FEF _{90%} , l/s	26.4 \pm 1.6	24.4–28.8
FEF _{95%} , l/s	23.4 \pm 2.2	20.4–27.0
<i>Plethysmographic data</i>		
Leith and Gillespie (16)		
FVC, liters ($n = 6$)	33	
PEF, l/s ($n = 4$)		60–90
Gillespie (9)		
FVC, liters ($n = 1$)	34	
PEF, l/s ($n = 1$)	90	
Leith (15)		
FVC, liters ($n = 1$)	36	
<i>Predicted measurements</i>		
VC (a), liters	32.8 \pm 3.0	27.0–37.1
VC (b), liters	30.3 \pm 2.7	25.0–34.1

$\Delta P_{L_{max}}$, maximum change in transpulmonary pressure; V_T, tidal volume; RL, pulmonary resistance; C_{dyn}, dynamic lung compliance; FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 s; PEF, peak expiratory flow; FEF_{75%}, flow at 75% of exhaled vital capacity; VC, a and b, vital capacity prediction according to Leith (15a) using equations from Stahl and Drorbaugh, respectively.

FE Maneuver

Protocol and safety. The maneuvers were well tolerated by the horses, and no deleterious side effects were detected with a Pr of -220 cmH₂O. Mild bronchial erythema and submucosal petechia were occasionally observed during bronchoscopic examinations, which were performed within 30 min of the last FE. Horses subjected to FE with Pr between -250 and -315 cmH₂O had evidence of moderate to severe submucosal petechiation. Mild hemorrhages within central airways (trachea, mainstem bronchi) were observed at the reservoir's lowest pressures. All abnormalities were resolved within 48 h.

Tracheal wall pressure imposed by the endotracheal tube cuff averaged 43 ± 14 cmH₂O. No adverse effects related to cuff pressure were noted during follow-up endoscopic examination.

Flow-volume curve data. Measurements derived from analysis of the FEFV curves are summarized in Table 2. The shapes of the flow-volume curves were similar among the nine horses and were characterized by an abrupt rise in flow to a peak value (PEF) immediately

after opening of the solenoid valve (Fig. 2). A plateau with small negative slope followed. Finally, flow decreased steeply when the last 10% of FVC was emptied from the lungs. Flow rates were almost identical among all horses up to 90% of FVC, suggesting that the nasotracheal tube constrained maximal flow rates during this portion of the FE.

FEFV curves obtained from the same horse were highly reproducible. Data collected from three consecutive FE in each of the nine horses yielded coefficients of variation <2.5% for FEV₁ and <9.5% for the other variables (Table 3). In addition, coefficients of variation for variables from nine FEFV curves obtained in three horses over a 3-wk period were <10% (Table 3).

In the group of control horses, body weight was significantly correlated with FEV₁ ($r = 0.83$), FEF_{90%} ($r = 0.76$), and FEF_{95%} ($r = 0.73$, $P < 0.05$); however, it was not significantly correlated with FVC. There was no significant correlation between horses' height and FEFV curve parameters.

Effect of driving pressure and tube size. FEF at 75, 80, 85, 90, 95% of forced expired volume increased as Pr was decreased from -60 to -250 cmH₂O. This is illustrated in Fig. 3, which represents data collected from one of the four horses tested. Decrease in Pr below 220–250 cmH₂O resulted in little or no increase in FEF values. FEF_{90%} appeared to plateau as Pr was decreased from -250 to -320 cmH₂O, whereas FEF_{95%} showed a marked decrease when Pr was decreased below 220 cmH₂O. This decrease was consistent with peripheral airway collapse at low lung volume. On the basis of these results, a Pr of -220 cmH₂O was chosen for the FE maneuvers. This was considered high enough to achieve flow limitation without precipitating excessive airway collapse or the possibility of airway injury.

FEFV curves obtained from a horse fitted successively with nasotracheal tubes of decreasing diameter showed that, for a constant driving pressure, maximal flow rates increased as tube diameter increased until

Table 3. Coefficients of variation calculated for parameters of 1) 3 consecutive flow-volume curves collected from 9 horses and 2) 9 flow-volume curves collected from 3 horses over a 3-wk period

	Coefficient of Variation, %			
	Parameter 1 (n=27)		Parameter 2 (n=27)	
	Mean	Range	Mean	Range
FVC	3.4	0–6.5	6.6	5.2–8.5
FEV ₁	1.0	0–2.2	4.7	1.5–8.4
PEF	2.0	0.5–3.5	4.8	3.6–7.3
FEF _{75%}	2.4	0.7–5.2	4.7	3.8–5.4
FEF _{80%}	2.9	0.8–6.0	4.8	3.6–6.2
FEF _{85%}	3.0	0.9–6.8	3.8	2.9–5.7
FEF _{90%}	2.7	0.4–4.5	5.2	4.6–6.1
FEF _{95%}	4.6	1.5–9.3	8.0	4.3–9.9

90% of FVC had been evacuated from the lungs (Fig. 4). During evacuation of the remaining volume, FEF did not change with tube diameter.

Bronchial Challenge

PC₂₀ values for the three horses were 41.7 (*horse 3*), 62.5 (*horse 9*), and 120.3 (*horse 1*) mg/dl histamine. PC_{20-x} values were 15–24% lower than PC₂₀ values. Dose-response curves for FEV_{1.3} and FEV_{1.5} provided the lowest provocative concentration of histamine and therefore were the most sensitive parameters to detect the onset of histamine-induced bronchoconstriction. A typical family of FEFV curves recorded during *horse 9* histamine challenge is shown Fig. 5. As the dose of histamine increased, the abrupt decrease in flow occurred at a progressively higher lung volume and FVC decreased. Maximal flow rates before the acute drop in flow were unaffected. At higher histamine concentrations, the terminal portion of the FEFV curve, after the steep decrease in flow, was characterized by a tail with slowly diminishing flow rates as a function of expired

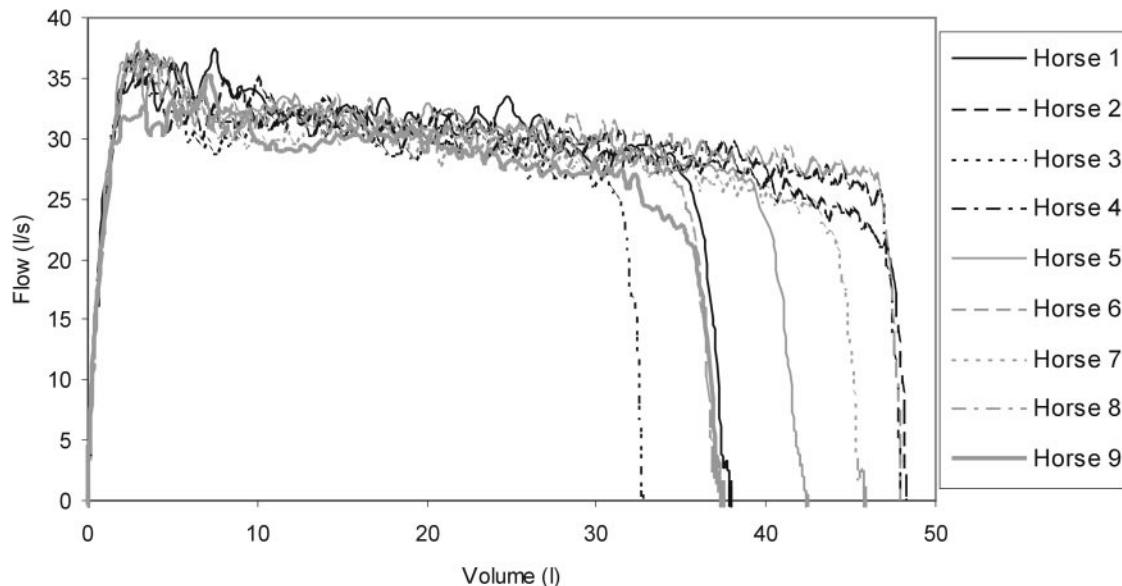


Fig. 2. Forced expiratory flow-volume curves recorded in 9 healthy horses.

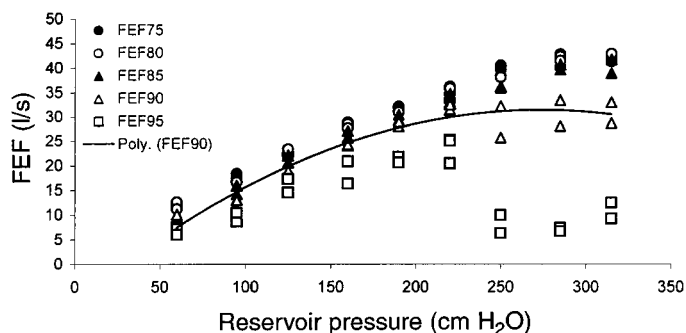


Fig. 3. Effect of reservoir pressure on forced expiratory flows (FEF) in 1 horse. Data from each of 2 forced expirations at each reservoir pressure are shown. Reservoir pressure is expressed in cmH_2O below atmospheric pressure. FEF_{75%}, flow at 75% of exhaled vital capacity; Poly (FEF90), curve that best fit data points for FEF_{90%}.

volume. After administration of the highest histamine concentration, FEV₁ decreased by 22, 54, and 41% for horses 1, 3, and 9, respectively.

COPD Horses

Pretreatment pulmonary mechanics data collected in horses A and B (Table 4) indicated that $\Delta P_{L_{\max}}$ was increased and C_{dyn} decreased compared with control data (Table 2). In addition, RL in the severely affected horse (horse B) was higher than in controls. Two weeks of oral prednisone therapy, combined with management changes, resulted in an improvement of clinical signs and lung mechanics data such as RL, C_{dyn}, and $\Delta P_{L_{\max}}$ in both horses (Table 4). After therapy, lung mechanics data from the mildly affected horse (horse A) were within the reference range and physical examination was unremarkable. FE data of horse A also revealed an improvement of all parameters after therapy; however, FEF_{90%} and FEF_{95%} were still below the reference range (Table 4, Fig. 6).

After therapy, lung mechanics data of the severely affected horse (horse B) were still outside of the reference range except for C_{dyn} (Table 4). Clinical examina-

tion revealed mild elevation in respiratory rate (20 breaths/min) and abnormal lung sounds. Ventilatory capacity parameters of horse B improved with therapy, but only FVC and FEV₁/FVC were within the reference range. Posttreatment FEF data were still dramatically reduced compared with control values.

DISCUSSION

The FE method is a promising method for detecting airflow obstruction in horses. FE maneuvers were safe, minimally invasive, and readily performed in sedated horses with or without respiratory disease. FEFV curves were repeatable and similar in shape to those in previous studies. In control horses, FEF was limited by nasotracheal tube diameter for up to 90% of FVC. During the later part of the FEFV curve, FEF was dependent on airway caliber and/or compliance and not driving pressure. Induced and naturally occurring peripheral airway obstruction was sensitively detected by analysis of the FEFV curve. Mild peripheral airway obstruction appeared as decreased FEF at low lung volume. Severe airway obstruction manifested as decreased FEV₁, FVC, and FEF over a broad range of lung volumes.

The main effects of sedation (xylazine, detomidine) on lung function appear to be related to an increase in upper airway resistance (13, 14, 24). In addition, xylazine administration reduces lower airway obstruction in ponies with acute signs of COPD but not during clinical remission or in healthy ponies (2). In our study, we used a combination of detomidine and butorphanol for sedation and a nasotracheal tube to bypass the upper airways. Therefore, we anticipated that, given the experimental conditions, sedation had little to no effect on FEFV curves obtained in our normal horses. In COPD horses or normal horses during histamine challenge, sedation may have attenuated airway smooth muscle contraction, resulting in an improvement of FE parameters.

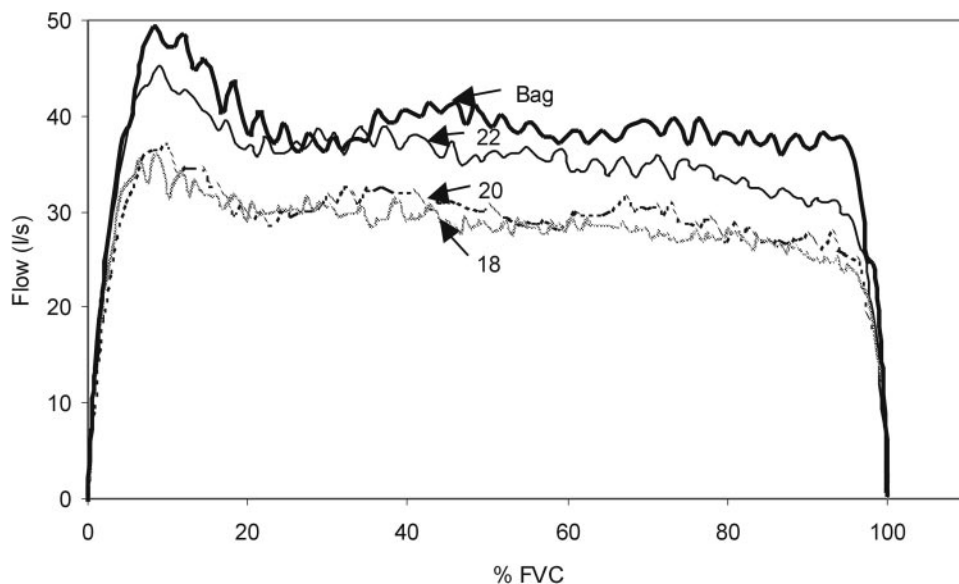


Fig. 4. Flow-volume curves (FVC) obtained in a horse fitted successively with nasotracheal tubes of decreasing diameter (22, 20, and 18 mm ID). A flow-volume curve obtained by emptying a 30-liter bag using the same setup (except nasotracheal tube) is shown for comparison purposes (trace labeled Bag).

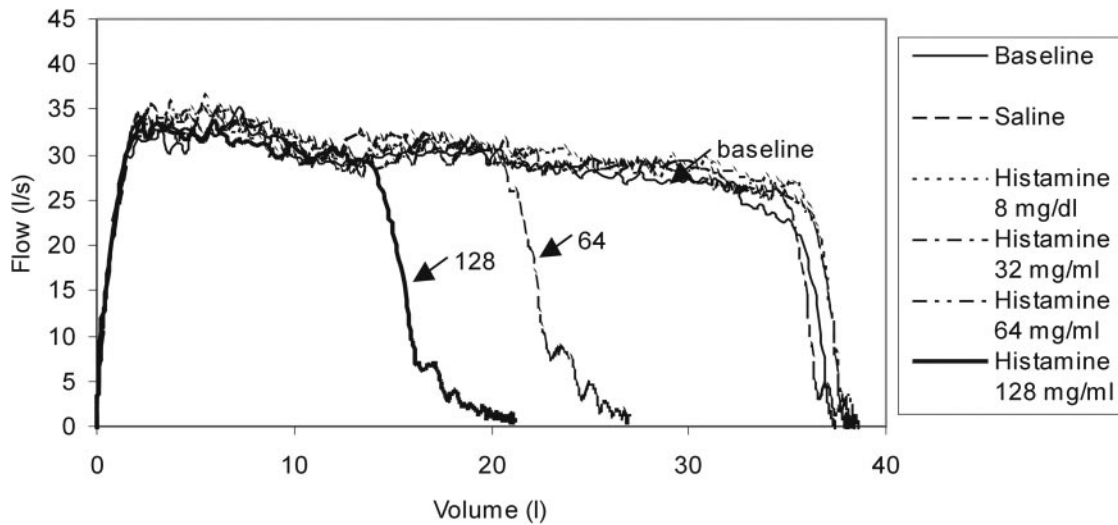


Fig. 5. Family of flow-volume curves obtained in a control horse (*horse 9*) during a bronchial challenge with 0–128 mg/dl histamine.

Flow-Volume Curves in Control Horses

The shape of the FEFV curve obtained with our method was similar to curves obtained by plethysmographic measurement of flow (9). Three different phases were recorded consistently in all horses. The first phase was a steep rise in flow to a peak value as soon as airways were exposed to the subatmospheric Pr. The second phase was a relative plateau with a mild negative slope over most of FVC. This was followed by a third and final phase characterized by a rapid decrease in flow. FEFV curves recorded in horses by plethysmography exhibit the same three phases (9).

The nasotracheal tube used in our study was the smallest cross section of the FE device and limited maximal flow rates during most of the FE (Fig. 4). Maximum expiratory flow rates recorded in our experi-

ment (23–37 l/s) were lower than the ones reported by Leith and Gillespie (60–90 l/s) (16). The main difference between the two methods is that we used a 20-mm ID nasotracheal tube and Leith and Gillespie used a tracheostomy tube of ~50-mm ID (personal communication, 1999). According to Bernoulli’s theorem, when tube diameter decreases from 50 to 20 mm, flow rate should be 84% lower. Maximum expiratory flow rates in our study were 38–74% lower than those reported by Leith and Gillespie. Therefore, differences in tube size accounted for most of the difference in FEF observed between the two studies.

The length of the flow plateau between PEF and FEF_{95%} was a result of flow limitation by the nasotracheal tube. In humans exhaling against a resistor, the FEFV curves display a similar plateau (11). The slight negative slope of the plateau observed in horses could be accounted for by the decrease in driving pressure occurring during the FE maneuver.

The descending limb of the FEFV curve, or third phase, is very steep in horses. This is sometimes the case in normal people and dogs, but, in addition, a fourth phase is generally recognized in which flow decreases at a much smaller rate down to zero (17, 21).

Table 4. Summary of pulmonary functions tests performed pre- and posttreatment in 2 horses with mild (*horse A*) and severe (*horse B*) chronic obstructive pulmonary disease

Parameters	Horse A		Horse B	
	Pre	Post	Pre	Post
<i>Lung mechanics during spontaneous breathing</i>				
$\Delta P_{L_{max}}$, cmH ₂ O	12.5	6.1	26.3	13.8
V _T , liters	5.0	5.0	3.4	3.4
R _L , cmH ₂ O · l ⁻¹ · s	0.92	0.5	2.56	2.17
C _{dyn} , l/cmH ₂ O	0.85	1.6	0.51	1.32
<i>Forced expiratory parameters</i>				
FVC, liters	37.6	37.9	27.6	38.4
FEV ₁ , liters	29.2	32.6	18.9	28.7
FEV ₁ /FVC	0.78	0.89	0.68	0.75
PEF, l/s	33.4	39.6	30.7	35.7
FEF _{75%} , l/s	27.0	29.6	6.4	11.9
FEF _{80%} , l/s	26.7	29.5	4.8	8.9
FEF _{85%} , l/s	19.6	28.3	4.1	5.6
FEF _{90%} , l/s	8.0	23.7	3.4	3.9
FEF _{95%} , l/s	1.8	4.9	2.6	1.8

Pre, before therapy; Post, after therapy.

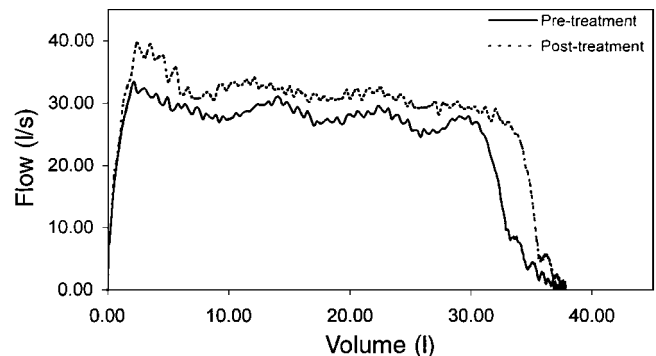


Fig. 6. Maximal flow-volume curves from a horse (*horse A*) with mild signs of chronic obstructive pulmonary disease before and after 2-wk therapy with prednisone and environmental changes.

Abrupt decrease in flow seems to be related to a sudden relocation of the choke point upstream, toward more peripheral airways (4, 21). The shape of the FEFV curve in horses suggests that the choke point remains in the trachea (or nasotracheal tube) until most of FVC has been exhaled. In this case, sudden relocation of the choke point to smaller airways would occur at lung volume close to residual volume explaining the rapid cessation of flow.

Recent studies show that the time course of the maximum inspiration preceding FE influences the FEFV curve (5). In particular, prolonged inspiration time and end-inspiratory pause decrease FEF. The effect is significant at high lung volume but decreases at lower lung volume. In our study, inspiration time and end-inspiratory pause were long, but, because airway narrowing affected mainly FEF measured at low lung volume, the effect of the preceding inspiration on FEF was probably limited.

Maximal Flow Rates

Families of isovolume flow-pressure curves obtained in horses demonstrated that, for driving pressures >200 cmH₂O, FEF did not increase further during evacuation of the last 10% of FVC (Fig. 3). This phenomenon of "effort independence" of flow has been well described in humans (11, 18). Therefore, during the last phase of the FEFV curve, flow is determined by airway characteristics of mainly caliber and compliance. A decrease in caliber or an increase in compliance of peripheral airways would tend to decrease FEF at low lung volume.

The size of the endotracheal tube used in this study (20 mm ID) was chosen because it was the largest tube that could be fitted through the nasal passages of most adult horses. Therefore, flow measurements recorded in the effort-dependent part of the FE would have likely been higher if they were obtained from horses fitted with larger tubes, i.e., after tracheostomy or oral intubation. In one horse, we recorded FEFV curves for three different tube sizes (18, 20, and 22 mm ID). We observed that, as expected, PEF and FEF at high lung volume increased with each increase in tube diameter. However, FEF were similar after 90% of FVC had been evacuated from the horse's lungs. These findings suggest that, in healthy horses, airway characteristics and not driving pressure mainly determine the last part of the FEFV curve obtained with our method.

Bronchial Challenge

Results of the bronchial challenge tests are consistent with histamine-induced peripheral airway narrowing. Administration of increasing doses of histamine reduced FVC and shifted the descending limb of FEFV curve toward higher lung volumes. Approximately 42% and 25% of the decrease in FVC recorded in *horses 3* and *9*, respectively, were a result of lower inspiratory volume and only at the highest histamine concentration. The absolute percent drop in FVC induced by the highest histamine concentration was $31.7 \pm 2.3\%$. This

corresponded to a $39.0 \pm 15.9\%$ fall in FEV₁. Maximal flows before the abrupt drop in flow were unchanged. During the late phase of FEFV curve, FEF decreased at a progressively slower rate as lung volume approached residual volume, which led to a prolonged end-expiration (fourth phase). Similar results have been seen in dogs in which histamine administration, by infusion or aerosol, resulted in a shortening of the flow plateau and a prolonged fourth phase of lung emptying (4, 17).

The concentrations of histamine used for the bronchial challenge (8–128 mg/ml) were high compared with those used in some equine studies of airway responsiveness (6, 8, 12). However, the histamine dose delivered to our horses (2–31 mg), which resulted in a decrease of FEV₁ by 20%, was comparable to histamine doses required to decrease C_{dyn} by 35% in normal horses or ponies (2–62.5 mg) (6, 8). In humans, histamine dose-response curves for FEV₁ discriminate better between normal and asthmatic subjects than dose-response curves for airway conductance (28). It is unknown if dose-response curves for FEV₁ would better discriminate between normal horses and horses with COPD than dose-response curves for C_{dyn}.

In several species, inflating normal lungs stimulates airway stretch receptors, resulting in a decrease in bronchomotor tone (20). Horses were mechanically ventilated before FE maneuvers were performed, which may have affected bronchomotor tone and the shape of the FEFV curve. However, this phenomenon should have little effect in normal equids because bronchial smooth muscle tone is minimal during tidal breathing (3). The effect of mechanical ventilation on bronchomotor tone may have been significant in horses with constricted airways, possibly delaying the onset of airway narrowing in response to histamine.

COPD Horses

Changes in lung mechanics of horses with COPD are characterized by a decrease in C_{dyn} and an increase in PL and RL (6, 23, 27). The clinical signs and baseline lung mechanics during tidal breathing of *horses A* and *B* were consistent with mild and severe COPD, respectively. After a 2-wk therapy with prednisone and environmental changes, clinical examination and lung mechanics data were within the normal range in *horse A* and were improved but still abnormal in *horse B*. Systemic corticosteroid and environmental changes are the cornerstone of COPD therapy. However, the effect of oral prednisone may be limited in cases of severe COPD or when horses are still exposed to allergens during treatment (25). Clinical signs and lung function tests normalize in most COPD horses after allergen avoidance such as pasture turnout (6). Improvement is usually noticed after at least 1 wk of environmental change. The incomplete recovery of *horse B* may have been the result of irreversible lung lesions, insufficient duration of treatment, or continued exposure to allergens other than organic dusts from hay or straw.

Pretreatment FE data in the two affected horses indicated a reduction in FEV₁, PEF, and FEF, particularly evident at low lung volume compared with post-

treatment values. A low maximum expiratory flow at lung volume equivalent to functional residual capacity was reported in one horse with COPD (16). FEFV curves in humans and animals with peripheral airway obstruction also exhibit reductions in FEV₁, PEF, and FEF (10, 19). According to clinical signs and spontaneous breathing lung mechanics data, the respiratory function of *horse A* appeared normal after therapy. However, the FEFV curve indicated a significant reduction in FEF_{95%}, suggestive of residual peripheral airway obstruction. Therefore, in this horse, analysis of the FEFV curve was more sensitive than other methods in detecting peripheral airway obstruction.

In conclusion, FEFV curves were readily and safely obtained in horses with the method presented in this study. This method of FE is minimally invasive, and measurements can be performed without requiring the horse to be anesthetized or placed in a body plethysmograph. The analysis of FEFV curves allowed us to detect airflow obstruction in histamine-challenged horses and in horses with COPD and was useful in evaluating the efficacy of prednisone therapy in two COPD horses. Therefore, FE parameters such as FEF as percentages of expired lung volume are early indicators of obstructive lung disease in the horse. FE may prove to be an effective diagnostic tool that can be used for clinical evaluations, screening, and research in horses. Future studies should investigate the sensitivity and specificity of FE parameters relative to other measures of pulmonary function in detecting and characterizing obstructive lung disease in horses.

APPENDIX

Calculation of Expired Volume From Tank Pressure

During FE, the instantaneous pressure was measured at a central position within one of the tanks. Data were sampled at 100 Hz and stored in a computer. Assuming the ideal gas law, before the FE is initiated, the number of moles of gas in the lungs (n_L) is given by

$$n_L = P_L V_L / R T_L \quad (A1)$$

where P_L is pressure in the lungs, V_L is volume of gas in the lungs, and R is the ideal gas constant.

Similarly, the number of moles of gas in the reservoir (n_r) is given by

$$n_r = P_r V_r / R T_r \quad (A2)$$

where V_r is volume of gas in the reservoir.

We next consider a volume of gas, dV_L , that leaves the horse's lungs during a period, dt , in the exhalation. As a first-order approximation, we assume that the temperature and pressure of gas in the lungs does not change in this process. The number of moles of gas leaving the lungs during dt is given by

$$dn_L = P_L dV_L / R T_L \quad (A3)$$

If we assume all molecules of gas leaving the horse go into the reservoir, then

$$dn_r = dn_L \quad (A4)$$

Taking differentials of both sides, we have

$$d(P_r V_r / R T_r) = P_L dV_L / R T_L \quad (A5)$$

$$V_r / R d(P_r / T_r) = P_L dV_L / R T_L \quad (A6)$$

$$(V_r / R) P_r / T_r (dP_r / P_r - dT_r / T_r) = P_L dV_L / R T_L \quad (A7)$$

$$dV_L = (V_r) (P_r / P_L) (T_L / T_r) (dP_r / P_r - dT_r / T_r) \quad (A8)$$

Over the entire exhalation, the changes in T and P are small relative to their initial values. Therefore, we can approximate the total exhaled volume as

$$\Delta V_L = (V_r) (P_r / P_L) (T_L / T_r) (\Delta P_r / P_r - \Delta T_r / T_r) \quad (A9)$$

where ΔP_r is final reservoir pressure – initial reservoir pressure, ΔV_L is volume of exhaled gas, and ΔT_r is final reservoir temperature – initial reservoir temperature.

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