Evaluation of head-out constant volume body plethysmography for measurement of specific airway resistance in conscious, sedated sheep

Daniela Bedenice, Dr Med Vet; Ephraim Bar-Yishay, PhD; Edward P. Ingenito, MD, PhD; Larry Tsai, MD; Melissa R. Mazan, DVM; Andrew M. Hoffman, DVM, DVSc

Objective—To evaluate the use of a modified whole body plethysmograph in awake sheep.

Animals—10 healthy adult sheep.

Procedure—Concurrent measurements of specific airway resistance (sR_{aw}) and pulmonary resistance (R_L) were obtained using a novel noninvasive head-out constant-volume plethysmograph and esophageal balloon-pneumotachography, respectively. All data were collected before and after external resistive loading with 1 and 5.6 cm H_20/L/s. Functional residual capacity (FRC) was measured by helium dilution for computation of airway resistance (R_{aw}) preloading ($R_{aw} = sR_{aw}$/FRC).

Results—The sR_{aw} and R_L were closely correlated in 10 adult sheep. Additionally, sR_{aw} and R_L accurately reflected the magnitude of added resistance. The mean FRC was 52 mL/kg and used to calculate R_{aw}. At baseline, the values for R_{aw} were significantly correlated with sR_{aw} and R_L.

Conclusions and Clinical Relevance—Precise measurements of sR_{aw} and R_{aw} at baseline and sR_{aw} after external resistive loading were obtained by use of this novel noninvasive plethysmographic technology. This method should have application to veterinary patients or animals used in research in which noninvasive rapid or serial measurements of sR_{aw} in the conscious state are required. (*Am J Vet Res* 2004;65:1259–1264)

Noninvasive yet precise measurements of **specific airway resistance (sR_{aw})** in awake small ruminants and large-breed dogs are not presently available. However, these would be valuable in the diagnosis and treatment of several common conditions in veterinary medicine, including laryngeal paralysis, tracheal stenosis, chronic bronchiolitis, allergic rhinitis, and asthma. Such a system would also be useful in preclinical research in large animal species.

A noninvasive technique of pulmonary function testing that has been explored extensively in laboratory animals is double-chamber plethysmography[1,2] for determination of sR_{aw}. Invasive methods include conscious intubation for whole body plethysmography in restrained sheep.[3] The advantage of the latter technique is removal of the upper respiratory tract (nasal passages, pharynx, and larynx) in the study. The disadvantage is the need for conscious intubation, which would not be possible in many veterinary species (eg, conscious dogs) and may be objectionable for repeated studies. Other methods for obtaining respiratory system resistance include monofrequency impulse or random noise oscillometry.[4,5] Although estimates of **airway resistance (R_{aw})** may be obtained in awake and anesthetized animals, these measurements are confounded by inclusion of thoracic wall resistance. Furthermore, oscillometric measurements in spontaneously breathing animals are distorted by artifacts introduced at the spontaneous breathing frequencies.

In small ruminants and large-breed dogs, a measure of R_{aw} and sR_{aw} would be most valuable, especially if serial or frequent measurements in 1 animal could be made to monitor progress. The purpose of the study reported here was to evaluate the use of a modified whole body plethysmograph in awake sheep. The technique is novel in that the animal's head is exteriorized, permitting handling and manipulation. The patient breathes from the plethysmographic chamber via a facemask and connector tubing as if breathing within the chamber. Similar considerations for whole body plethysmography in humans apply, although panting is not voluntarily obtained in sheep, necessitating a different approach to the analysis of box pressure-flow plots during quiet tidal breathing.[6] Sheep underwent external loading with known resistors positioned within the connector tubing, similar to other studies[7,8] that have used known resistances for validation. A direct comparison between R_{aw}[6] and **pulmonary resistance (R_L)**[9,10] was obtained by simultaneous recording of these variables.

Materials and Methods

Sheep—Ten healthy 3-year-old nonpregnant female sheep weighing 52 to 62 kg (mean, 56.9 kg) were used in the study. All procedures were approved by the Institutional Animal Care and Use Committee at the School of Veterinary Medicine, Tufts University.

Study design—Food was withheld from sheep for 12 hours. All sheep were sedated with midazolam (0.2 to 0.3 mg/kg, IV), and their heads were maintained in a horizontal position while standing during the concurrent plethysmographic measurement of sR_{aw} according to Agrawal and

Received November 25, 2003.
Accepted January 28, 2004.
From the Department of Clinical Sciences, School of Veterinary Medicine, Tufts University, North Grafton, MA, 01536 (Bedenice, Mazan, Hoffman); Pulmonary Function Testing Laboratory, Hadassah University Hospital, PO Box 12000, Jerusalem 91120, Israel (Bar-Yishay); and Pulmonary/Critical Care Medicine, Brigham and Woman's Hospital, 75 Francis St, Boston, MA, 02115 (Ingenito, Tsai).
Partially supported by the National Institutes of Health (NIH grant No. HL062266-02).
Presented in part at the 20th Veterinary Comparative Respiratory Society Meeting, Boston, October 2002.
The authors thank Keith Harrington for technical assistance.
Address correspondence to Dr. Bedenice.

Agrawal[11] and determination of R_L.[9] All data were collected by use of a pneumotachygraph[a] for 2 minutes during preloading and for 2 minutes during external resistive loading with 1 cm H_20/L/s and 5.0 cm H_20/L/s.[b] The actual added external resistances measured via lateral pressures and constant flow (0.5 L/s) through a rotameter were 1 and 5.6 cm H_20/L/s. Sheep were lightly sedated with midazolam (0.2 mg/kg, IV). **Functional residual capacity (FRC)** was obtained with the sheep outside the plethysmograph, via multiple breath helium dilution for computation of R_{aw} ($R_{aw} = sR_{aw}/FRC$).

Physical properties of the constant-volume head-out plethysmograph—An enamel and silicon sealed wooden, head-out constant-volume (330 L) plethysmograph was used. Two doors permitted exteriorization of the head and entrance and exit of the sheep (**Figure 1**). The head was exteriorized through a double-layer latex shroud with a sealed perimeter that contained a 1.5-cm layer of plastic beads (3 mm). The stiffness of the shroud was increased by applying negative pressure to the inside of the 2 latex sheets. Silicone lubricant was used to enhance the seal around the neck of the sheep. With the head exteriorized, sheep were fitted with a solid plastic facemask with its own latex seal 5 to 8 cm behind the external nares. The dead space in the mask with the sheep fitted was 50 mL.

The nosepiece of the mask was affixed to a pneumotachograph[a] for measurement of nasal flow and distally to a 75-cm-long insulated breathing tube[c] with an inner diameter of 5 cm. The breathing tube was attached directly to the plethysmographic chamber, which permitted rebreathing of expired gases from the tubing and the plethysmographic chamber. The pneumotachograph was connected to a differential pressure transducer[d] and calibrated by use of the electronic integration of flow introduced through the pneumotachograph with a precision syringe.[e] A second, separate differential pressure transducer[f] was used to measure the pressure differential between the atmosphere and the interior plethysmograph (box pressure). The constant volume plethysmographic pressure was calibrated by injecting a known volume of room air (60 mL) into the sealed plethysmograph either as a step or an oscillatory signal. Changes in box pressure were therefore recorded as a **volume signal (V_{box})** and used in the calculation of sR_{aw}. All data acquisition was performed by use of a carrier demodulator amplifier, data acquisition card,[g] and commercial software analyzer.[h]

To minimize thermal drift, a controlled leak (mean time constant, $\tau = 12.02$ seconds) and a thermal sink with a 15 × 20 × 50-cm conglomeration of copper mesh were introduced. Variable frequency (0.5, 1, 2, 5, and 10 Hz) sinusoidal waveform oscillation[12] of the sealed constant volume plethysmograph revealed that peak pressure, resistance, and elastance of the plethysmographic chamber were independent of the input frequency. The resistance of the assembled tubing system of the rebreathing circuit was considered negligible at 0.0291 cm H_20/L/s.

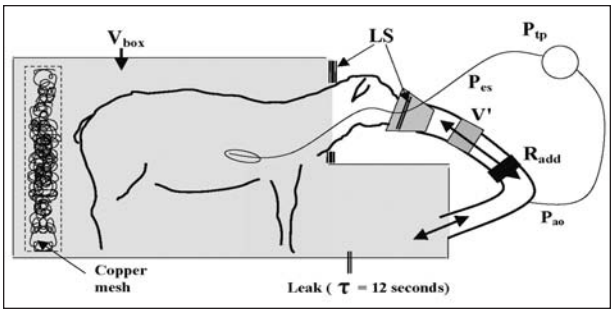

Figure 1—Illustration of the head-out constant-volume (330 L) plethysmograph. Sheep were acclimated in the box for 5 minutes before testing to permit equilibration of temperature, which was attenuated by use of a slow leak and the insertion of copper mesh. FM = Facemask. LS = Latex seals for facemask and neck. P_{es} = Esophageal balloon catheter. P_{ao} = Airway opening pressure catheter. P_{tp} = Transpulmonary pressure. V' = Pneumotachograph. V_{box} = Volume of box. R_{add} = Added resistor. τ = Mean time constant.

Measurement of sR_{aw}—The sR_{aw} was measured during quiet breathing or panting, according to Agrawal and Agrawal,[11] by use of the constant volume head-out plethysmograph. Briefly, the association between airflow at the nares and box pressure changes calibrated as a **volume shift (ΔV_{box})** was acquired in the form of an X - Y (V_{box} - V'_{ao}) plot. Historically, sR_{aw} is derived from conventional plethysmographic measurements of R_{aw} and FRC, with a subject panting against a closed shutter, under consideration of the following formula[8]:

$$sR_{aw} = R_{aw} \times FRC$$
$$\approx [(\Delta P_{ao}/\Delta V_{box}) \times (\Delta V_{box}/V'_{ao})] \times [\Delta V_{box}/\Delta P_{ao} \times (P_B - P_{H20})]$$
$$\approx (\Delta V_{box}/V'_{ao})] \times (P_B - P_{H20})$$

where ΔP_{ao} is the pressure change at the airway opening during occlusion, V'_{ao} is the flow at the airway opening, and $P_B - P_{H20}$ is the barometric pressure minus the water vapor pressure.

Similarly, sR_{aw}, according to Agrawal and Agrawal,[11] was computed from the X - Y (V_{box} - V'_{ao}) plots as follows:

$$sR_{aw} = 1/\tan\theta \times (P_B - P_{H20}) \times Cf \times [(\text{box volume} - \text{body weight})/\text{box volume}]$$

The $\tan\theta$ is the slope of the (V_{box} - V'_{ao}) plot measured during the transition phase from expiration to inspiration ($V' = \pm 0.5$ L/s; **Figure 2**) in which temperature and humidity artifacts are minimized. The correction factor (Cf) was defined as the arithmetic ratio of values recorded between equal distances (1 cm) on the abscissa and ordinate.

Results of data analyses were compared at 2 different time points, with early (baseline) and late (hyperpnea) recordings obtained within 30 seconds and between 1.5 to 2 minutes of data collection, respectively. Hyperpnea was achieved because of rebreathing of exhaled CO_2 in the rebreathing circuit. The CO_2 concentrations within the rebreathing circuit were monitored continuously and compared between baseline (before attachment to the facemask) and the end of the recording period (before detachment of the mask).

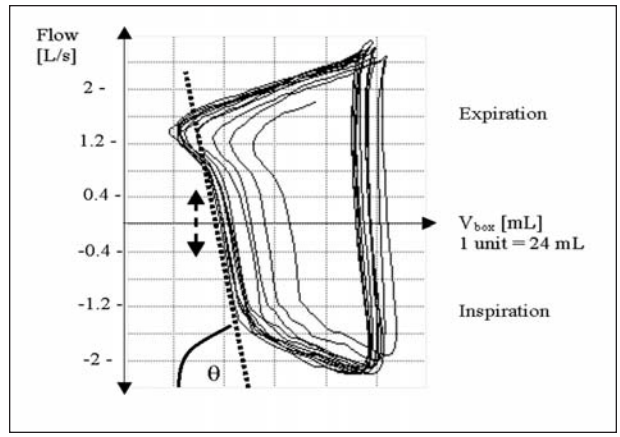

Figure 2—Representative box volume (V_{box}) versus nasal flow (V') X - Y plots in a healthy sheep during hyperpnea (preloading) obtained by head-out constant volume plethysmography. The dotted line ($\tan\theta$) is the slope of the V_{box} - V' plot measured during the transition phase from expiration to inspiration (± 0.5 L/s; indicated by interrupted arrow). The solid curve represents the outline of the angle ≤ c.

Measurement of R_L—Transpulmonary pressure (esophageal-circuit pressure distal to the resistor; Figure 1) was measured with an esophageal balloon catheter composed of a balloon 10 cm in length with a perimeter of 3.8 cm and a wall thickness of 0.8 mm sealed over the distal end of a polypropylene catheter (inner diameter, 4 mm; outer diameter, 5 mm; length, 100 cm).[9] The esophageal balloon catheter was passed to the level of the middle of the thorax, and the balloon inflated with air (2 mL). Positioning was determined on the basis of the recording of maximum negative transpulmonary pressure excursions. The proximal end of the catheter was exited through an airtight latex seal on the distal end of the facemask. The esophageal balloon catheter was connected to a differential pressure transducer[i] that was calibrated statically against a water U-manometer. The signals derived were amplified, sampled at 167 Hz, and digitized for processing by commercial software.[h] Frequency (f), **tidal volume** (V_T), peak flows, **minute ventilation (MV)**, and pleural pressures were recorded continuously and displayed by the computer on a breath-by-breath basis. Total R_L was computed by the isovolume method[9] for comparison to sR_{aw} (with and without **added resistance** [R_{add}]) or R_{aw} (without R_{add}). The mean data of 5 to 10 successive breaths at baseline (time point, 0 to 30 seconds) or during hyperpnea (time point, 1.5 to 2 minute) were obtained at each resistive load. No phase delay or signal attenuation of the pressure and flow sensors was observed up to 10 Hz.

Helium dilution measurement of FRC—Functional residual capacity[13] was measured with the sheep outside the plethysmograph while standing unsupported in a cart. Sheep were fitted with a low dead space (50 mL) clear plastic facemask sealed at the rear with a latex shroud. The mask was connected to a low dead space 3-way angled tap (120°) stopcock[j] and nondiffusible gas collection bag containing 500 mL of breathable test gas (0.3% **carbon monoxide** [**CO**], 10% **helium** [**He**], 20% O_2, and 69.7% N_2). The starting concentrations of He and CO were verified by use of specified analyzers.[k,l] At the end of expiration, the tap was turned connecting the sheep to the test gas for rebreathing. Expired gas collected at end-expiration, following a rebreathing period of 35 seconds, was analyzed. Observations in similar sized dogs indicated > 95% equilibration of He in 30 seconds.[13] The dilution of He (a nonexchangeable gas) gave a measure of FRC according to the following formula:

$$FRC = \{[He_i \times 0.625/He_f \times (1 - CO_2)] - [0.625 + DS_{ins}]\} \times 1.11\ L$$

where He_i was the starting concentration of He, He_f was the concentration of He following rebreathing, and DS_{ins} was the instrument dead space.[13] Three consecutive measurements were obtained, and the mean for each sheep was calculated.

Statistical analyses—The effects of added resistance on ventilatory variables and sR_{aw} were tested by use of a 1-way ANOVA. Paired-sample *t* tests were used to compare data obtained at baseline and during hyperpnea. Nonparametric testing (Spearman rank correlation) was used to determine the correlation between sR_{aw} and R_L or R_{aw} and R_L across all added resistances. Values of $P < 0.05$ were considered significant.

Results

Baseline plethysmography, R_L, and FRC measurement—The preloading values for R_L, sR_{aw}, and R_{aw} at baseline and during hyperpnea were determined (**Table 1**). Hyperpnea developed in all sheep within the 2-minute recording period, significantly ($P < 0.001$) increasing the measured V_T, peak expiratory flow, peak inspiratory flow, and MV at all levels of R_{add}, compared with baseline (**Table 2**). Values for R_L and R_{aw} at baseline were 2.19 and 2.05 cm H_20/L/s and during hyperpnea were 2.33 and 2.07 cm H_20/L/s, respectively.

Table 1—Mean ± SD values during preloading for pulmonary resistance (R_L), specific airway resistance (sR_{aw}), and airway resistance (R_{aw}) obtained via head-out plethysmography with different levels of added resistors (R_{add}) in 10 healthy sheep.

	R_{add}	Baseline		Hyperpnea	
Resistance	**(cm H_20 /L/s)**	**Mean ± SD**	**CV (%)**	**Mean ± SD**	**CV (%)**
R_L (cm H_20/L/s)	0	2.19 ± 0.57	26.03	2.33 ± 0.7	30.04
R_L (cm H_20/L/s)	1	3.39 ± 0.86	25.37	4.59 ± 0.96*	20.92
R_L (cm H_20/L/s)	5.6	7.19 ± 0.64	8.90	8.07 ± 0.91*	11.28
sR_{aw} (cm H_20 • s)	0	6.04 ± 1.6	26.49	6.11 ± 2.36	38.63
sR_{aw} (cm H_20 • s)	1	11.48 ± 2.88	25.09	12.29 ± 2.57	20.91
sR_{aw} (cm H_20 • s)	5.6	30.76 ± 9.65	31.37	34.34 ± 8.74	25.45
R_{aw} (cm H_20/L/s)	0	2.05 ± 0.4	19.51	2.07 ± 0.79	38.16

*Significantly ($P < 0.05$) different from baseline value.
CV = Coefficient of variation.

Table 2—Mean ± SD values for ventilatory variables obtained during hyperpnea via head-out plethysmography with different levels of added resistors (R_{add}) in 10 healthy sheep.

Time point	R_{add} (cm H_20/L/s)	f (per min)	V_T (L)	PEF (L/s)	PIF (L/s)	MV (L/min)	Ti (s)	Te (s)
Baseline	0	46.07 ± 17.56	0.45 ± 0.15	1.18 ± 0.38	1.29 ± 0.41	18.64 ± 6.43	0.51 ± 0.12	1.01 ± 0.31
Hyperpnea	0	44.02 ± 10.88	0.85 ± 0.16*	1.86 ± 0.36*	2.06 ± 0.42*	36.39 ± 7.79*	0.55 ± 0.1	0.89 ± 0.21
Baseline	1	42.46 ± 17.97	0.39 ± 0.12	0.79 ± 0.12	1.01 ± 0.18	15.04 ± 3.16	0.56 ± 0.16	1.04 ± 0.36
Hyperpnea	1	39.13 ± 8.02	0.71 ± 0.12*	1.2 ± 0.17*	1.59 ± 0.39*	27.54 ± 6.14*	0.62 ± 0.11	0.98 ± 0.21
Baseline	5.6	37.04 ± 9.96	0.35 ± 0.1	0.53 ± 0.13	0.86 ± 0.2	12.49 ± 3.27	0.61 ± 0.13	1.12 ± 0.32
Hyperpnea	5.6	34.35 ± 5.26	0.59 ± 0.12*	0.78 ± 0.16*	1.19 ± 0.35*	19.79 ± 4.38*	0.7 ± 0.1*	1.1 ± 0.23

*Significantly ($P < 0.05$) different from baseline value.
f = Respiratory frequency. V_T = Tidal volume. PEF = Peak expiratory flow. PIF = Peak inspiratory flow. MV = Minute ventilation. Ti = Inspiratory time. Te = Expiratory time.

Hence, R_L was 6.5% higher than R_{aw} at baseline and 11.2% higher during hyperpnea. The coefficient of variation (between sheep) was lowest for R_{aw} (19.5%) at baseline, compared with R_L (26%) and sR_{aw} (26.5%). In the absence of external resistive loading, values for R_L were significantly correlated with sR_{aw} (r = 0.77; P = 0.01) and R_{aw} (r = 0.72; P = 0.02; **Figure 3**) for the group of 10 sheep during hyperpnea. Mean FRC measured in 10 awake sheep via He dilution was 2.95 ± 0.51 L or 52 mL/kg.

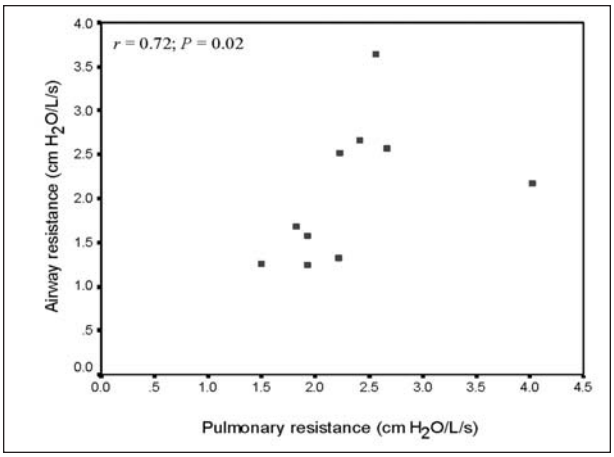

Figure 3—Correlation between pulmonary resistance and airway resistance before resistive loading during hyperpnea as measured by head-out constant-volume plethysmography in 10 healthy sheep.

Correlation between sR_{aw} and R_L during resistive loading—Resistive loading was associated with a significant reduction in frequency, V_T, peak expiratory flow, peak inspiratory flow, and MV and an increase in inspiratory time and expiratory time (Table 2). Resistive loading (1 and 5.6 cm H_20/L/s) caused significant ($P < 0.001$) increases in sR_{aw} and R_L that were different between added resistors (Table 1). Although hyperpnea was associated with higher values for sR_{aw} during resistive loading, these changes were not significant. In contrast, R_L increased significantly ($P < 0.05$) from baseline to hyperpnea during resistive loading with 1 and 5.6 cm H_20/L/s. Hyperpnea was also associated with a significant increase in the rebreathing circuit CO_2 concentration from 0.08% CO_2 (± 0.04) at baseline to 6.89% CO_2 (± 1.09) at the end of 2 minutes during hyperpnea. Baseline and hyperpneic CO_2 values within the rebreathing circuit were similar over all levels of added resistance.

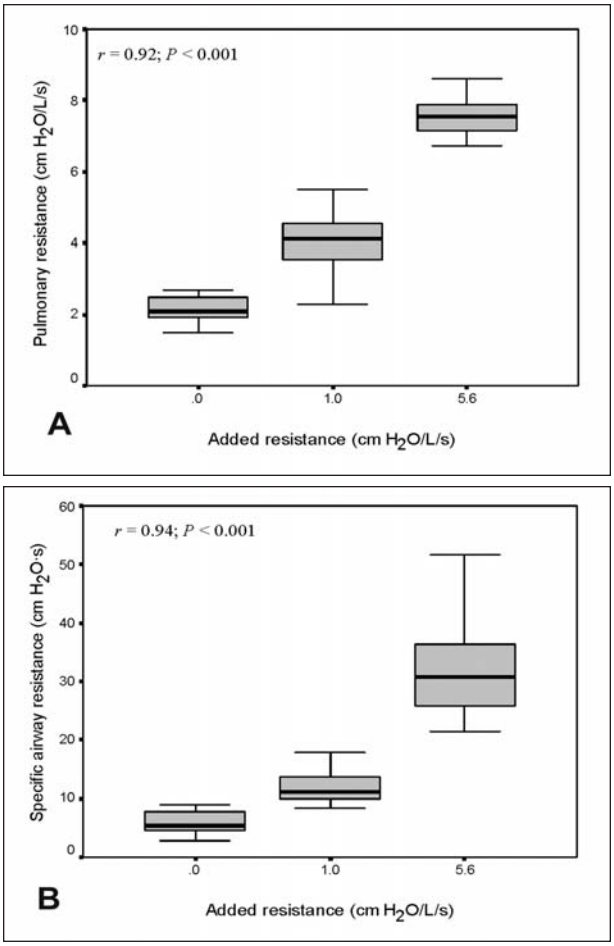

Figure 4—Boxplots depicting the correlation between pulmonary resistance (A) or specific airway resistance (B) and the magnitude of added resistance as measured by head-out constant volume plethysmography in 10 healthy sheep. Error bars represent SD. Boltines represent mean specific airway resistance.

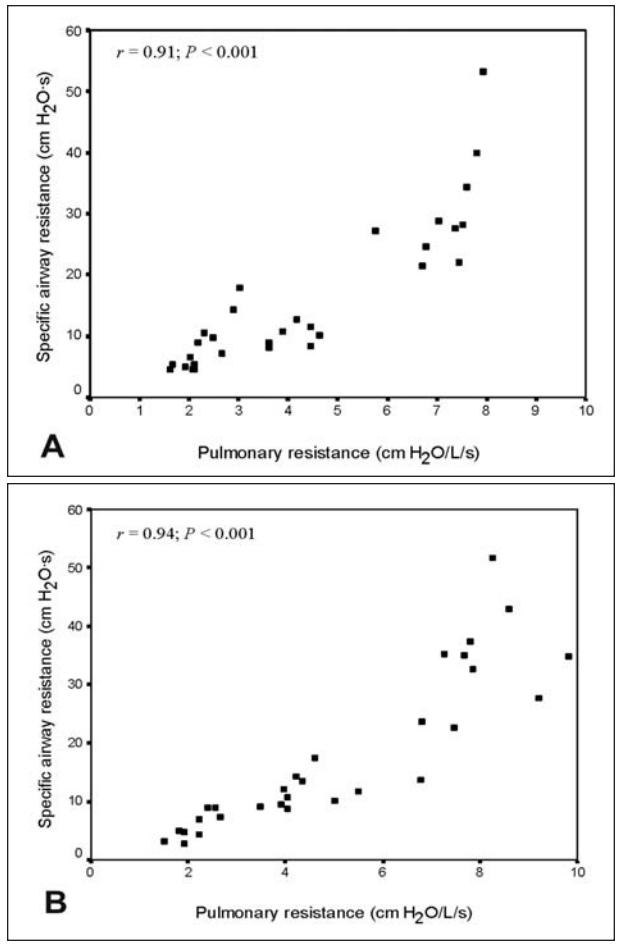

Figure 5—Scatterplots of specific airway resistance and pulmonary resistance data recorded simultaneously before and during resistive loading during the quiet breathing (baseline; A) and hyperpneic (B) phases as measured by head-out constant volume plethysmography in 10 healthy sheep.

Pulmonary resistance was significantly ($P < 0.001$; $r = 0.92$) correlated with the magnitude of R_{add} (1 and 5.6 cm H_20/L/s; **Figure 4**), although external resistive loading with 5.6 cm H_20/L/s was slightly underestimated by use of the isovolume method, according to Mead et al.[14] The sR_{aw} was significantly ($P < 0.001$; $r = 0.94$) correlated with external resistive loading. A plot of all values for sR_{aw} and R_L during preloading and after resistive loading indicated a strong correlation between these variables at baseline ($r = 0.91$; $P < 0.001$) and during hyperpnea ($r = 0.94$; $P < 0.001$; **Figure 5**).

Discussion

Results of our study indicated the validity of head-out constant volume plethysmography for measurement of sR_{aw} as a function of added external resistances in sheep. This method permitted a rapid, noninvasive determination of sR_{aw} and, when coupled with measurement of FRC, a derivation of R_{aw}. This technique is applicable for repeated measurements in sheep without the confounding effects of anesthesia and without significant stress or restraint associated with intubation. The disadvantage of this system is that the resistance of the upper airways is incorporated into the measurement of sR_{aw}. Therefore, the contribution of individual segments of the airways to constriction cannot always be determined.

Several limitations of the study are worthy of consideration. Because of the complexity of the animal's response (ie, progressive hyperpnea) and duration of the study (30 to 40 minutes for completion of all plethysmographic measurements for each sheep), we were unable to measure FRC serially at each resistive load. Therefore, it was not possible to directly compare R_{aw} (calculated as sR_{aw}/FRC) to R_L, except at baseline. We did not observe shifts in FRC as a function of hyperpnea or resistive loading (ie, as evidenced by the absence of drift in the loops along the abscissa); therefore, we do not believe that shifts in FRC were notably confounding our results, but we cannot make conclusions concerning R_{aw} at higher resistive loads. The comparison between R_L and R_{aw} during external loading can further be complicated by underestimation of R_{add} by use of the isovolume method to determine R_L.[7] We therefore restricted our correlations between R_{aw} and R_L to baseline measurements. Within the narrow range of normal R_{aw} and R_L measurements at baseline, a significant correlation between these variables was observed, suggesting that head-out plethysmography provided an accurate estimation of airway patency. One sheep had a markedly higher R_L (4.03 cm H_20/L/s), compared with all other sheep (mean, $R_L = 2.33 \pm 0.7$ cm H_20/L/s). The cause of the discrepancy could not be determined, and this measurement of R_L may be considered as an outlier.

Another limitation of the study reported here related to the use of the method of Agrawal[15] in the determination of sR_{aw}. This method was validated in guinea pigs[15] and humans[6] rather than sheep. Humans were prompted to breathe in a slow, regular pattern within a prescribed range of flows, increasing the precision and reproducibility of the resistances added, which may be flow dependent. In contrast to studies in humans, ventilation was not standardized in our dogs. However, because the values for R_{aw} preloading and sR_{aw} at any resistive load did not differ with hyperpnea, this problem was considered negligible. The whole body plethysmographic system used by Krell et al[6] also differed from the head-out system in that no tubing or mask was required. Results of our study indicated that the resistive properties of the tubing were minimal. However, the tubing and mask have the potential to impose a variable resistance and inertance (opposition to airflow) on the subject.

Our measurements of R_{aw} and R_L were close (within 6.5% at baseline and 11% during hyperpnea). That R_L was greater than R_{aw} was supported by results of other studies. For example, Dubois et al[8] used whole body plethysmography for measurement of R_{aw} and found that R_L was greater than R_{aw} by 33%; however, the difference between R_L and R_{aw} was smaller in other studies.[16,17] Tissue resistance is believed to explain the differences between R_{aw} and R_L.

In our study, the mean value for R_L (2.19 ± 0.57 cm H_20/L/s) was comparable with results of Wanner and Reinhart[3] ($R_L = 2.4 \pm 0.7$ cm H_20/L/s) with whole body plethysmography. However, it is not entirely accurate to compare our data for R_L with that of Wanner and Reinhart[3] because in that study, intubation was used to bypass the upper airways in sheep. Furthermore, sheep in that study were smaller (36 vs 57 kg). In our study, FRC was higher (2.95 ± 0.51 L [52 mL/kg]) than in other studies. Wanner et al[18] reported FRC values of 1.35 ± 0.78 L (37.5 mL/kg), and Mundie et al[19] reported FRC values of 25.7 to 44 mL/kg. It is possible that FRC values are higher when measured in standing, nonintubated sheep, compared with sheep in the supine position. Additionally, the breed of sheep we used may have had a higher FRC per kilogram, or a certain degree of hyperinflation may have been present at the onset of helium rebreathing. Additionally, a small leak may have contributed to an error in helium dilution in certain sheep, although the seal had been checked prior to the study by use of CO_2 monitoring around the mask, and the results were highly repeatable. An error in the timing of end-expiration (ie, the manual switching to helium rebreathing) may have induced minor increases in FRC, but these would be too small to explain the discrepancy in our FRC data when compared with that of others.

In addition to the unique head-out design used by this constant-volume plethysmograph, we used a method for the analysis of X - Y (V_{box} - V'_{ao}) plots that differs from whole body plethysmography in panting humans. This method was adopted from Agrawal[15] in guinea pigs and later used in humans.[6] In guinea pigs, recordings were made during uncontrolled spontaneous breathing (before and after histamine challenge) and similar polygon-shaped plots were obtained from which sR_{aw} was derived by the slope of the expiratory to inspiratory side of the polygon.[15] Results of our study support the validity of this method for quiet breathing subjects. Krell et al[6] also found that sR_{aw} measurements obtained by the quiet breathing method were similar to measurements obtained by the standard method requiring panting in humans with normal and

abnormal respiratory function. In our study, data obtained at baseline and hyperpnea were similar and comparable to results of Krell et al.[6] That sR_{aw} can be measured during quiet or hyperpneic breathing with similar accuracy and reproducibility is highly relevant to the application of head-out plethysmography in spontaneously breathing animals because many animals with disease, provocation, or excitement may be hyperpneic or panting.

Furthermore, the values obtained during preloading were similar to values obtained with the isovolume method at baseline and during hyperpnea. Specific airway resistance appears to be less distorted than R_L by hyperpnea after external resistive loading with 1 cm H_20/L/s, perhaps because of distortion of tissues at higher lung volumes.[20] Measurements of R_L after external resistive loading with 5.6 cm H_20/L/s had a small coefficient of variation between sheep, compared with all other recordings, which may have clarified the effect of hyperpnea on R_L.

In the study reported here, respiratory frequency, V_T, MV, and inspiratory time were similarly affected by external resistive loading as described in humans during normal sleep and were related to hypoventilation evoked by inspiratory flow limitation.[21] Peak inspiratory flows, V_T, and MV decreased significantly in all sheep after sustained (2 minute) external resistive loading, despite a concurrent prolongation of inspiratory time. In contrast, Weigand et al[21] indicated that minute ventilation was well maintained with acute and sustained resistive airway loading during wakefulness in humans. The ventilatory response to an added external resistive load in conscious humans depends on cortical, intrinsic muscle, and reflex factors. Increased diaphragm tension induced by inspiratory loading is believed to initiate the load compensation response.[22] Similar studies have not been performed in conscious sheep. However, in our study, the loss of ventilation as a function of resistive loading may have impacted our measurements by influencing the lung volume-dependent aspects of R_L and may confound comparisons between similar studies in humans.

Precise measurements of sR_{aw} and R_{aw} at baseline and sR_{aw} after external resistive loading were obtained with this novel noninvasive plethysmographic technology. This method should have application to veterinary patients or animals used in research (eg, small ruminants and large-breed dogs > 15 kg) in which noninvasive rapid or serial measurements in the conscious state are required.

[a]Fleisch #2 pneumotachygraph, Original Equipment Manufacturer, Lenoir, NC.
[b]Resistors, Hans Rudolf, Kansas City, Mo.
[c]Series 9000, Hans Rudolf, Kansas City, Mo.
[d]DP45-14, Validyne Engineering, Northbridge, Calif.
[e]3-L syringe, Hans Rudolph, Kansas City, Mo.
[f]SCXL004, Invensys Sensor Systems, Milpitas, Calif.
[g]6024E, National Instruments, Austin, Tex.
[h]XA Biosystems, version 2.2, Buxco Electronics, Sharon, Conn.
[i]DP45-28, Validyne Engineering, Northbridge, Calif.
[j]Stopcock, Hans Rudolph, Kansas City, Mo.
[k]Helium analyzer, PK Morgan, Chatham, Kent, UK.
[l]Carbon monoxide analyzer, CD-3A, Amtek, Pittsburgh, Pa.

References

1. Pennock BE, Cox CP, Rogers RM, et al. A noninvasive technique for measurement of changes in specific airway resistance. *J Appl Physiol* 1979;46:399–406.
2. Johanson WG Jr, Pierce AK. A noninvasive technique for measurement of airway conductance in small animals. *J Appl Physiol* 1971;30:146–150.
3. Wanner A, Reinhart ME. Respiratory mechanics in conscious sheep: response to methacholine. *J Appl Physiol* 1978;44:479–482.
4. Reinhold P, Macleod D, Lekeux P. Comparative evaluation of impulse oscillometry and a monofrequency forced oscillation technique in clinically healthy calves undergoing bronchochallenges. *Res Vet Sci* 1996;61:206–213.
5. Hellinckx J, Cauberghs M, De Boeck K, et al. Evaluation of impulse oscillation system: comparison with forced oscillation technique and body plethysmography. *Eur Respir J* 2001;18:564–570.
6. Krell WS, Agrawal KP, Hyatt RE. Quiet-breathing vs. panting methods for determination of specific airway conductance. *J Appl Physiol* 1984;57:1917–1922.
7. Bergman NA, Waltemath CL. A comparison of some methods for measuring total respiratory resistance. *J Appl Physiol* 1974;36:131–134.
8. DuBois AB, Botelho SY, Comroe JH Jr. A new method for measuring airway resistance in man using a body plethysmograph: values in normal subjects and in patients with respiratory disease. *J Clin Invest* 1956;35:327–335.
9. Amdur M, Mead J. Mechanics of respiration in unanesthetized guinea pigs. *Am J Physiol* 1958;192:364–368.
10. Mead J, McIlroy MR, Selverstone NJ, et al. Measurement of intraesophageal pressure. *J Appl Physiol* 1955;7:491–495.
11. Agrawal A, Agrawal KP. Body plethysmographic measurement of thoracic gas volume without panting against a shutter. *J Appl Physiol* 1996;81:1007–1011.
12. Lutchen KR, Yang K, Kaczka DW, et al. Optimal ventilation waveforms for estimating low-frequency respiratory impedance. *J Appl Physiol* 1993;75:4783–488.
13. Amis TC, Jones HA. Measurement of functional residual capacity and pulmonary carbon monoxide uptake in conscious Greyhounds. *Am J Vet Res* 1984;45:1447–1450.
14. Mead J, Whittenberger JL. Physical properties of human lungs measured during spontaneous respiration. *J Appl Physiol* 1953;5:779–796.
15. Agrawal KP. Specific airways conductance in guinea pigs: normal values and histamine induced fall. *Respir Physiol* 1981;43:23–30.
16. McIlroy MB, Mead J, Selverstone NJ, et al. Measurement of lung tissue viscous resistance using gases of equal kinematic viscosity. *J Appl Physiol* 1955;7:485–490.
17. Ferris BG Jr, Mead J, Opie LH. Partitioning of respiratory flow resistance in man. *J Appl Physiol* 1964;19:653–658.
18. Wanner A, Mezey RJ, Reinhart ME, et al. Antigen-induced bronchospasm in conscious sheep. *J Appl Physiol* 1979; 47:917–922.
19. Mundie TG, Dodd KT, Lagutchik M. Relationship of functional residual capacity to various body measurements in normal sheep. *Lab Anim Sci* 1992;42:589–592.
20. Strieder DJ, Laguarda R, Stigol LC, et al. Increased lung recoil during hypoxia in dogs. *Respir Physiol* 1974;21:193-201.
21. Wiegand L, Zwillich CW, White DP. Sleep and the ventilatory response to resistive loading in normal men. *J Appl Physiol* 1988;64:1186–1195.
22. Lavietes MH, Sanchez CW, Tiersky LA, et al. Psychological profile and ventilatory response to inspiratory resistive loading. *Am J Respir Crit Care Med* 2000;161:737–744.