Experiences with Whole-Body Plethysmography in Horses with Obstructive Pulmonary Disease

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Summer pasture-associated obstructive pulmonary disease (SPAOPD) is a condition that occurs in horses in the southeastern region of the United States.[1] The condition is seasonal in that it occurs only in the warm, humid summer months of June through September. Horses gradually recover during the late fall months and are not affected during the cooler months of winter and early spring. Once an animal has shown signs of the disease, a reoccurrence can be expected each successive summer.

SPAOPD is characterized by coughing, exercise intolerance and a double expiratory effort or heave line. Treatment consists of removal of horses from pasture and administration of various bronchodilators such as β_2 stimulators, xanthine derivatives and parasympatholytic agents. Although treatment is helpful, affected horses still retain clinical signs to such a degree that they are not useful for athletic endeavors. Brood mares will often abort their fetuses before term if severely affected. Owners become quite frustrated with these animals and often have them euthanatized or sold for slaughter.

Whole-body plethysmography was investigated as a tool for studying changes in lung function in horses with SPAOPD. It was also used to determine the efficacy and duration of action of various types of therapy for SPAOPD.

Whole-body plethysmography has been successfully used for respiratory measurements in people since the mid-fifties. At that time *DuBois* et *al.*[2,3] solved several of the problems that had limited the usefulness of this technique for making respiratory measurements. Since that time plethysmography has been refined and developed to the point that whole-body plethysmographs with on-line computers are commercially available for making respiratory measurements in man and laboratory animals.

Some of the respiratory measurements which can be succesfully completed using body plethysmography include: 1) absolute gas volumes (e. g. thoracic gas volume), 2) alveolar pressure, 3) airway resistance, and 4) spirometric displacements during breathing which allow measurement of a) pulmonary subdivisions b) pulmonary mechanics, and c) maximum expiratory flow-volume curves.

Alveolar pressures (P_{ALV}) are generated as a consequence of airway resistance to airflow during inspiration and expiration during tidal breathing. Airway obstruction will cause these pressures to become increasingly more negative during inspiration and increasingly more positive during exhalation. P_{ALV} is then a reflection of obstructive events occurring in the airways and can be used to monitor changes in airway conductance. P_{ALV} changes in horses with SPAOPD will be the subject of the remainder of this manuscript.

The foundation for using plethysmography to measure P_{ALV} was laid by *Bert* in 1870.[4] He recognized that during quiet breathing chest volume changes differ from tidal volume measured at the mouth because alveolar gas is compressed and decompressed during airflow. *Sonne* used this principle in 1923 when he performed plethysmographic experiments in man.[5] He was not able to analyze his records quantitatively, however, because thermal and water vapor exchange between lung air and tidal air produced volume changes in the plethysmograph which were in the same direction as those produced by P_{ALV} variations.

Plethysmography as a tool for making P_{ALV} measurements was largely ignored until 1956 when *DuBois* et *al.*[2,3] solved the thermal exchange problem by having the subjects pant. This limited the tidal excursion to less than the volume of the mouthpiece-flowmeter assembly and thus minimized heat exchange between the lungs and gas in the plethysmograph. Using this method is was possible to measure P_{ALV}. Even though this represented a significant breakthrough, measurement of P_{ALV} was restricted to panting frequencies. Various methods have been used to eliminate the thermal exchange problem at other than panting frequencies and volumes. The methods tried have included electronic compensation,[6] rebreathing from a bag containing gas at BTPS[7] and graphical methods.[8]

Plethysmographic estimates of P_{ALV} have not been reported in horses. Because there is no other method for obtaining these measurements, their values in horses are currently unknown.

Materials and Methods

Plethysmograph

The plethysmograph (box) consisted of a set of stocks welded to a square steel tubing chassis which in turn was incorporated by an airtight box. The wall and ceiling of the box were made from 19 mm thick plywood attached to an

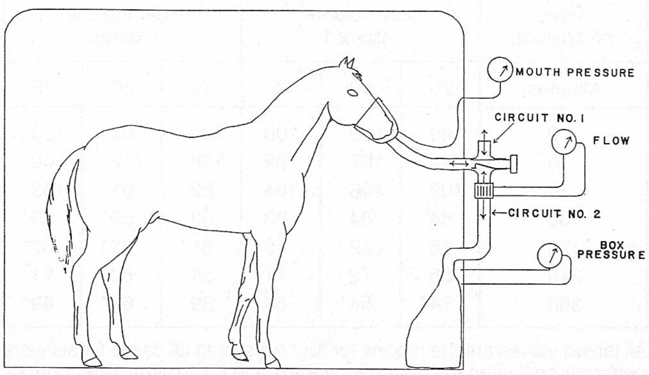

Fig. 1: Box for body plethysmography

Table 1: Alveolar pressure in control and SPAOPD horses (cm of HOH).

	% tidal volume inspired			% tidal volume expired		
Horses	25	50	75	25	50	75
Control	−1.60	−2.47	−3.26	0.14	0.28	1.84
SPAOPD	−12.81**	−16.11**	−17.55**	8.16**	13.85**	25.44**

All values in the table are the means for four horses. ** indicates statistical significance at $t_{.995,6}$ when compared to corresponding mean for control group.

angle iron framework. The floor was made from 6 mm thick steel plating. A large door was incorporated into one side of the box to accomodate entry and removal of horses and a hinged window was attached to one end to facilitate attachment of the facemask assembly to the breathing circuit. The door and window were sealed when closed by means of specially designed gaskets constructed from closed cell foam rubber weatherstripping material coated with latex rubber.

Two breathing circuits were built into the box. These are labeled as circuits 1 and 2 in Figure 1. Circuit 1 opened to the outside of the box and was used for making measurements of thoracic gas volume (TGV). To facilitate making this measurement an electronic shutter was used to close this circuit to the atmosphere at the appropriate time. Circuit 2 was connected to a large rubber bag inside the box and was used for making measurements of P_{ALV}. Circuit 2 contained a pneumotachograph for making flow measurements and heat tapes to regulate the temperature of air in the bag and breathing circuit to that in the lungs during measurements.

Calibration Syringe

A 0−5 liter calibration syringe was constructed from an aluminum cylinder fitted with ends made from acrylic plastic. The plunger was made from an acrylic plastic disc and a handle fashioned from galvanized pipe. Injection of a volume of gas from the syringe into the box while a horse was in the box allowed calibration of the box for each individual horse.

Table 2: Alveolar pressure (% of control); removal from pasture only.

Time off pasture	% tidal volume inspired			% tidal volume expired		
Minutes	25	50	75	25	50	75
0	100	100	100	100	100	100
15	113*	107	109	108	112	100
30	109	106	104	82	91	103
60	84	84	83	53	59*	63*
120	75	72	72	61*	63*	57*
240	66	72	71	51	62	51*
360	64*	64*	64	39*	63*	49*

All tabled values are the means for four horses. In all cases t tests were performed between the treatment means and their respective controls. * Indicates significance at $t_{.975,6}$.

Signal Detection and Recording

Two differential pressure transducers were used to measure pressure signals from the apparatus during a test. Flow across the pneumotachometer in circuit 2 was measured with a Statham PM5E differential pressure transducer. A Statham PM97 differential pressure transducer was used to measure box pressure. Frequency responses for the transducers and attached tubing were in phase up to signal frequency of 6 Hz.

An Electronics for Medicine electronic recorder equipped with a pulmonary function studies module was used to make a paper recording of pressure and flow signals associated with a horse breathing in the box. This recorder was also used to electronically integrate the flow signal to determine tidal volume.

Facemask

Each horse wore a facemask fashioned from a plastic wastebasket. Air entered and exited the mask via a 6.35 cm diameter hole in the bottom. A plastic fitting was inserted into the hole for attachment to the breathing circuits in the box. A flexible membrane was used to attach the mask to a horse. This membrane was made by painting stretch nylon with latex rubber. The facemask was taped to the horse's muzzle using plastic tape to help prevent leaks and held in place on the horse's head using a nylon halter.

Horses

Eight horses were used as subjects in the box for the present study. Four were considered normal with regard to pulmonary function while 4 were diagnosed as having SPAOPD. The control horses ranged in age from 3 to 15 years and their weights varied from 300 to 450 kg. The SPAOPD horses ranged in age from 13 to 19 years and in weight from 413 to 495 kg. Acceptance of the box by the experimental subjects was quite good. The primary factor affecting acceptance of the box by a horse was the amount of experience the horse had with being loaded and transported in trailers.

Alveolar Pressure Measurements

Measurement of alveolar pressure during normal tidal breathing was performed according to the methods of *Jonson* and *Bouhuys*.[8] These measurements required that TGV be known. TGV was measured as described by *DuBois* et *al.*[2]

Experiment One

P_{ALV} was measured in each of the control and SPAOPD horses. Their values were then averaged to determine a value for alveolar pressure in the two groups. Measurements were made at 25, 50 and 75% of tidal volume inspired and at 25, 50 and 75% of tidal volume expired.

Experiment Two

P_{ALV} was measured in SPAOPD horses immediately after removal from pasture and after being off pasture for 15, 30, 60, 120, 240, and 360 minutes. As described above, measurements were made at 25, 50 and 75% of tidal volume inspired and at 25, 50 and 75% of tidal volume expired.

Experiment Three

P_{ALV} was measured in these same SPAOPD horses at 0, 15, 30, 60, 120, 240 and 360 minutes after removal from pasture plus receiving intravenous clenbuterol. The dosage of clenbuterol was 0.8 µg/kg. Measurements were made at three points during inhalation and exhalation as outlined above.

Statistical Analysis

Student's t-test was used according to methods described by Ostle to determine differences between means.[9]

Results and Discussion

Table 1 shows the results of experiment one. P_{ALV} became negative during inspiration and positive during expiration. It can also be seen that the most negative values occurred at 75% tidal volume inspired and the most positive values occurred at 75% tidal volume expired. The overall magnitude of these values was dramatically higher in SPAOPD horses than in control horses.

Table 2 shows the results of experiment 2. Values in the table are expressed as a % of control or zero value to decrease variability in the data. The source of variability was both within horses and between horses. The reasons for such variability were 1) SPAOPD becomes more severe in an individual as the season progresses, 2) daily variations in weather influence the severity of the condition and 3) different horses are affected more or less severely than others. The values decreased greatly after one hour off pasture and decreased only gradually thereafter. It can also be seen that the values during expiration tended to decrease more quickly than those taken during inspiration.

Removal from pasture is a standard recommendation for treating horses with SPAOPD. The results in Table 2 show that this was indeed effective in decreasing alveolar pressure. The reason for this decrease is unknown. However, it could be due to a decrease in bronchoconstriction associated with the condition. This bronchoconstriction could be the result of either airway irritation or specific mediator release associated with immunologic reactions in the lungs. The statistically significant reductions in P_{ALV} especially during 75% tidal volume exhaled, are probably due to a decrease in the dynamic compression of the airways during exhalation after removal from pasture. Clinically these horses would have less of a heave line.

Table 3 shows the results of treatment with intravenous clenbuterol in addition to removal from pasture. Treatment with clenbuterol at the 0.8 µg/kg level did not significantly influence P_{ALV} during inspiration. However, it did cause significant reductions in P_{ALV} during exhalation over those seen with removal from pasture only. This occurred at both 30 minutes and 120 minutes after administration of the drug. These data indicate that clenbuterol was an effective bronchodilator in SPAOPD horses at the 0.8 µg/kg dosage and its effects were additive to those of removal from pasture. It is interesting to note that the reduction in P_{ALV} occurred earlier than that seen by removal from pasture only.

Conclusions

Whole body plethysmography proved to be an effective tool for studying P_{ALV} in horses with SPAOPD. Such studies revealed that horses with SPAOPD had a dramatic rise in P_{ALV} over that seen in normal horses.

Removal from pasture was effective in reducing P_{ALV} in horses with SPAOPD. Administration of intravenous clenbuterol produced an even greater effect in reducing P_{ALV} than removal from pasture only. These beneficial effects of clenbuterol at a dosage of 0.8 µg/kg in horses with SPAOPD support the work of others who have reported beneficial effects with this drug in horses with obstructive pulmonary disease.[10–12]

Table 3: Alveolar pressure (% of control); removal from pasture plus 0.8 µg/kg clenbuterol IV.

Time after injection	% tidal volume inspired			% tidal volume expired		
Minutes	25	50	75	25	50	75
0	100	100	100	100	100	100
15	95	97	90	64	67	62
30	101	91	97	40	52*	64*
60	110	104	100	70	109	80
120	95	90	91	22*	30*	47
240	90	82	78	43	54	56
360	68	72	70	20	32	48

All tabled values are the means for four horses. In all cases t tests were performed between the treatment means and their respective values in table 2. * Indicates significance at $t_{.975,6}$.

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