Telemetric analysis of breathing pattern variability in recurrent airway obstruction (heaves)–affected horses

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Objective—To use noninvasive respiratory inductance plethysmography (RIP) to investigate differences in breathing patterns between horses with and without recurrent airway obstruction (RAO) during the onset of airway obstruction induced through confinement to stables.

Animals—12 horses with no history or clinical signs of respiratory disease (control horses) and 7 RAO-affected horses.

Procedures—The study involved 2 phases. In phase 1, the optimal position of RIP bands for recording pulmonary function was investigated in 12 control horses. In phase 2, 7 RAO-affected and 7 control horses were confined to stables. Respiratory inductance plethysmography bands were applied to horses for 24 h/d to record respiratory rate and total displacement in 4-hour periods for 7 days or until RAO-affected horses developed signs of severe RAO that persisted for 2 consecutive days. Lung function was measured once daily.

Results—In phase 1, thoracic and abdominal cavity displacements were best represented by RIP bands positioned at intercostal spaces 6 and 17, respectively. In phase 2, pulmonary function indicated airway obstruction in the RAO-affected group on the final 2 days of stable confinement. Respiratory rate and total degree of respiratory displacement measured by RIP did not differ between the RAO-affected and control groups, but the SDs of these decreased significantly within 8 hours after stable confinement began in RAO-affected horses. Respiratory inductance plethysmography and pulmonary function findings became highly correlated as severity of disease progressed.

Conclusions and Clinical Relevance—The decrease in the SDs of RIP measurements indicated a lower degree of variability in breathing patterns of RAO-affected horses. This loss of variability may provide an early indicator of airway inflammation. (*Am J Vet Res* 2013;74:925–933)

Recurrent airway obstruction (heaves) is an airway disease that becomes clinically evident in susceptible middle-aged horses.[1] The obstruction by bronchospasm and excess mucoid secretions is a consequence of inflammation that develops within a few hours after inhalation of organic dust. To understand the sequence of events in airway obstruction, a close relation between airway dysfunction and the accompanying inflammatory events must be established. For the past 40 years, changes in the severity of airway obstruction in RAO have been assessed through various lung functions tests that are slightly invasive because they require use of a face mask pneumotachograph system with or without an esophageal balloon.[2,3] Given the invasiveness of the equipment, evaluations of respiratory function with this method can be performed only in cooperative horses and for limited periods, making it difficult to continually study respiration during the onset and progression of RAO.

Clinicians evaluate respiratory system function by observing the breathing pattern, which incorporates the effort, respiratory rate, and timing of inspiration and expiration. In horses with severe RAO, the abdominal effort is greater than typical, expiration is prolonged, and there is little variability in respiratory pattern among breaths. By contrast, in healthy horses, breathing can be difficult to discern and the pattern can be quite variable as horses sniff and sigh in among more regular breaths. Horses with less severe RAO can be dif-

Abbreviations

RAO	Recurrent airway obstruction
RIP	Respiratory inductance plethysmography

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ficult to distinguish from horses without RAO, even though the biphasic breathing pattern characteristic of healthy horses is not clinically discernible and peak inspiratory flow rate is higher than usual.[4] To monitor such respiratory changes during the onset of RAO, a noninvasive method is needed that can be used to continually monitor breathing patterns. Respiratory inductance plethysmography provides such a tool.

Respiratory inductance plethysmography is noninvasive, does not require use of a face mask, and when used with telemetry, continually records respiratory movements without interfering with a horse's behavior. The technique measures changes in the cross-sectional area of the thorax and abdomen by means of 2 cotton bands containing a coiled metal wire through which an alternating electrical current passes, thereby creating a magnetic field. The respiratory movements of the thoracic and abdominal compartments change the shape of the magnetic field, resulting in a measurable opposing electric current proportional to the change in the cross-sectional area.[5] The sum of the output of the 2 bands provides the total degree of respiratory displacement, which is an indicator of tidal volume, and the reciprocal of the total duration of each breath provides an instantaneous value of frequency (respiratory rate) for each breath.

In human medicine, RIP has been used to assess respiration in young children intolerant of the placement of the face mask–pneumotachograph system. A study[6] of 37 children in which tidal breathing variables such as ratio of inspiratory duration to total breath duration and respiratory frequency were compared revealed good agreement between results of noncalibrated RIP and a face mask–pneumotachograph system when performed simultaneously. A similar comparison indicated that RIP could be used to accurately monitor breathing pattern in sheep with and without provoked bronchospasm.[7] In horses, simultaneous recordings of breathing via RIP and pneumotachography have been made for approximately 50 breaths during a short-term exacerbation of heaves,[8] but to our knowledge, no one has monitored respiration continually in healthy or RAO-affected horses throughout the day. Our hypothesis was that RIP could be used to detect differences between RAO-affected and clinically normal horses within a few hours after confinement to stables.

Materials and Methods

Animals—Two groups of control horses and 1 group of RAO-affected horses were used in the study. The first control group included 11 mares and 1 gelding with no history or clinical signs of respiratory disease, with a mean ± SD age of 11.9 ± 4.3 years and a mean body weight of 489 ± 35 kg. The second control group included 6 mares and 1 gelding with no history or clinical signs of respiratory disease, with a mean age of 9.1 ± 5.0 years and mean body weight of 486 ± 33 kg. The RAO-affected group, which fulfilled the phenotype definition of organic dust–induced airway obstruction reversed by a change in environment and administration of bronchodilators,[1] included 4 mares and 3 geldings, with a mean age of 21.0 ± 6.4 years and mean body weight of 503 ± 36 kg. All horses were maintained on pasture and received a supplemental pelleted diet except during periods of stable confinement and exposure to hay and straw bedding to induce short-term exacerbations of RAO.[9] The All-University Committee for Animal Use and Care of Michigan State University approved the study protocol.

Study design—The investigation involved 2 phases. In phase 1, the first group of control horses was used to determine the optimal position for placement of abdominal RIP bands for measurement of respiratory variables. In phase 2, RIP in the configuration determined in phase 1 was used to investigate differences in breathing pattern between the second control group and RAO-affected horses during the onset of airway obstruction induced by stable confinement.

RIP instrumentation and phase 1—For RIP, 2 RIP bands[a] were connected to a transmitter box (2 × 5 × 4 cm) that telemetrically sent the RIP signal to a receiver connected to an analog-to-digital converter. The transmitter box was attached to a breast collar and the mane so that it hung on the left side of the neck adjacent to the cranial border of the left scapula. For phase 1 of the experiment, 1 RIP band was fixed at intercostal space 6 in each horse in the first control group and the second band was randomly moved among intercostal spaces 9, 11, 13, 15, and 17 and over the abdomen just caudal to the 18th rib.[8] These positions were chosen because, anatomically, intercostal space 6 encompasses only the thorax, whereas subsequent intercostal spaces surround increasing amounts of abdomen. At each location, RIP output was recorded for 1 minute.

Computer software[a] was used to analyze the RIP output from the 2 bands in real time on a breath-to-breath basis. The variables of interest were instantaneous respiratory frequency and abdominal, thoracic, and total degree of inspiratory displacement. The respiratory rate was calculated as the inverse of breath duration, and the degree of displacement was calculated from the change in the cross-sectional area of the thoracic and abdominal compartment. Displacement data were summed to calculate the total degree of respiratory displacement measured via RIP. No attempt was made to correlate displacement measurements with independently measured changes in absolute lung volume.

Phase 2—After the optimal position of RIP bands had been established, phase 2 of the experiment began in which RIP output and pulmonary function were compared between the second control group and the RAO-affected group. For phase 2, pairs were made of control and RAO-affected horses, which were brought in from pasture for baseline (nonconfined) scoring of clinical signs and measurement of pulmonary function.

Clinical signs of disease in all horses were scored daily by the same individual on the basis of observed degree of nasal flaring and abdominal effort (each scored 1 to 4). Scores for both characteristics were summed to obtain the clinical score.

Pulmonary function testing was conducted with an esophageal balloon sealed over the end of a 240-cm-long polypropylene catheter (internal diameter, 2.4 mm; external diameter, 4 mm) in which 5 lateral holes had been drilled in the portion covered by the

balloon. The balloon was passed in each horse via nasogastric tube and positioned in the intrathoracic portion of the esophagus, caudal to the heart base but cranial to the thoracic diaphragm. Pressure changes within the balloon were measured by a pressure transducer[a] that was calibrated daily against a water manometer.[10] A plastic face mask[b] was placed over the horse's nose and sealed around the face by means of a rubber cuff and electrical tape. A pneumotachograph attached to a pressure transducer was inserted into the front of the face mask for measurement of airflow.

Calibration of the pneumotachograph was performed daily with a rotameter flow meter. The transducer signals for esophageal pressure and flow were passed through an analog-to-digital converter and amplifier and then analyzed with the aid of a software program.[a] Outputs recorded included change in pleural pressure during inspiration, pulmonary resistance calculated by the isovolume method at 75% tidal volume, and dynamic compliance. Because the software could not be used to calculate maximal change in pleural pressure, that variable was manually calculated from the printed output of the esophageal pressure signal. For each variable, data were collected for 20 breaths > 1 L.

When the clinical score and mean maximal change in pleural pressure were ≤ 4 of 8 and < 10 cm H_2O, respectively, phase 2 began and horses were housed in pairs in stalls with straw bedding and fed poor-quality hay. The same individual assigned clinical scores on a daily basis as previously described throughout phase 2. Each horse pair was housed in the same 9-horse stable (each horse in an individual stall) for 7 days unless the RAO-affected horse developed a clinical score of 8 (severe nasal flaring and respiratory effort on a scale from 1 to 8) that persisted for 2 consecutive days.

For continual RIP assessment during the confinement period, RIP bands were placed on each horse when it was brought into the stable as established in phase 1 and secured with loosely applied horizontal mattress sutures that passed over but allowed free stretching of the bands. Respiratory inductance plethysmography data were recorded as described for phase 1 throughout the day, except for approximately 1 hour around 9 AM, when lung function was measured as previously described. The daily RIP output was divided into 6 periods, which were equivalent to 10 AM to 2 PM, 2 PM to 6 PM, 6 PM to 10 PM, 10 PM to 2 AM, 2 AM to 6 AM, and 6 AM to 9 AM. Each period contained approximately 4,000 breaths. The means and SDs for respiratory rate and total degree of respiratory displacement measured by RIP were calculated for each 4-hour period.

Statistical analysis—For phase 1, comparisons of RIP output between the fixed thoracic band and the abdominal band placed at the various intercostal spaces were performed via ANOVA,[c] with horse as a random effect and intercostal space as a fixed effect, and the Tukey highly significant difference test. Values of $P < 0.05$ were considered significant.

For phase 2, data representing lung function (change in pleural pressure, maximal change in pleural pressure, pulmonary resistance, and dynamic compliance) and RIP output (respiratory rate and total degree of respiratory displacement measured by RIP) were analyzed via split-plot repeated-measures ANOVA,[c] with values of $P < 0.05$ considered significant. Factors evaluated for an influence on lung function were horse, disease status (control and RAO), and study day (baseline [at introduction to stable confinement], after 1 day of confinement, on the second-to-last day of confinement, and on the final day of confinement). first day of confinement, penultimate day of confinement, and final day of confinement). Factors in the RIP analyses were the same as for lung function, with an additional factor of period (6/d). The Pearson correlation coefficient (r) was calculated to examine the relationship between lung function and RIP variables. Data are reported as mean ± SD.

Results

Optimal RIP band placement—Experimentation with RIP band positioning in the control horses in phase 1 involved abdominal band movement among intercostal spaces 9 through 17, revealing that the degree of inspiratory displacement increased with increasing distance from the thoracic band at intercostal space 6 (**Figure 1**). The degree of inspiratory displacement determined at intercostal spaces 11 through 17 was significantly different from that determined at intercostal space 9, but only the displacement at intercostal space 17 was different from that at intercostal spaces 9 and 11. Additionally, the degree of inspiratory displacement measured at spaces 11 through 17 all differed significantly from that at the thoracic band positioned at intercostal space 6.

When the abdominal band was placed caudal to the 18th rib, the degree of inspiratory displacement measured did not differ from that measured at intercostal space 17, but the variability of displacement (mean ± SD, 1.4 ± 1.1 relative L) was greater. Because intercostal space 17 had the largest degree of inspiratory displacement and the inspiratory displacement there differed significantly from intercostal space 6 and was less variable than caudal to the 18th rib, intercostal space 17 was selected as the position for abdominal RIP band placement in phase 2 of the

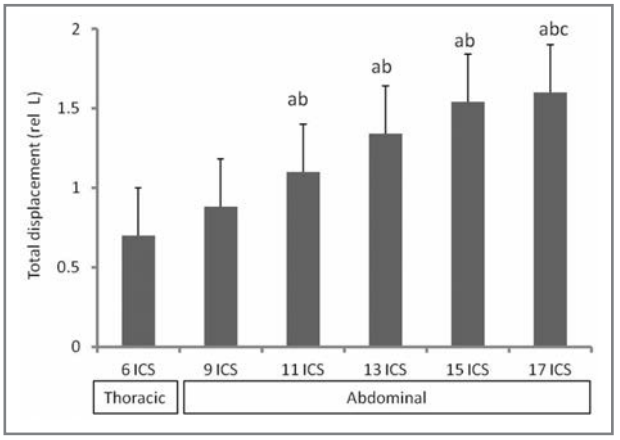

Figure 1—Mean ± SE total degree of displacement of a thoracic RIP band that remained at intercostal space 6 and an abdominal RIP band that was moved among intercostal spaces 9 through 17 in healthy horses (n = 12). ICS = Intercostal space. rel L = Relative liters. [a–c]Values are significantly ($P < 0.05$) different from those for spaces 6 (a), 9 (b), and 11 (c).

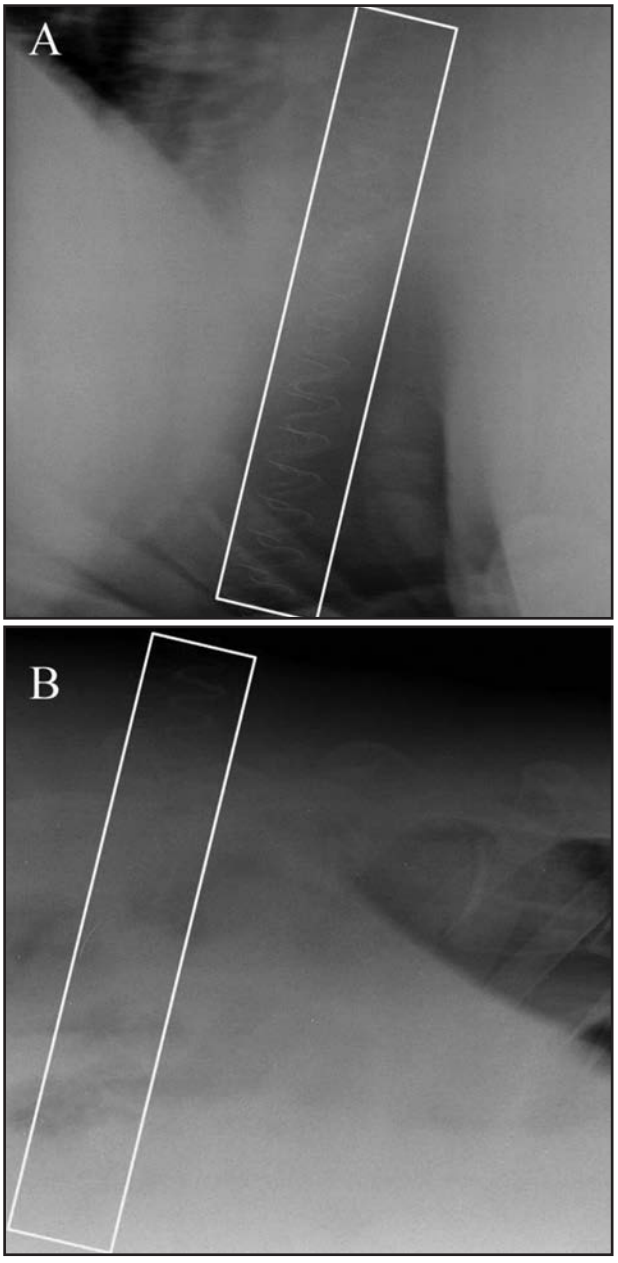

Figure 2—Thoracic radiographic views of a clinically normal horse (head to the right; tail to the left) showing RIP bands (highlighted region) at intercostal spaces 6 (A) and 17 (B). The bands at intercostal spaces 6 and 17 overlie the thorax and abdomen, respectively.

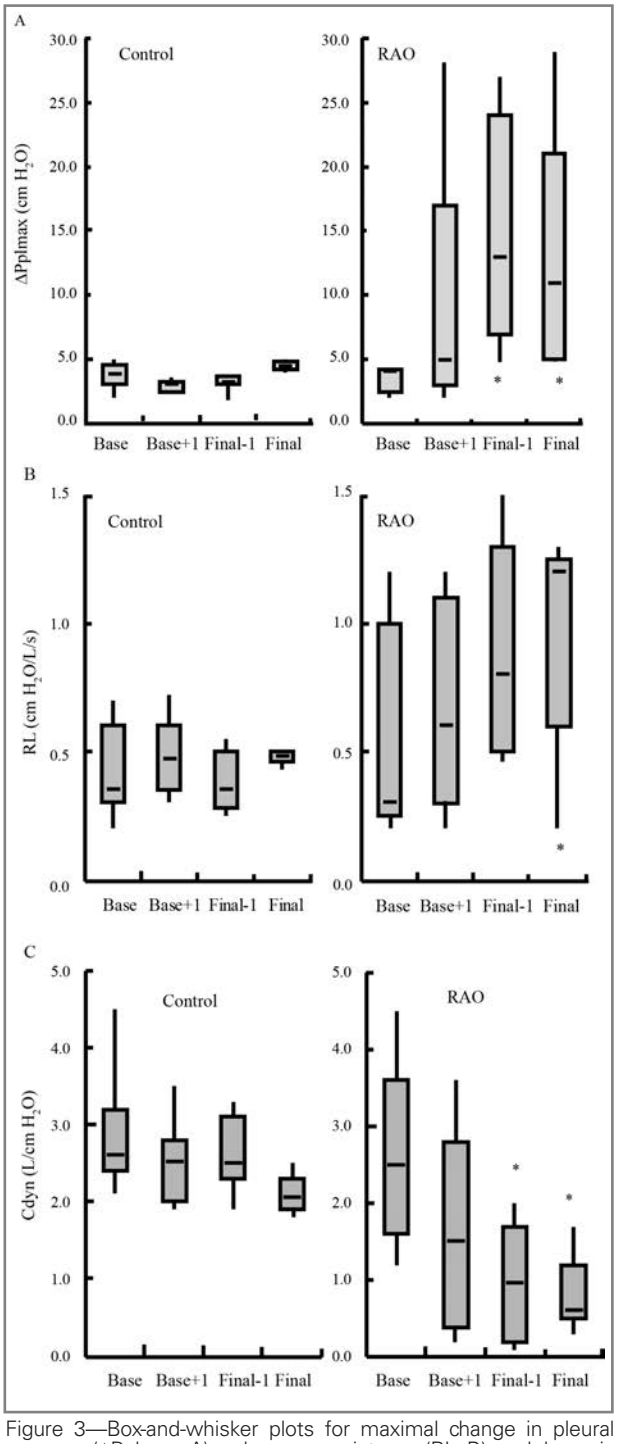

Figure 3—Box-and-whisker plots for maximal change in pleural pressure (ΔPplmax; A), pulmonary resistance (RL; B), and dynamic compliance (Cdyn; C) over time in RAO-affected (n = 7) and control (7) horses housed in an RAO-inducing environment. Base represents measurement on initial confinement; base +1, the second day of confinement; final –1, the penultimate day; and final, the final day (maximum period of confinement, 7 days). The line in the center of each box represents the mean, top and bottom boundaries of the box represent the 25th and 75th percentile, and the whiskers indicate maximum and minimum values. *Value is significantly ($P < 0.05$) different from the control value on the same day.

study. Furthermore, radiography revealed that intercostal space 6 was totally within the thorax and intercostal space 17 was caudal to the diaphragm (**Figure 2**).

RIP output in RAO-affected versus control horses—Confinement in an RAO-inducing environment led to clinical airway obstruction in the RAO-affected horses at varying rates. Four pairs of horses completed the 7-day exposure. The remaining 3 pairs had to be removed from the stables after 4 days because of the severity of obstruction in the RAO-affected horses. For this reason, the decision was made to analyze the data at the following 4 time points: baseline (at introduction to stable confinement), after 1 day of confinement, on the second-to-last day of confinement, and on the final day of confinement. The maximal change in pleural pressure of RAO-affected horses increased during stable confinement and differed sig-

nificantly from control values on the second-to-last ($P = 0.007$) and final ($P = 0.004$) days of confinement (**Figure 3**). Likewise, dynamic compliance significantly decreased from baseline in the RAO-affected group and was significantly different from the control group on the second-to-last ($P = 0.004$) and final ($P \leq 0.001$) days of confinement. Although it was lower than baseline on the second-to-last and final days in the RAO-affected group, pulmonary resistance only differed significantly ($P = 0.012$) from control values on the final day. Stable confinement had no significant effect on maximal change in pleural pressure, pulmonary resistance, or dynamic compliance in the control group.

RIP—Although neither total degree of respiratory displacement measured nor respiratory rate as measured by RIP differed between groups or changed during stable confinement (**Figure 4**), considerable differences were identified in breathing patterns between RAO-affected and control horses (**Figure 5**). In control horses, the amplitude and duration of each breath varied over time: short and quick breaths were often followed by bigger, less frequent breaths. In contrast, the RAO-affected horses had a repetitive pattern in both amplitude and duration with little variability among breaths. This variability was evaluated through calculation of the SDs of total degree of respiratory displacement and respiratory rate measured by RIP for the 6 time blocks in each day. In the first 24 hours of stable confinement, SDs of total degree of respiratory displacement and respiratory rate measured by RIP did not change in control horses but decreased in the RAO-affected animals. The 2 groups differed significantly by 8 hours after stable confinement (**Figure 6**). These significant differences between groups persisted during the final 24 hours of confinement.

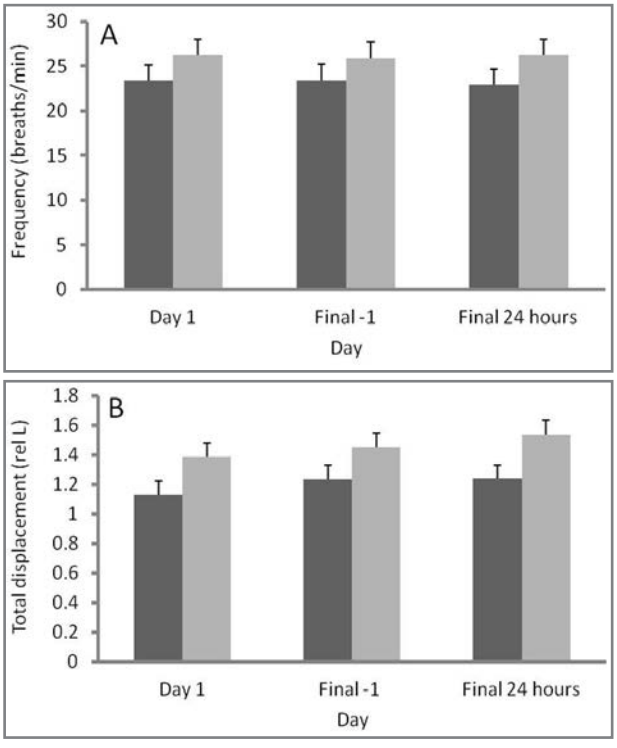

Figure 4—Mean ± SE respiratory frequency (A) and total degree of respiratory displacement (B) as measured via RIP for the control (black bars) and RAO-affected (gray bars) horses in Figure 3. There were no significant differences between groups or over time. rel L = Relative liters. *See* Figure 3 for remainder of key.

A circadian rhythm was evident in the SDs of total degree of respiratory displacement and respiratory rate measured by RIP (**Figure 7**). For this reason, split-plot ANOVA was performed to examine the main effect of time period during the day. The results were the same for both variables. In control horses, the SDs of total degree of respiratory displacement and respiratory rate measured were significantly ($P < 0.05$) greater during the period 6 AM to 9 AM than during all other periods. In the RAO-affected horses, the circadian variation was more obvious, with a significant nadir between 6 PM and 2 AM. At all periods except 10 AM to 2 PM, the SDs of total degree of respiratory displacement and respiratory rate were significantly less in RAO-affected than in control horses.

To determine the relationship between both RIP variables (SDs of total degree of respiratory displacement and respiratory rate) and the pulmonary function variables, correlation analysis was performed with the period 2 PM to 6 PM, which was the first period during which groups significantly differed after stable confinement, used as the comparison period. The correlation between lung function and the RIP variables was initially poor, but over the final 24 hours of confinement, values were highly correlated (**Table 1**). Generally, both RIP variables were better correlated with maximal change in pleural pres-

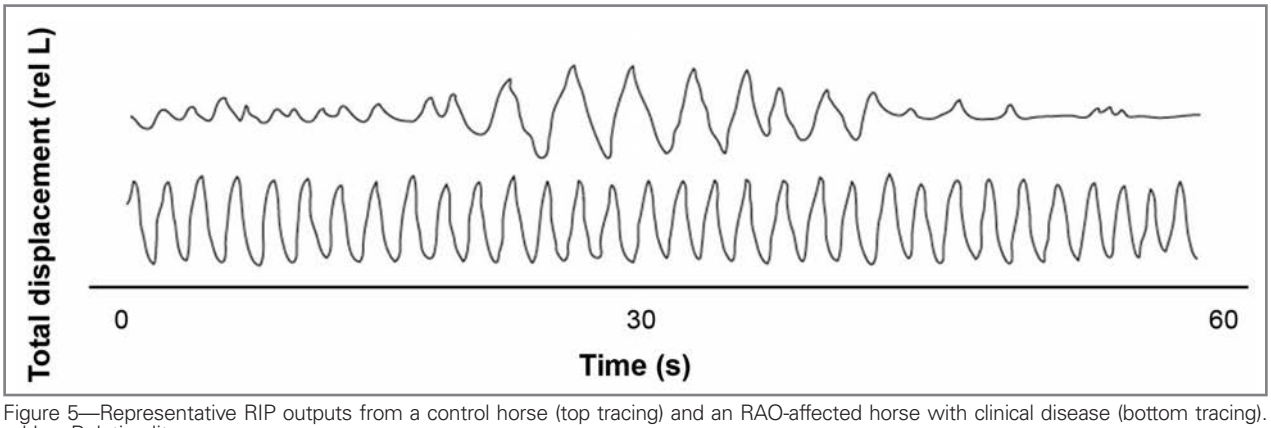

Figure 5—Representative RIP outputs from a control horse (top tracing) and an RAO-affected horse with clinical disease (bottom tracing). rel L = Relative liters.

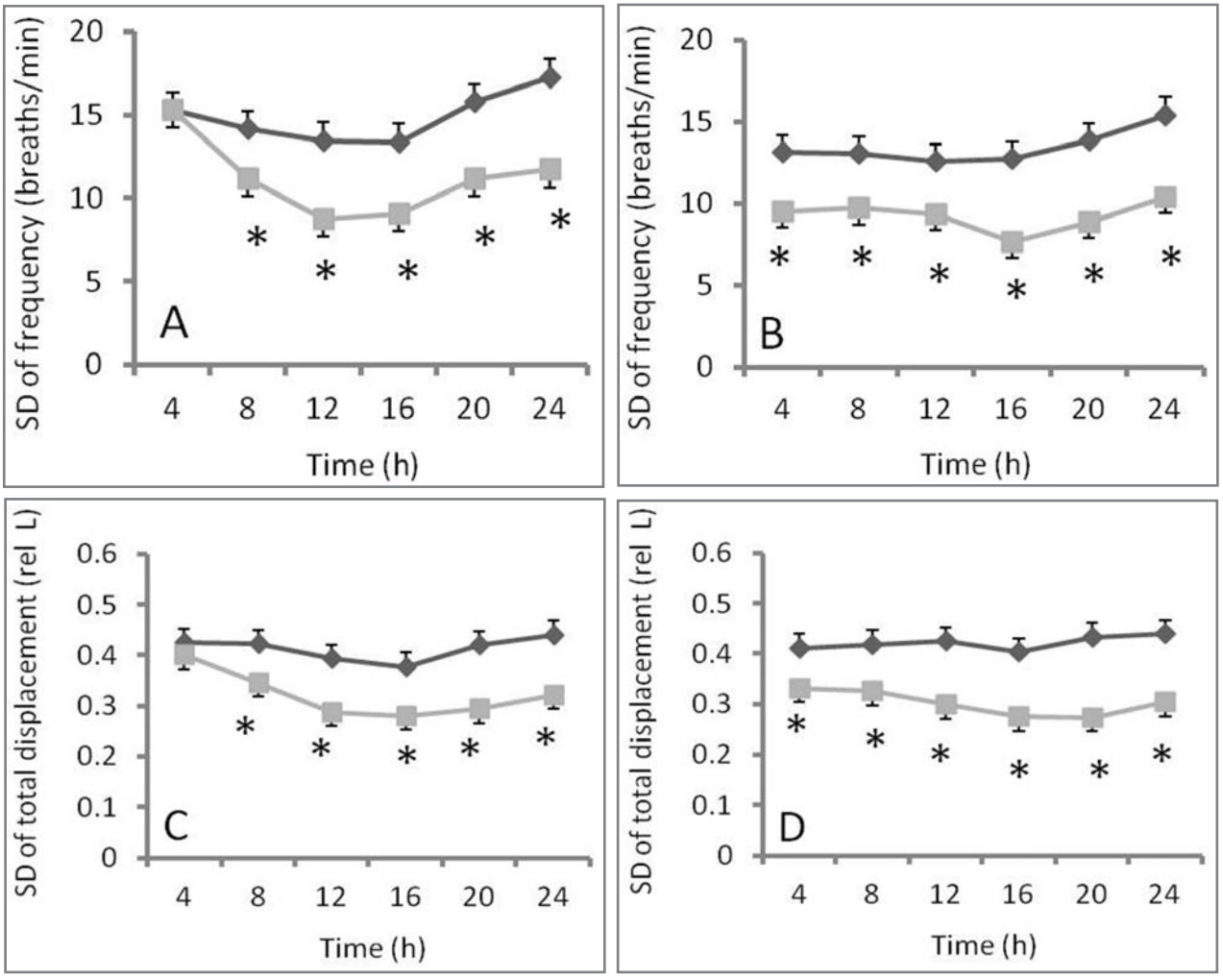

Figure 6—Mean ± SE SD of respiratory frequency (A and B) and total degree of respiratory displacement (C and D) as measured over time via RIP for the control (diamonds) and RAO-affected (squares) horses in Figure 3. Data were measured at 4-hour intervals throughout the first (A and C) and final (B and D) 24 hours of stable confinement. *Value is significantly ($P < 0.05$) different from the control value at the same period of day. rel L = Relative liters.

sure than with pulmonary resistance or dynamic compliance.

Discussion

To our knowledge, the present study is the first in which continuously monitored breathing patterns were characterized in unrestrained horses for > 24 hours. The cumbersome nature of conventional pulmonary function testing limits the ability to analyze sequential breathing patterns over an extended period. Therefore, a less invasive method, RIP, was used, revealing significant decreases in breathing pattern variability within 8 hours after stable confinement in RAO-affected horses, well before significant changes were evident in maximal change in pleural pressure, pulmonary resistance, or dynamic compliance.

Respiratory inductance plethysmography can be used to measure changes in the cross-sectional area of the thorax and abdomen. During inhalation, the cross-sectional area of the rib cage increases; in addition, contraction of the thoracic diaphragm displaces the abdominal contents, resulting in an increase in the cross-sectional area of the abdomen. For this reason, it was necessary to sum the degree of displacement of the thoracic and abdominal compartments during inspiration to provide an indication of the total volume inhaled.

Relating RIP band data to absolute changes in volume measured at the nares can be problematic, particularly in horses with airway obstruction. This is because of gas trapping within the lung, which results in gas compression and phase differences between RIP and volumes measured at the external nares.[8] Because we were interested solely in use of RIP to detect the presence of lung disease and not in phase relationships, RIP bands were not calibrated to absolute volumes and therefore the total degree of displacement was only reported as relative volume. Traditionally, respiratory frequency is determined by counting breaths per minute, but in the present study, the frequency was calculated breath-by-breath as the reciprocal of the duration of each breath. Calculation of the frequency from each breath provided greater power to examine variability.

The main objective of the first phase of the study was to determine optimal RIP band placement for mea-

surement of the thoracic and abdominal components of respiration. One RIP band was maintained at intercostal space 6, and the second was randomly placed over the more caudal odd-numbered spaces. The anatomy of the thoracic diaphragm and rib cage provides the basis for understanding the effect of intercostal space on the degree of inspiratory displacement. Because the cranial ribs form part of the pectoral girdle, their respiratory movements are limited. The asternal and floating ribs have more freedom of movement and therefore contribute more to the total degree of respiratory displacement.[11] Intercostal space 6 was chosen as the position of the thoracic band because it encloses only the thoracic contents and is between 2 sternal ribs. As the abdominal band was placed more caudally, 2 factors contributed to the increasing degree of inspiratory displacement: the movement of the asternal ribs and the outward movement of the abdominal wall that results from caudal displacement of the diaphragm.

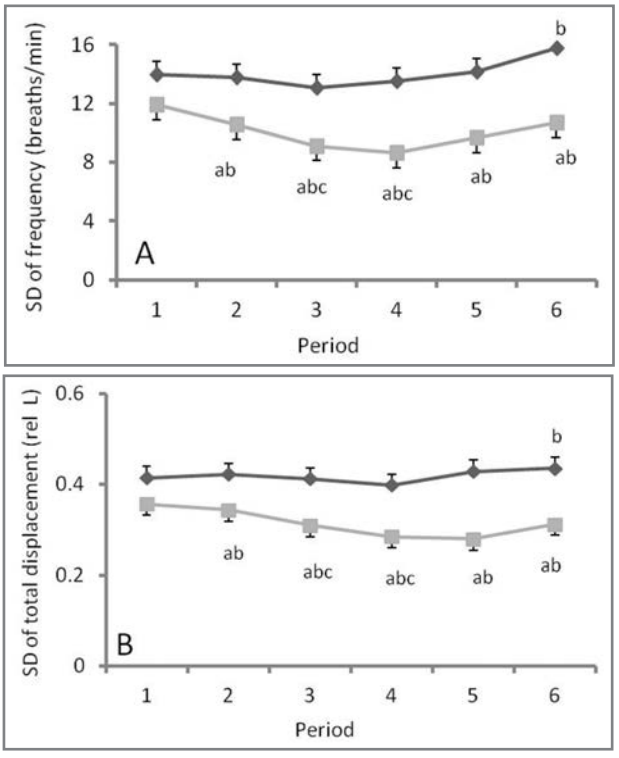

Figure 7—Mean ± SE SD of respiratory frequency (A) and total degree of respiratory displacement (B) by time of day (ie, circadian rhythm) for the control (diamonds) and RAO-affected (squares) horses in Figure 3. Starting around 10 AM (period 1), data are summarized in 4-hour periods, with the exception of period 6, which comprised only 3 hours. [a]Value is significantly ($P < 0.05$) different from that during 10 AM to 2 PM (period 1) in RAO-affected horses. [b]Value is significantly ($P < 0.05$) different between control and RAO-affected horses at the indicated point. [c]Value is significantly ($P < 0.05$) different between period 3 (2 PM to 6 PM) and period 6 (6 AM to 9 AM).

Significant differences between the degree of inspiratory displacement for thoracic band at intercostal space 6 and the abdominal band were not evident until the abdominal band was placed at intercostal space 11. The mean degrees of displacement for intercostal spaces 13 to 17 were not significantly different from one another. Because intercostal space 17 was totally over the abdomen and was the position of abdominal band placement that yielded the greatest degree of inspiratory displacement, it was selected as the optimal location to measure abdominal excursions. In another study,[8] the abdominal RIP band was positioned behind the 18th rib; however, our study showed that data from this location were highly variable and only significantly different from values at intercostal spaces 6 and 9.

Results of pulmonary function tests performed during stable confinement of RAO-affected and control horses indicated that airway obstruction developed in the RAO-affected but not in the control horses. The maximal change in pleural pressure and pulmonary resistance increased and dynamic compliance decreased with time in the RAO-affected horses and became significantly different from control values on the final 2 days of stable confinement.

The RIP output of total degree of inspiratory displacement and frequency did not differ between groups or throughout stable confinement. A previous study[4] involving use of pulmonary function tests showed that tidal volume is unaffected by stable confinement in both control and RAO-affected horses. On the other hand, reports[4,12] of changes in breathing frequency with stable confinement are conflicting.

Although breathing frequency did not differ between groups nor change with confinement, further examination of the 24-hour RIP traces revealed a considerably different pattern of breathing between the control and RAO-affected animals (Figure 5). To quantitatively express this difference, we calculated the SDs of total degree of respiratory displacement and respiratory rate measured by RIP. In contrast to mean total de-

Table 1—Pearson correlation coefficients for associations between RIP output and pulmonary function variables in RAO-affected (n = 7) and control (7) horses housed in an RAO-inducing environment.

Variable	Maximal change in pleural pressure	Pulmonary resistance	Dynamic compliance
SD of respiratory rate measured via RIP			
Baseline	0.56	0.73	0.51
Next-to-last day of stable confinement	0.78	0.35	0.64
Final day of stable confinement	0.90	0.75	0.58
SD of total degree of respiratory displacement measured via RIP			
Baseline	0.63	0.51	0.52
Next-to-last day of stable confinement	0.81	0.32	0.44
Final day of stable confinement	0.82	0.73	0.56

gree of respiratory displacement measured by RIP and respiratory rate, these 2 values in RAO-affected horses significantly decreased by 8 hours after confinement but remained unchanged in the control horses. This significant difference between RAO-affected and control horses persisted throughout confinement. The only way to explain a decreased SD in breathing frequency and total displacement in the absence of a change in mean values is that breathing pattern of RAO-affected horses became less variable as the disease became more severe. As breathing approached a regular wave form, each breath approached the same degree of inspiratory displacement (tidal volume) and lasted for the same duration.

The loss in breathing pattern variability likely involves respiratory control mechanisms, the main goal of which is maintenance of gas exchange.[13] For the latter to occur, the brain receives sensory input from various sources and appropriately adjusts the rate and depth of breathing. These sources include chemoreceptors, airway and intrapulmonary receptors, and muscle stretch receptors. Peripheral chemoreceptors monitor changes in Pa_{O_2}, Pa_{CO_2}, and blood pH. Pulmonary and airway receptors innervated by vagal afferent nerves include stretch, irritant, and C-fiber receptors that can activate bronchoconstriction, initiate cough, and change rate and depth of breathing. Intercostal muscle stretch receptors adjust the force of contraction to maintain ventilation during airway obstruction and other changes in the mechanical properties of the lung.

In horses with RAO, all of the aforementioned factors may provide input to respiratory control because inflammation leads to airway obstruction, which causes ventilation-perfusion mismatching, resulting in hypoxemia.[14] However, it is unlikely that low Pa_{O_2} is an important regulator of the breathing pattern because administration of oxygen in a dose-dependent manner to increase Pa_{O_2} to ≥ 100 mm Hg has no effect on respiratory rate.[15] In contrast, vagal afferents may play an important role in the regulation of breathing in RAO-affected horses. When ovalbumin sensitization and exposure is used to model RAO in ponies, vagal blockade leads to a decrease in respiratory rate.[16] This observation is in agreement with the considerable evidence for the involvement of vagal afferent fibers, such as pulmonary C-fibers and rapidly adapting stretch receptors, as contributors to dyspnea in people.[17] The sensitivity of these pulmonary afferents to stimuli increases in the presence of airway inflammation, and these changes can last well beyond the period of acute inflammation.[18] Therefore, we propose that the recurrent bouts of inflammation characteristic of RAO upregulate pulmonary afferent sensory function, thereby causing alterations in breathing patterns. Interestingly, in RAO-affected horses, neutrophil influx into the lung occurs within 6 hours of stable confinement,[19] which coincides with the onset of decreased variability of breathing identified in our study. Absence of a similar decrease in control horses was presumably because the transient inflammation that occurs during confinement is of much lesser magnitude than in the RAO-affected horses.[20]

A circadian rhythm in the breathing variability was particularly evident in the RAO-affected group, with the significant nadir in the SDs of the 2 RIP variables occurring in the evening and early night (6 PM to 2 AM). This circadian variation in the breathing patterns coincides with the diurnal variation in serum cortisol concentration. In clinically normal horses, serum cortisol concentration peaks during the morning (6 AM to 9 AM) and reaches a trough in the evening hours (7 PM to 11 PM).[21–24] Because of the anti-inflammatory properties of cortisol, it is possible that as the cortisol concentration decreases, the degree of pulmonary inflammation increases. Pulmonary inflammation increases activity in pulmonary afferent nerves and alters the breathing pattern of RAO-affected horses. In healthy horses, evidence of a circadian rhythm was minimal, most likely because the airway inflammation was minimal. Support of our hypothesis will require concurrent measurement of breathing variability, inflammatory markers, and serum cortisol concentrations.

Comparisons were made between the SDs of RIP values and pulmonary function test values (maximal change in pleural pressure, pulmonary resistance, and dynamic compliance) to determine the relationship between the 2 types of measurements. Although the SDs of the RIP values became significantly less in RAO-affected than control horses within 8 hours after stable confinement began, significant differences in pulmonary function between the 2 groups were not evident until the final 2 days of confinement. This explains the poor correlation between breathing variability and lung function during the early stages of the disease. However, as the severity of airway obstruction increased, the SDs of the RIP values became highly correlated with the lung function values, particularly with maximal change in pleural pressure. Overall, these observations indicated that the SDs of total degree of respiratory displacement and respiratory rate measured by RIP are early indicators of RAO exacerbations.

In the study reported here, telemetric RIP was found to be a noninvasive technique that could be used to measure variations in breathing patterns of clinically normal and RAO-affected horses over long periods. The technique allowed monitoring of horses in a challenge environment during the onset and progression of RAO. If, as we postulate, airway inflammation alters sensory nerve activity resulting in the early changes in breathing patterns, then use of RIP will provide new insights into the inflammatory mechanisms involved during the onset of the disease.

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b. Aeromask, Trudell Medical International, London, ON, Canada.
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