Surfactant alterations in horses with recurrent airway obstruction at various clinical stages

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**Objective**—To evaluate the phospholipid composition and function of surfactant in horses with recurrent airway obstruction (RAO) at various clinical stages and compare these properties with findings in horses without RAO.

**Animals**—7 horses with confirmed RAO and 7 without RAO (non-RAO horses).

**Procedures**—Pairs of RAO-affected and non-RAO horses were evaluated before, during, and after exposure to hay. Evaluations included clinical scoring, lung function testing, airway endoscopy, and bronchoalveolar lavage fluid (BALF) absolute and differential cell counts. Cell-free BALF was separated into crude surfactant pellet and supernatant by ultracentrifugation, and phospholipid and protein concentrations were determined. Phospholipid composition of crude surfactant pellets and surface tension were evaluated with high-performance liquid chromatography and a pulsating bubble surfactometer, respectively. Findings were compared statistically via mixed-effects, repeated-measures ANOVA.

**Results**—Total phospholipid concentration in BALF was lower in RAO-affected versus non-RAO horses at all sampling times. In the RAO-affected group, total phospholipid concentration was lower during exposure to hay than before or after exposure. There were no significant differences in BALF protein concentration, percentages of phospholipid classes, or surface tension between or within groups of horses.

**Conclusions and Clinical Relevance**—All clinical stages of RAO-affected horses were characterized by low surfactant concentration in BALF. Exacerbation of RAO led to an additional decrease in surfactant concentration. Causes for low surfactant concentration in RAO-affected horses remain to be determined. Low phospholipid concentration may render RAO-affected horses more susceptible than unaffected horses to surfactant alterations and contribute to clinical disease status and progression. (Am J Vet Res 2010;71:468–475)

Lung surfactant, produced by type 2 alveolar cells, lines the epithelial surface of the lung. Lung surfactant is largely (> 80%) composed of phospholipids with a smaller portion of surfactant-specific proteins (approx 10%) and neutral lipids (5% to 10%). Phospholipids confer to surfactant its ability to lower surface tension in the alveoli (biophysical function). Surfactant proteins A and D participate primarily in innate immune defense mechanisms of the lung (immunologic function), whereas surfactant proteins B and C contribute considerably to biophysical surfactant functions.

Phospholipids are molecules with an amphipathic structure consisting of a hydrophilic head region (determining phospholipid class) and 2 hydrophobic tails (composed of fatty acid chains). This molecular structure allows achievement of low surface tension and prevention of alveolar collapse. Surfactant deficiency in premature animals leads to neonatal respiratory distress
syndrome. This syndrome is prevented and treated by administration of exogenous surfactant to premature infants. Surfactant dysfunction (high surfactant surface tension) contributes to the pathophysiology of acute respiratory distress syndrome.

Airway surfactant, which is derived from alveolar surfactant, is essential to maintain airflow in small conducting airways. It promotes mucociliary clearance, opposes airway edema, and reduces the sensitivity and overall magnitude of the bronchocinetic response. Surfactant alterations have been described for various airway diseases, including asthma in humans. Endobronchial allergen challenge in asthmatic humans induces surfactant dysfunction, serum protein infiltration, and alteration in the distribution of surfactant aggregates and phospholipid composition. Protein inhibition is considered one of the main mechanisms of surfactant dysfunction in asthmatic patients. Hydrolysis of surfactant phospholipids via secretory phospholipase A2 further contributes to surfactant dysfunction.

Surfactant alterations in RAO-affected horses with clinical disease exacerbation have been reported. Such surfactant abnormalities include decreases in surfactant phospholipid concentration, changes in surfactant aggregate ratio, and changes in the ratio between PC and PG, the 2 main phospholipid classes of surfactant. Changes in surfactant composition and function that develop as a result of short-term exposure to a challenge environment have not been evaluated in RAO-affected horses. The purpose of the study reported here was to examine surfactant composition and function in RAO-affected horses at various clinical stages and to compare these properties with findings in horses without RAO.

Materials and Methods

Horses—Two groups of horses were used in the study: those affected with RAO (RAO-affected horses; n = 7) and those without RAO (non-RAO horses; 7). The non-RAO horses were part of the Virginia-Maryland Regional College of Veterinary Medicine equine teaching herd. The group included 6 mares and 1 gelding of various breeds; mean ± SD age was 14 ± 4 years. These horses were housed on pasture all year long, had no history or clinical signs of respiratory disease for at least 1 year, and were known not to develop clinical signs of airway obstruction when housed in a barn and fed hay. The RAO-affected horses were part of a preexisting herd of RAO-affected horses maintained at the teaching hospital. The group included 4 mares and 3 geldings of various breeds; mean age was 17 ± 3 years. These horses were known to develop airway inflammation and obstruction when housed in a barn and fed moldy hay and to enter remission once pastured or stabilized in a controlled environment.

Experimental protocol—Each RAO-affected horse was paired with a non-RAO horse, and the horse pairs (n = 7) were subjected to the same sample collection procedures and environments in parallel. Whenever possible, horse pairs were of comparable age and body weight. Sample collection times for each horse pair were determined by the clinical stage in RAO-affected horses.

Before the beginning of the study, all horses were maintained on pasture for a minimum of 6 weeks to induce remission. A baseline sample of BALF was collected from a horse pair when the RAO-affected horse was in remission and had a clinical score < 4 according to a scoring system described elsewhere. Briefly, the sum of scores for nasal flare (1 to 4; from lowest to highest degree) and abdominal component to breathing (1 to 4) was calculated, resulting in a total score of 2 for no signs, 3 or 4 for mild signs, 5 or 6 for moderate signs, and 7 or 8 for severe signs of RAO. One week later, horses were brought into the research barn. Horses were housed in pairs in juxtaposed stalls and maintained in similar environmental conditions. Horse pairs were bedded on dusty straw and fed dusty hay to create an environmental challenge for the RAO-affected horses. This environmental challenge was continued until RAO-affected horses developed evident clinical signs of airway obstruction with a clinical score > 4 or horses had been in the barn for > 3 days; at that point, the exposure BALF sample was collected. Horse pairs were then released back to a pasture environment. The postexposure BALF sample was collected when RAO-affected horses were in recovery from airway obstruction, as indicated by a decrease in clinical score by 2 points or after housing on pasture for > 1 week.

Data and sample collection—Physical examination and clinical score assessment of each horse were performed twice a day by the same investigator (UC). At each sample collection point, a series of tests and procedures was performed and included measurement of ∆Ppl\textsubscript{max} during tidal breathing, airway endoscopy with mucus score assessment, and BALF collection and analysis.

Change in pleural pressure during tidal breathing was measured with techniques described elsewhere. Briefly, an esophageal balloon (length, 10 cm; perimeter, 3.5 cm; and wall thickness, 0.06 cm) was sealed over the end of a polypropylene catheter (internal diameter, 3 mm; and external diameter, 4.4 mm). The tubing was passed into the distal third of the esophagus and attached to a low-range differential pressure transducer calibrated before each set of measurements by means of a water manometer. The position of the esophageal balloon was adjusted to obtain the ∆Ppl\textsubscript{max}. Pleural pressure during breathing was obtained by a computer equipped with lung function software. At each data collection time, values for at least 15 consecutive breaths were averaged.

Horses were sedated with butorphanol (0.01 mg/kg, IV) and detomidine (0.01 mg/kg, IV), and an endoscope was passed through the nasal passages and into the trachea. Endoscopic examination of the trachea was subsequently performed. The amount of mucus visible in the trachea was graded on a scale of 0 to 5, as described elsewhere (0 = no mucus; 1 = a few small blobs; 2 = moderate amount; 3 = marked amount, stream forming; 4 = large amount, pool forming; and 5 = marked amount). Bronchoalveolar lavage fluid was collected by passing a cuffed BALF collection tube through the nasal passages and into the trachea. A 0.4% lidocaine solution (approx 60 mL) was infused to reduce
coughing, the tube was advanced until wedged, and the cuff was inflated with 6 mL of air. Three aliquots of 100 mL (total volume, 300 mL) of prewarmed sterile saline (0.9% NaCl) solution were infused and reaspirated manually by use of a syringe. Aspirated fluid for each horse was mixed, pooled in a sterile specimen cup, and placed on ice. Recovered fluid volume and quality were recorded. When possible, mucus strands were carefully removed from samples by use of a pipette. Fluid samples were processed within 20 minutes after collection.

Analysis of BALF—Samples of BALF were centrifuged at 400 × g for 10 minutes. Cell-free supernatant was removed and further processed for isolation of surfactant. A differential cell count was evaluated on slides prepared with a cytocentrifuge and stained with a modified Wright stain. At least 400 cells/specimen were counted with a light microscope by use of oil immersion at a magnification of 100X.

Isolation and analysis of surfactant—Cell-free BALF supernatant was centrifuged at 40,000 × g for 1 hour to allow its separation into 2 fractions: CSP and surfactant supernatant. Surfactant supernatant was isolated, and the CSP was washed twice with 100 mL of saline solution (pH, 7.4). The CSP was then resuspended in a known volume of saline solution (depending on the pellet diameter). Aliquots of surfactant supernatant and CSP were stored at −80°C.

Phospholipids from the CSPs and surfactant supernatant were isolated by organic extraction, and their lipid phosphorus concentration was measured. Protein concentrations in CSP and surfactant supernatant were measured by use of the bicinchoninic acid method with bovine serum albumin as the standard. Total phospholipid and protein concentrations were calculated as the sum of their respective contents in each CSP and surfactant supernatant. In preparation for high-performance liquid chromatography, samples of CSP underwent extraction, and their lipid phosphorus concentration was measured. Protein concentrations in CSP and surfactant supernatant were measured by use of the bicinchoninic acid method with bovine serum albumin as the standard. Total phospholipid and protein concentrations were calculated as the sum of their respective contents in each CSP and surfactant supernatant.

In analysis of phospholipid classes in each CSP were determined: PC, PG, phosphatidylethanolamine, PI, and sphingomyelin. In preparation for high-performance liquid chromatography, samples of CSP underwent extraction, and their lipid phosphorus concentration was measured. Protein concentrations in CSP and surfactant supernatant were measured by use of the bicinchoninic acid method with bovine serum albumin as the standard. Total phospholipid and protein concentrations were calculated as the sum of their respective contents in each CSP and surfactant supernatant.

A pulsating bubble surfactometer was used to measure surface tension–lowering activity of the surfactant. Surfactant from CSP was brought to a final concentration of 0.5 mg of phospholipid/mL with buffered saline solution containing 1.5mM CaCl₂. Samples (40 μL) were analyzed at 20 cycles/min for 10 minutes at 37°C in the surfactometer, as described. Results are reported as γₐₘₜₑₓₚₑ₉.

In addition, commercial surfactant was incubated with surfactant supernatant at 37°C from 10 minutes to 4 hours. This incubation was performed to assess whether surface tension of commercial surfactant was affected by inhibitors potentially present in the supernatant from the various horses. Following incubation, samples were prepared and evaluated as described previously.

Statistical analysis—For all responses measured on a continuous scale (ie, phospholipid and protein concentrations, γₐₘₜₑₓ, and percentage of phospholipid class), a mixed-effects, repeated-measures ANOVA was used to test for main effects of disease and sample collection time as well as their interaction. Disease status (RAO or no RAO), sample time (date), and their interaction were included in the model as fixed effects; horse pairs (block) were included as random effects. Repeated measures (date) were specified for horses within each horse pair and disease state. The Tukey-Kramer adjustment was used for multiple comparisons. Data for cell counts and phospholipid and protein concentrations were log-transformed for statistical analysis to fulfill model assumptions. Data are reported as mean ± SE or geometric mean (95% confidence interval). Adjusted values of P < 0.05 were considered significant.

Results
Clinical evaluation—In non-RAO horses, a clinical score < 4 was maintained throughout the study and no significant differences existed in clinical scores among sample collection times (Figure 1). In the RAO-affected horses, the clinical score was significantly different at each sample collection time. All RAO-affected horses had a clinical score < 4 at baseline (before exposure to the challenge environment), the clinical score increased to 5 or 6 (moderate disease) in 4 horses and to 7 or 8 (severe disease) in 3 horses during exposure, and the postexposure score was intermediate (between baseline and exposure values). Furthermore, the clinical scores of RAO-affected horses during and after exposure were significantly higher than those of non-RAO horses at any sample collection time.

When values for all sample collection times were combined, tracheal mucus scores were significantly higher in RAO-affected horses versus non-RAO horses. When values for both horse groups were combined, tracheal mucus scores were significantly higher during exposure and after exposure to the challenge versus before exposure. The mucus scores of non-RAO horses were < 1.5 at all sample collection times, and no significant differences existed among sample collection times (Figure 1). Mucus scores of RAO-affected horses at the various sample collection times ranged from 1 to 3 and were significantly higher after versus before exposure.

For ΔPpl₀-₉ₐₓ, a significant interaction existed between the effect of RAO status and sample collection time. In the non-RAO group, no significant differences existed in ΔPpl₀-₉ₐₓ between sample collection times. All non-RAO horses had a ΔPpl₀-₉ₐₓ < 8 cm of H₂O at all sample collection times (Figure 1). In RAO-affected horses, the ΔPpl₀-₉ₐₓ was significantly higher during exposure than before or after exposure. It was ≤ 8 cm of H₂O at baseline, was ≥ 20 cm of H₂O in most (6/7) horses during exposure, and reached an intermediate value after exposure. Values of ΔPpl₀-₉ₐₓ only increased to 8 cm of H₂O in 1 RAO-affected horse during exposure. Compared with values in non-RAO horses at any sample collection time, values of ΔPpl₀-₉ₐₓ in RAO-affected horses were significantly higher during exposure.

When data for both horse groups were combined, results of differential cell counts indicated
the percentage of neutrophils in BALF was significantly higher during exposure than at baseline and after exposure. In the non-RAO group, no significant differences existed in neutrophil percentage between sample collection times. The neutrophil percentage in BALF from non-RAO horses was < 5% at baseline, < 25% during exposure, and < 10% after exposure. In the group of RAO-affected horses, the neutrophil percentage was significantly higher during exposure than at baseline and after exposure. Among the RAO-affected horses, the neutrophil percentage in BALF was < 10% at baseline and after exposure (Table 1). Six RAO-affected horses had a neutrophil percentage > 15% during exposure. In one of the RAO-affected horses, the neutrophil percentage reached only 11% during exposure. Analysis of data from macrophage and lymphocyte percentages revealed no significant interaction between the effect of RAO status and sample collection time. When data from all sample collection times were combined, macrophage percentage was significantly higher in non-RAO horses versus RAO-affected horses. When data from both horse groups were combined, macrophage and lymphocyte percentages were significantly lower during exposure than before or after exposure.

The percentage of BALF that was recovered after the lavage procedure was calculated and compared between non-RAO and RAO-affected horses. Percentages did not differ significantly between the groups with baseline, exposure, and postexposure values in non-RAO and RAO-affected horses.

Table 1—Mean ± SE percentage of neutrophils, macrophages, and lymphocytes among leukocytes harvested from BALF of horses with (RAO-affected; n = 7) and without (non-RAO; 7) RAO before (baseline), during, and after exposure to an environmental challenge with dusty straw and hay. Data on ΔPplmax for non-RAO horses at baseline include values for 5 horses only because 2 horses were too uncooperative for measurement. Lower limits for some error bars are truncated because none of the data included negative values. *Values differ significantly (P < 0.05) between non-RAO horses at baseline and RAO-affected horses at the indicated sample collection time. †Values differ significantly between non-RAO horses during exposure and RAO-affected horses at the indicated sample collection time. ‡Values differ significantly between non-RAO horses after exposure and RAO-affected horses at the indicated sample collection time. §, ‖Similar symbols represent significant differences between sample collection times within the group of RAO-affected horses.

<table>
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<th>Component</th>
<th>Non-RAO Baseline</th>
<th>Non-RAO Exposure</th>
<th>Non-RAO Postexposure</th>
<th>RAO-affected Baseline</th>
<th>RAO-affected Exposure</th>
<th>RAO-affected Postexposure</th>
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</thead>
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<tr>
<td>Neutrophils</td>
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<td>3.7 ± 4.5</td>
<td>6.1 ± 4.5</td>
<td>33.3 ± 4.5†‡</td>
<td>5.1 ± 4.5‡</td>
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<td>40.6 ± 4.0</td>
<td>49.7 ± 4.0</td>
<td>41.3 ± 4.0</td>
<td>28.6 ± 4.0‡</td>
<td>36.6 ± 4.0</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>48.6 ± 6.7</td>
<td>41.0 ± 4.7</td>
<td>46.4 ± 6.7</td>
<td>52.6 ± 4.7†</td>
<td>36.1 ± 4.7§</td>
<td>55.7 ± 4.7</td>
</tr>
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*Values differ significantly (P < 0.05) between non-RAO horses at baseline and RAO-affected horses at the indicated sample collection time. †Values differ significantly between non-RAO horses after exposure and RAO-affected horses at the indicated sample collection time. §, ‖Similar superscripts represent significant (P < 0.05) differences between pairs of sample times within the group of RAO-affected horses.
non-RAO horses of 64.3 ± 4.7%, 59.4 ± 4.7%, and 54.9 ± 4.7%, respectively, and values in RAO-affected horses of 50.9 ± 4.7%, 52.8 ± 4.7%, and 57.7 ± 4.7%, respectively. Similarly, the percentage of BALF recovered did not differ from one sample collection time to another. There were no other significant differences.

Surfactant analysis—When data from all sample collection times were combined, phospholipid concentration in cell-free BALF and its subfractions (CSP and surfactant supernatant) was significantly higher in non-RAO horses than in RAO-affected horses. When data for both horse groups were combined, phos-
lipid concentration in cell-free BALF and its subfractons was significantly higher at baseline than during exposure. In addition, phospholipid concentration in surfactant supernatant was significantly higher at baseline than after exposure.

Examination of differences in means revealed that the phospholipid concentration in cell-free BALF and CSP was significantly higher at all sample collection times in non-RAO versus RAO-affected horses (Figure 2; Table 2). Phospholipid concentration in surfactant supernatant was significantly higher in non-RAO horses at baseline than during or after exposure in RAO horses. In the RAO-affected horses, cell-free BALF phospholipid concentration was higher at baseline than during exposure. No significant differences existed among sample collection times in the group of non-RAO horses.

When data from both horse groups were combined, total protein concentration in BALF was significantly higher at baseline than during exposure. No significant differences existed among sample collection times in the group of non-RAO horses. In the RAO-affected horses, cell-free BALF phospholipid concentration was significantly higher at baseline than after exposure. No other significant differences were evident (Figure 2; Table 2).

Sample collection time and horse group had no significant effect on the percentage of various phospholipids in the phospholipid fraction of CSP (Table 3). When data from all sample collection times were combined, the mean percentage of PG was lower in RAO-affected horses (6.0 ± 0.2%) versus non-RAO horses (6.7 ± 0.2%) but the difference was not significant (P = 0.06). Similarly, when data from all sample collection times were combined, the mean percentage of PI appeared higher in RAO-affected horses (1.1 ± 0.1%) versus non-RAO horses (0.9 ± 0.1%) but the difference was not significant (P = 0.054). Concentrations of PC, PI, and PG in the phospholipid fraction of BALF were significantly higher in non-RAO horses versus RAO-affected horses at all sample collection times; no interaction existed between RAO status and sample collection time (data not presented).

No significant differences were found in γ_y between RAO and non-RAO horses or between sample collection times. Similarly, no significant differences existed in surface tension of commercial surfactant incubated with sample surfactant supernatant (Figure 3).

**Discussion**

The study reported here yielded evidence that BALF phospholipid concentration is lower not only in RAO-affected horses versus non-RAO horses during clinical exacerbation of RAO (as has been reported) but also in RAO-susceptible horses lacking clinical signs. Exposure of RAO-affected horses to a challenge environment of dusty straw and hay and the ensuing clinical exacerbation led to a further decrease in BALF phospholipid concentration. In a previous study, we evaluated RAO-affected horses with variable duration of clinical disease and exposure to hay. The age range of RAO-affected horses in that study was comparable to that in the present study. In contrast to findings from our previous study and another study, no abnormalities were detected in surfactant phospholipid composition or function in the present study.

Causes of lung surfactant alterations include decreased surfactant synthesis and secretion by type 2 alveolar cells, increased surfactant degradation via inflammatory enzymes or cells, or inhibition of surfactant function through accumulation of edema fluid or inflammatory exudate. Depending on the type and severity of inflammation present, several or all of these mechanisms may exist.

Even though changes in surfactant aggregate ratio develop in asthmatic humans, an overall decrease of surfactant in those patients has not been reported. However, repeated allergen challenge in a model of asthma in guinea pigs reportedly induces a decrease in amounts of surfactant and PC synthesis. Focal alveolar changes do develop in RAO-affected horses and include alveolar hyperinflation and fibrosis.
tural changes in type 2 alveolar cells from RAO-affected horses include various degrees of cellular degeneration and accumulation of lamellar bodies (the cellular storage form of surfactant). It is conceivable that these morphological changes in type 2 alveolar cells are accompanied by altered surfactant metabolism.

Inflammatory enzymes such as phospholipases and proteases have the ability to degrade surfactant components. In addition, the capacity of macrophages and neutrophils to degrade surfactant increases during inflammation. Our group and others have reported decreased amounts of PG in horses clinically affected with RAO, indicating possible degradation of this specific surfactant component. Similar findings have also been detected in asthmatic patients with surfactant dysfunction. In the present study, the percentage of PG in the phospholipid fraction of surfactant appeared to be lower in RAO-affected horses than in non-RAO horses but the difference between groups was not significant. The number of horses in our study may have been too low to reveal a significant difference. It is also possible that short duration of exposure to the allergen and development of only moderate airway neutrophilia in RAO-affected horses failed to induce changes in surfactant composition. Alternatively, a nonspecific degradation of surfactant components by macrophages and neutrophils may have taken place in RAO-affected horses.

Endobronchial allergen challenge leads to pronounced airway inflammation, surfactant dysfunction, and increased BALF protein concentration in human asthmatics. A relationship between airway inflammation and surfactant dysfunction (as indicated by high surface tension) was not evident in our study. Specifically, no significant difference in surfactant function was detected between groups of horses or at different sample collection times within groups of horses. In our study, in vitro surfactant function was measured at a constant phospholipid concentration (0.5 mg/mL). However, the in vivo effect of a generalized reduction in phospholipid concentration was not examined. Incubation of commercially available surfactant with surfactant supernatants (that typically contain inhibitory proteins) also did not yield evidence of surfactant inhibition by proteins in RAO-affected horses. Similar to findings in our study, disease in nonchallenged asthmatic humans does not induce a significant increase in BALF protein concentration or in vitro surface tension.

In the present study, low phospholipid concentration in BALF persisted at all clinical stages in RAO-affected horses and was exacerbated following exposure to hay. Horses with RAO reportedly maintain a degree of airway obstruction (measured with forced expiratory flow) during remission. These changes were believed to be related to persistent airway remodeling in affected horses. Such horses likely maintain a chronic degree of airway inflammation during remission (even in the absence of evident BALF neutrophilia) similar to that in asthmatic human patients. For example, an increase in myeloperoxidase (an enzyme released from neutrophils during respiratory burst) concentration in BALF without concurrent BALF neutrophilia was detected in RAO-affected horses 2 months after they were returned to pasture. Our findings provided evidence that surfactant alterations also persist during remission in RAO-affected horses.

The nature of our results (decreased phospholipid concentration in the BALF of RAO-affected horses without changes in phospholipid composition and function) may raise the question of whether sample recovery from the bronchoalveolar region was less efficient in RAO-affected versus non-RAO horses. For example, airway inflammation, bronchoconstriction, and excessive mucus accumulation in RAO may influence recovery of surfactant from the alveoli to the conducting airways. In addition, mucus accumulation may trap surfactant and prevent efficient recovery. However, percentage BALF recovery was not significantly different between groups of horses in our study; and the small differences detected were not enough to explain measured changes in phospholipid concentration. Measurement of dilutional markers has been suggested as a means of evaluating dilution of pulmonary epithelial lining fluid during BALF collection. Concentrations of dilutional markers (ie, urea or albumin) are influenced by dwell time, pulmonary permeability, and other factors and are therefore not considered reliable indicators of pulmonary epithelial lining fluid dilution. Current recommendations for reporting acellular components in BALF are to standardize BALF recovery volumes and to report results in amount per milliliter of BALF recovered or as a ratio or proportion of the components measured. In our study, results were reported according to those recommendations.

We speculate that low phospholipid concentration in BALF from RAO-affected horses may render these horses more susceptible to surfactant alterations and dysfunction when airway inflammation develops. In the absence of considerable amounts of surfactant inhibitors (ie, protein), a substantial decrease in surfactant phospholipid concentration must take place to result in dysfunction. In vitro experiments have revealed that surfactant maintains low surface tension at phospholipid concentrations as low as 0.3 mg/mL. Surfactant in healthy animals exists in abundance, offering protection against the effect of possible surfactant-altering or-inhibiting agents. Changes in surfactant phospholipid concentration can impact mucus wettability and mucociliary transport and may contribute to increased mucus accumulation in RAO-affected horses. Low amounts of surfactant may also affect bronchial hyperresponsiveness by 2 mechanisms: unmasking of irritant receptors and impaired smooth muscle relaxation.

In the study reported here, less surfactant was present in BALF from horses with RAO at all clinical stages of the disease than in horses without RAO. Exposure of RAO-affected horses to hay led to a further decrease in BALF phospholipid concentration. It is likely that low BALF phospholipid concentration in RAO-affected horses was related to a combination of factors including changes in surfactant synthesis, secretion or passage of surfactant to the conducting airways, degradation of surfactant, and perhaps inhibition of surfactant function. Our study did not yield evidence of a specific cause of the low amount of surfactant in RAO-affected horses nor of an injury to a specific surfactant com-
ponent other than a reduction in PG concentration. Additional studies, which will likely require a larger cohort of RAO-affected horses, are needed to clarify these mechanisms. Furthermore, the influence of surfactant alterations on the pathophysiologic aspects of RAO remains unclear. We speculate that low amounts of surfactant phospholipids result from ongoing inflammation and remodeling in RAO-affected horses and may contribute to the clinical manifestation of RAO, including impaired mucus clearance and bronchial hyperresponsiveness.

References


