Effect of aerosolized albuterol sulfate on resting energy expenditure determined by use of open-flow indirect calorimetry in horses with recurrent airway obstruction

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Objective—To evaluate effects of sedation on stability of resistance of the respiratory system (RRS) and measures of resting energy expenditure (REE) by use of open-flow indirect calorimetry (IC) and treatment with aerosolized albuterol on REE in horses with recurrent airway obstruction (RAO).

Animals—9 clinically normal horses and 8 horses with RAO.

Procedure—In phase 1, RRS was measured by using forced oscillometry (FOT) in 5 clinically normal horses before and after sedation with xylazine. In phase 2, REE was measured in 4 clinically normal horses between 20 and 25 minutes and again 35 to 40 minutes after sedation with xylazine. In phase 3, IC was performed between 20 and 25 minutes and FOT was performed between 30 and 35 minutes after xylazine administration in 8 horses with RAO; after administration of 450 μg of albuterol, IC and FOT were repeated.

Results—In phase 1, RRS values were significantly lower 5 and 10 minutes after sedation. In phase 2, diminishing sedation did not significantly affect REE. In phase 3, there was a significant decrease in mean RRS (1.15 ± 0.25 vs 0.84 ± 0.14 cm H_20/L/s) and REE (30.68 ± 17.89 vs 27.46 ± 16.54 kcal/kg/d) after albuterol administration.

Conclusions and Clinical Relevance—FOT and IC are useful in obtaining repeatable measurements of RRS and REE, respectively, in sedated horses. Concurrent bronchodilation and decreased REE after albuterol administration suggest that increased work of breathing as a result of airway obstruction may contribute to increased energy demands in horses with RAO. (*Am J Vet Res* 2003;64:235–242)

Recurrent airway obstruction (RAO), also known as heaves, is a common disease in horses and is characterized by reversible airway obstruction, bronchospasm, and airway hyperreactivity attributable to inflammation of the airways, production of mucus, and thickening of airway walls.[1] Horses that have an exacerbation of RAO are characterized by flared nostrils, coughing, and marked expiratory efforts. Even during clinical remission, horses with RAO have residual, subclinical airway inflammation, obstruction, and hyperresponsiveness.[2] Clinicians have mentioned that a subset of horses with RAO tends to be thin, perhaps even cachectic. It is unknown whether increased work of breathing contributes to weight loss in these horses.

Although the pathologic mechanism of **chronic obstructive pulmonary disease (COPD)** in humans differs substantially from that of horses with RAO,[2] it may be useful to mention that humans with COPD similarly have frequent weight loss. There are multiple putative contributors to COPD-associated weight loss, including increased **resting energy expenditure (REE)** attributable to increased oxygen consumption needed to overcome airway obstruction.[3,4] Pharmacologic treatment with β_2-agonist bronchodilators, however, increases REE in humans with COPD despite bronchodilation.[5-6,a] Horses with RAO are frequently treated with **β_2-agonist receptors (β_2-ARs)**, especially aerosolized albuterol, to effect bronchodilation.[7] Despite several studies on the effects of β_2-ARs on airway and lung mechanics in horses with airway obstruction during rest[7-9] and in clinically normal horses during exercise,[10-12] to the authors' knowledge, there have not been any studies on the effect of β_2-ARs on oxygen consumption or REE in horses with airway obstruction during rest. Airway obstruction in humans with COPD is often largely irreversible,[13] as opposed to horses with RAO. Therefore, it may be that albuterol sulfate (as a β_2-AR bronchodilator) has a greater ability to reverse airway obstruction in horses with RAO, which consequently results in decreased energy expenditure in these horses.

Open-flow indirect calorimetry, a modification of open-flow respirometry that has been commonly used in exercising horses,[12,14-17] has been used extensively to measure REE in humans that are in critical condition[18] and as a tool to investigate weight loss in humans with COPD. By measuring **oxygen consumption ($\dot{V}O_2$)** and **carbon dioxide production ($\dot{V}CO_2$)**, the abbreviated Weir formula[19] can be used to calculate the number of kilocalories needed by a resting individual each day. This technique is considered to be extremely accurate and reliable in humans,[18,20] but its use in veterinary medicine has been largely confined to small animals.[21,22] Its use for measurement of REE in large animals at rest[23,24] has been hampered by technical limitations of sensitivity and reproducibility.

We had 3 objectives in the study reported here. First, because of the difficulty of inserting instruments

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and conducting lung function tests in untrained horses, we intended to assess the effect of the α_2-agonist, xylazine hydrochloride, on measurements of RRS and REE. Xylazine is an ideal sedative, but it has physiologic effects on airways.[25-28] Because our ultimate objective was to determine the effect of albuterol sulfate on REE and work of breathing attributable to respiratory resistance, stability of measurements in sedated horses was an important determination. Second, we intended to assess the short-term repeatability of open-flow indirect calorimetry measurements in sedated horses. Third, we intended to measure the calorimetric responses of 8 horses with various degrees of airway obstruction after administration of aerosolized albuterol sulfate. We theorized that there were 2 possible outcomes. One possible outcome was that despite the potential thermogenic effect, stimulation by a β_2-AR bronchodilator would result in a decrease in work of breathing and, thus, a decrease in REE. The second possible outcome was that thermogenic effects would predominate, and despite a possible decrease in work of breathing, REE would increase.

Materials and Methods

Animals—Seventeen adult horses were used in the 3-phase study. None of the horses had been treated with any corticosteroid for 4 weeks or bronchodilators for 2 weeks before phase 3 of the study. The Institutional Animal Care and Use Committee at Tufts University School of Veterinary Medicine approved all procedures.

In phase 1, 5 clinically normal Standardbred mares (mean ± SD age, 5 ± 2 years; mean weight, 385 ± 50 kg) were used to determine the effects of xylazine on the stability of baseline total **resistance of the respiratory system (RRS)** as measured by use of **forced oscillometry (FOT)**. In phase 2, 4 clinically normal Standardbred mares (mean age, 9 ± 2 years; mean weight, 450 ± 52 kg) were used to assess the effects of changes in sedation on REE measurements within a 1-hour period. In phase 3, 8 horses (3 geldings and 5 mares; mean age, 13 ± 5 years; mean weight, 442 ± 77 kg; mean body condition score 5 ± 1 [scale of 1 to 9]) of various breeds that had RAO were used to determine REE and RRS before and after albuterol administration. Horses for phase 3 were chosen on the basis that they had a documented history of RAO (per a referring veterinarian) that included recurring reversible episodes of increased expiratory effort, nasal flaring, coughing, and nasal discharge without evidence of infection. Bronchoalveolar lavage was performed after testing was completed to further characterize the extent of RAO in each horse.

Body condition scoring—The procedure was adapted from that described by Henneke et al.[29] It involved the use of a combination of visual inspection and palpation to rate each horse from extremely thin (score of 1) to extremely fat (score of 9), with a score of 5 being ideal.

Indirect calorimetry—The open-flow indirect calorimetry system used in the study was a modification of a system used for exercise testing.[12] Briefly, air was drawn through a rigid open facemask via flexible airway tubing (outside diameter, 7 cm) by use of a vacuum[b] located outside the building. Flow was regulated with a rotameter equipped with a control valve.[c] Flow through the system was between 450 and 650 L/min, depending on the size of the horse, and allowed all of the expired air to be drawn into the mask. A 200-L mixing chamber was interposed between the horse and gas analyzers. Air was drawn through the mixing chamber, traversing 2 plastic plates spaced 30 cm apart. Each plate contained 9 circular openings that were 8 cm in diameter and located at regular intervals. An aliquot of the mixed-air sample emanating from the mixing chamber was diverted to a sampling pump with a flowmeter-needle valve assembly to control sample flow through the sensor cells,[d] which was then split and metered to the carbon dioxide analyzer[e] and oxygen analyzer.[f] Before entering the oxygen analyzer, the air sample traversed a column of ascarite to remove carbon dioxide and a drying column that consisted of anhydrous calcium sulfate. Oxygen and carbon dioxide fractions were measured continuously. The $\dot{V}O_2$ was calibrated by use of the Fedak nitrogen dilution technique, which has an accuracy of ± 3%,[30] whereas $\dot{V}CO_2$ was calibrated by infusing a known flow of carbon dioxide into the mask. All volumes were corrected on the basis of standard temperature and pressure (dry). A minimum of 10 minutes was allowed for equilibration, at which time the system had reached a steady state. Once a steady state was achieved, data were collected for an additional 5 minutes for use in calculating mean $\dot{V}O_2$ and mean $\dot{V}CO_2$ for the period. Analogue signals from the gas analyzers were digitized and processed by use of a computer and custom-written software.[g]

Values for REE were calculated from mean $\dot{V}O_2$ and $\dot{V}CO_2$ by use of the abbreviated Weir equation[31]: REE = $(3.94\dot{V}O_2 + 1.1\dot{V}CO_2) \times 1.44$. The equation ignores urine nitrogen content and, therefore, does not take into account protein metabolism; this is considered to contribute a negligible amount of error to the calculation of the value in humans.[19] The Weir equation assumes that each liter of oxygen consumed will generate 3.94 kcal and that each liter of carbon dioxide produced will generate 1.1 kcal.

Measurement of lung mechanics—Lung mechanics determined by use of FOT were used for determination of total respiratory system impedance, which was measured automatically over the frequency range of 1 to 3 Hz in 1-Hz increments by use of a personal computer and purpose-built controller and digital signal processing system,[h] using a previously described technique.[12,32-34] In brief, a sinusoidal airflow of the desired frequency was generated by use of a proportional pneumatic valve[i] connected to a compressed air source (517.1 kPa) and applied to each horse's respiratory system via a rigid, low-dead-space, shrouded facemask. A resistor (approx 2 cm H_2O/L/s) attached to a side arm diverted most of the oscillating airflow into the horse's nostrils while allowing the horse to breathe quietly. Flow at the mask opening was measured by use of a pneumotachograph[j] attached to a differential pressure transducer.[k] Another differential pressure transducer[l] was used to measure pressure generated at the airway opening, and the difference between mask and atmospheric pressures was determined. Amplified pressure and flow signals were digitized at 25.6 Hz for 10 seconds. Signals were filtered (0.4-Hz-wide passband centered at the measurement frequency and 80-decibel stopband attenuation) and divided into consecutive 5-second epochs with 50% overlap; total respiratory system impedance was calculated from those epochs.[35] Coherence values were also calculated to provide an indication of the signal-to-noise ratio and linearity of the system. Only values with coherence ≥ 0.90 were stored for analysis.

The forced oscillation apparatus was calibrated daily by use of the wave tube principle. An open-ended polyvinyl chloride tube with dimensions similar to those of the FOT measurement head (length, 6.3 m; inside diameter, 52.3 mm) was used as a reference impedance. **Impedance of the tube (Zm)** was measured by use of the forced oscillatory apparatus; **theoretical impedance of the tube (Zref)** was calculated by use of the equations of Franken et al,[36] and a **complex correction factor (*k*)** was derived at each frequency by use of the

following equation: k = Zref/Zm. Subsequent **corrected values of Zm (Zcorr)** were calculated by use of the value of k appropriate for the measurement frequency, using the following equation: Zcorr = Zm × k. Total RRS was derived from total respiratory system impedance by use of the following equation that models the respiratory system as a series electrical circuit:

$$Zcorr = (R^2 + [2\pi f - 1/2\pi fC]^2)^{1/2}$$

where R is the resistance, f is oscillatory frequency, and C is compliance.[32] During all testing, the head of each horse was maintained in a neutral position (parallel to or slightly above the horizontal plane) to minimize the effects of sedation.[27]

Administration of aerosolized drug—Albuterol[m] was administered by use of an aerosolizing facemask system.[n] This system consisted of a well-fitting facemask with inhalation and exhalation valves and a holding chamber. Before administration of albuterol (90 µg of albuterol/activation), the metered-dose inhaler was shaken for 1 minute, followed by a single primer activation. The metered-dose inhaler was then attached to the holding chamber and activated at end-expiration. A total of 5 activations were performed with 30-second intervals between each activation (total of 450 µg of albuterol delivered). This dose is in accordance with other doses that have been effective in eliciting bronchodilation in horses with RAO.[7]

Bronchoalveolar lavage—A sterile tube[o] was used to perform bronchoalveolar lavage, as described elsewhere.[37] The volume of sterile saline (0.9% NaCl) solution used was 500 mL, which was instilled in two 250-mL aliquots and removed by use of suction (–10 cm H_2O). Samples were transferred to tubes that contained EDTA and prepared for cytologic examination (centrifugation of 600 µL at 600 × g for 5 minutes). Air-dried smears were stained with a Wright-Giemsa solution, and 800 cells were classified under high magnification (1,000×) as alveolar macrophages, lymphocytes, neutrophils, metachromatic (mast) cells, or eosinophils; values for each were expressed as percentages of the total count.

Assessment of sedation—During measurements of lung mechanics, sedation was judged to be adequate when each horse allowed its head to be maintained in a fully extended position and was unresponsive to minor movements and noises within the laboratory. During measurement of REE, sedation was judged to be adequate when there was a lack of voluntary movement (eg, head tossing, foot movements, tail swishing) throughout the testing period and when horses were quiet and nonresponsive to minor noises and movements within the laboratory.

Phase 1—The RRS was measured at frequencies of 1 to 3 Hz in 5 clinically normal, unsedated horses. Although the head of each horse was maintained in the most fully extended position possible, voluntary movement was noticeable in the unsedated horses. Each horse was then sedated by administration of xylazine hydrochloride[p] (0.5 mg/kg, IV), the head was maintained in an extended position, and RRS was measured at frequencies of 1 to 3 Hz at 5, 10, 20, 30, and 45 minutes after injection of xylazine.

Phase 2—Food was withheld for 4 hours from 4 clinically normal horses, and each horse was then administered xylazine (0.5 mg/kg, IV). Ten minutes after administration of xylazine, indirect calorimetry was performed during a 15-minute period (10-minute equilibration period and 5-minute measurement period of REE). The mask was removed for 10 minutes, and a second 10-minute equilibration period began 35 minutes after administration of xylazine, which was immediately followed by a second 5-minute measurement period of REE.

Phase 3—All testing in phase 3 took place during a 3-month period. Food was withheld for 12 hours from each of 8 horses with RAO before testing began. Each horse was administered xylazine (0.5 mg/kg, IV) and allowed to rest for 10 minutes. Then, REE was measured by use of indirect calorimetry during a 15-minute period (10 minutes for equilibration and 5 minutes for data collection). Thirty minutes after administration of xylazine, lung mechanics were measured by use of FOT during a period of 3 to 5 minutes. At 35 minutes after administration of xylazine, albuterol was administered via a metered -dose inhaler during a period of approximately 5 minutes, and horses were then allowed to rest quietly for 10 minutes.

Ten minutes after administration of albuterol, each horse was again administered xylazine (0.5 mg/kg, IV) and allowed to rest for 10 minutes. Then, REE was again measured by use of indirect calorimetry during a 15-minute period (10 minutes for equilibration and 5 minutes for data collection). At 30 minutes after administration of albuterol, lung mechanics were measured by use of FOT during a period of 3 to 5 minutes. At 35 minutes after administration of albuterol, each horse was again administered xylazine (0.5 mg/kg, IV), and bronchoalveolar lavage was performed. Each horse was subsequently allowed to recover from sedation in a stall.

Statistical analysis—In phase 1, a repeated-measures ANOVA was used to examine the effect of time for each frequency. The Bonferroni test was used to perform pairwise multiple comparisons. In phase 2, a paired t-test was performed to detect differences in REE, $\dot{V}O_2$, $\dot{V}CO_2$, and **respiratory quotient (RQ)** with decreases in sedation. In phase 3, REE, RRS, $\dot{V}O_2$, $\dot{V}CO_2$, and RQ before and after administration of albuterol were compared by use of a 2-tailed paired t-test. The Pearson correlation coefficient was used to test for associations between REE and RRS, $\dot{V}O_2$, $\dot{V}CO_2$, and RQ before and after albuterol administration. The Pearson correlation coefficient was also used to test for associations between percentages of cells in bronchoalveolar lavage fluid and measures of indirect calorimetry and lung function during the period before as well as the period after albuterol administration. The Spearman correlation coefficient[q] was used to test for associations between measures of lung function, body condition score,[29] REE, and results of cytologic examination of bronchoalveolar lavage fluid. An independent-samples t-test was used to compare REE, $\dot{V}O_2$, and $\dot{V}CO_2$ among horses between 20 and 25 minutes after administration of xylazine in phases 2 and 3.

Data were reported as mean ± SD. Significance for all statistical analyses was assumed at a value of $P < 0.05$.

Results

Phase 1—We did not detect a significant effect of xylazine on RRS conducted at a frequency of 1 Hz (**Fig 1**). There was a significant decrease (maximum, –34%) in RRS conducted at a frequency of 2 Hz at 5 and 10 minutes, compared with the measurement obtained prior to sedation; there were no significant differences between the value obtained prior to sedation and values for any subsequent measurements (20, 30, and 45 minutes) or between the value at 10 minutes and the value for any subsequent measurements. There was a significant decrease in RRS conducted at 3 Hz (maximum, –39%) at 5, 10, 20, and 30 minutes.

Phase 2—During all indirect calorimetry tests, there appeared to be a steady plane of sedation as

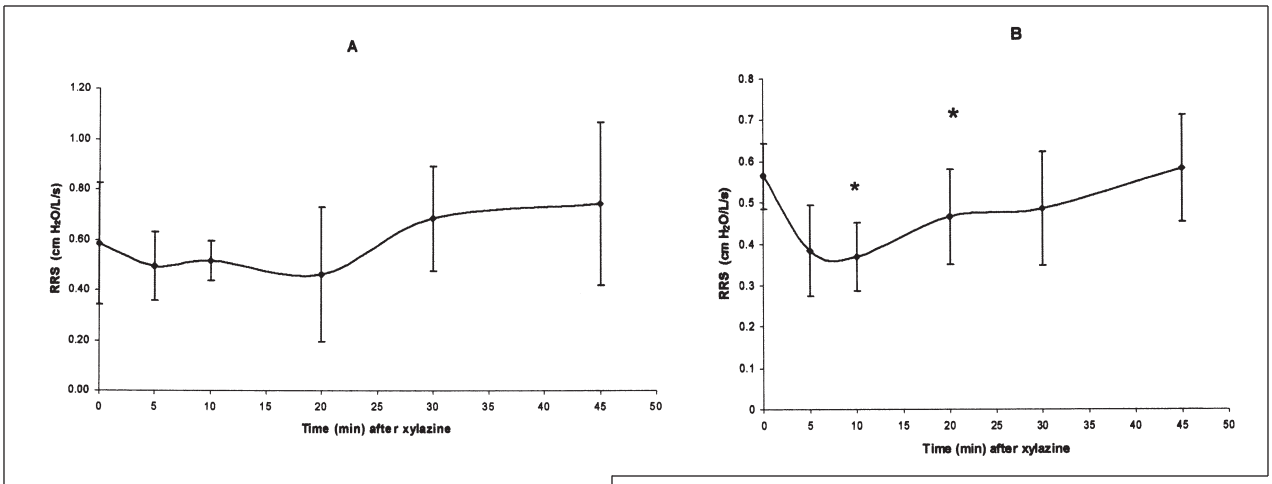

Figure 1—Mean ± SD values in phase 1 for resistance of the respiratory system (RRS) in 5 clinically normal horses before (time 0) and 5, 10, 20, 30, and 45 minutes after administration of xylazine (0.5 mg/kg, IV). Measurements were obtained at frequencies of 1 (A), 2 (B), and 3 (C) Hz, respectively. *Within a frequency, value differs significantly ($P < 0.05$) from value at time 0.

judged by lack of voluntary movements (eg, head tossing, foot movements, tail swishing) throughout the testing period. Horses were quiet and nonresponsive to minor noises and movements within the laboratory. Room temperature remained between 19.0 and 21.0°C. We did not detect significant differences between the 2 measurements made on each horse within a 1-hour period. Group means at the initial measurement were 4.00 ± 0.83 mL/kg/min for $\dot{V}O_2$, 2.71 ± 1.04 mL/kg/min for $\dot{V}CO_2$, and 26.65 ± 5.47 kcal/kg of body weight/d for REE, whereas means for the final measurement were 4.09 ± 1.08 mL/kg/min for $\dot{V}O_2$, 2.31 ± 0.40 mL/kg/min for $\dot{V}CO_2$, and 26.60 ± 6.34 kcal/kg of body weight/d for REE.

Phase 3—During all indirect calorimetry tests, the plane of sedation appeared to be steady as determined by visual inspection of horses. Room temperature remained between 19.0 and 21.0°C. During testing with the FOT at 1 Hz, coherence was not > 0.9 for all horses; therefore, data at 2 Hz were used for analysis.

Neutrophils were found in high numbers in bronchoalveolar lavage fluid in all horses (mean ± SD, 57 ± 30%). Mean baseline respiratory resistance (ie, measurements obtained between 30 and 35 minutes after injection) was higher (after xylazine, 1.15 ± 0.25 H_2O/L/s; after albuterol, 0.84 ± 0.14 cm H_2O/L/s) than that established as a reference value by our laboratory group[37] (< 0.65 cm H_2O/L/s; **Fig 2**). Response to albuterol varied. There was a significant mean decrease in RRS for all horses after albuterol administration (24.20 ± 19.01%); however, 3 horses had a decrease in RRS of < 10%. Only 1 horse had marked respiratory distress, although RRS was not markedly increased in that horse (1.03 cm H_2O/L/s).

Resting values (ie, values obtained between 20 and 25 minutes after injection) ranged from 2.57 to 4.49 mL/kg/min for $\dot{V}O_2$ and from 17.94 to 32.2 kcal/kg/d for REE, except for 1 horse that had values of 12.41 mL/kg/min for $\dot{V}O_2$ and 77.25 kcal/kg/d for REE; all values except those for that 1 horse were within the published ranges.[23,38,39] The horse that had values outside the published ranges was smaller than its cohorts (290 kg, compared with a mean of 442 kg).

Oxygen consumption in response to albuterol varied. There was a significant decrease in mean $\dot{V}O_2$ after administration of albuterol (4.59 ± 2.98 mL/kg/min before vs 4.10 ± 2.78 mL/kg/min after). There was not a significant change in $\dot{V}CO_2$ after administration of albuterol (3.27 ± 1.27 mL/kg/min before vs 2.99 ± 1.06 mL/kg/min after; **Fig 3**). There were 3 horses that had a decrease of < 10% for each of those variables. The REE after administration of albuterol also varied; however, there was a significant mean decrease in REE (before albuterol, 30.68 ± 17.89 kcal/kg/d; after albuterol, 27.46 ± 16.54 kcal/kg/d; a decrease of 10.36 ± 12.27%; **Fig 4**). We did not detect a significant difference between RQ measurements before and after albuterol (0.78 ± 0.15 and 0.79 ± 0.14, respectively). There was not a significant correlation between REE or RRS before or after albuterol administration.

When values for the 4 clinically normal horses in phase 2 were compared with values for the 8 horses with RAO in phase 3, we did not detect significant differences between $\dot{V}O_2$, $\dot{V}CO_2$, or REE, regardless of

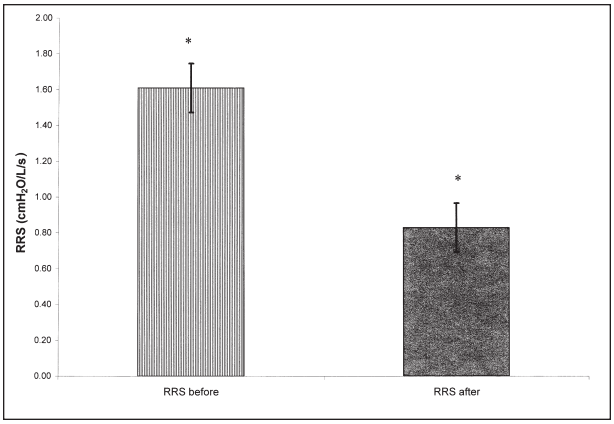

Figure 2—Mean ± SD values during phase 3 for RRS in 8 horses with recurrent airway obstruction (RAO) before and 40 to 45 minutes after aerosolized administration of 450 µg of albuterol. Measurements were obtained by use of forced oscillometry at a frequency of 2 Hz 30 to 35 minutes after administration of xylazine (0.5 mg/kg, IV). *Values differ significantly ($P < 0.05$).

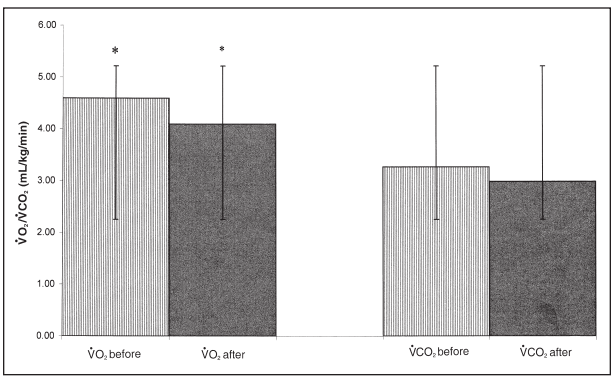

Figure 3—Mean ± SD values during phase 3 for oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) in 8 horses with RAO before and 30 to 35 minutes after aerosolized administration of 450 µg of albuterol. Measurements were obtained by use of open-flow indirect calorimetry 20 to 25 minutes after administration of xylazine administration. *See* Figure 2 for key.

whether it was before or after albuterol administration. We did not detect any other significant correlations between measures of lung function, indirect calorimetry, or age.

Discussion

Although xylazine causes bronchodilation in ponies with pre-existent airway obstruction,[26] we documented in the study reported here that stable measurements for RRS can be obtained for > 45 minutes in horses sedated with xylazine, and clinical observations suggested that the degree of sedation was consistent. Although RRS performed at a frequency of 2 Hz did decrease significantly between time of injection and 10 minutes, there was not a significant difference thereafter between RRS at baseline and at later measurements. This finding suggests that, depending on the frequency used for measurement, the bronchodilatory effects of the α_2-agonist, xylazine hydrochloride, are short-lived. However, it is important to mention that there was a significant decrease in RRS performed at a frequency of 3 Hz for up to 30 minutes after injection; thus, stability of measurement was only determined for measurements made at 1 and 2 Hz.

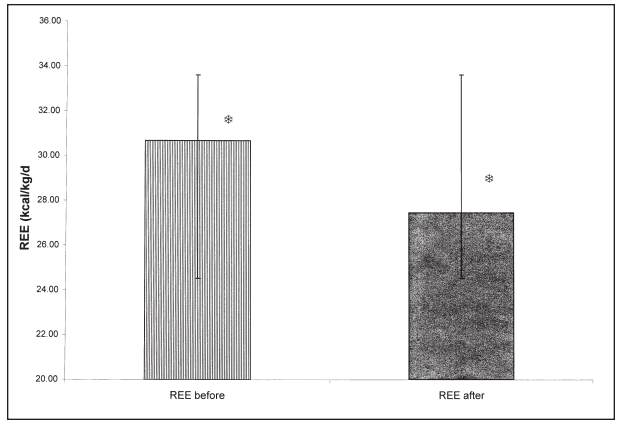

Figure 4—Mean ± SD values during phase 3 for resting energy expenditure (REE) in 8 horses with RAO before and 30 to 35 minutes after aerosolized administration of 450 µg of albuterol. The REE was calculated by use of the Weir equation: REE = $(3.94\dot{V}O_2 + 1.1\dot{V}CO_2) \times 1.44$. Values for $\dot{V}O_2$ and $\dot{V}CO_2$ were obtained by use of open-flow indirect calorimetry 20 to 25 minutes after xylazine administration . *See* Figure 2 for key.

Open-flow respirometry, which is based on the same principles as open-flow indirect calorimetry, has been used extensively in exercise studies in horses[12,17,39]; however, there are little published data on energy expenditure in resting horses.[23,24] It would seem intuitive that REE would be higher in animals with RAO than in control animals because of the increased work of breathing incurred by the disease. When we compared $\dot{V}O_2$, $\dot{V}CO_2$, and REE between clinically normal horses and horses with RAO before or after albuterol administration, there was not a significant difference between the 2 groups, although mean values were greater at the initial measurement for horses with RAO ($\dot{V}O_2$, 4.59 ± 2.98 mL/kg/min; $\dot{V}CO_2$, 3.27 ± 1.27 mL/kg/min, REE, 30.68 ± 17.89 kcal/kg/d) than for clinically normal horses ($\dot{V}O_2$, 4.00 ± 0.83 mL/kg/min; $\dot{V}CO_2$, 2.71 ± 1.04 mL/kg/min; REE, 26.65 ± 5.47 kcal/kg/d) before albuterol administration. However, the higher values for horses with RAO were influenced by values for 1 horse; without the values for that horse, the mean would have been closer to the mean value of the clinically normal horses. As reflected by the lung mechanics, most of the horses with RAO in phase 3 were mildly affected; thus, it is likely that REE will need to be examined in a greater number of clinically normal horses and horses severely affected with RAO to fully determine whether horses with RAO have REE that is higher than in healthy horses.

One horse had a noticeable increase in REE, compared with values for the other horses. Pagan and Hintz[23] did not detect a difference in REE when allometric scaling was used; nonetheless, smaller animals tend to have greater energy expenditure even when scaling on the basis of mass.[40] It seems intuitive that the visibly greater respiratory effort observed in this horse would have contributed to an increase in REE; however, RRS was not markedly increased in that horse. This may have been attributable to greater compliance of the pharyngeal region, causing decreased values for

RRS[41] or harmonics in the breathing signal that were the same frequency as the oscillatory signal.[42] It is also possible that the higher values for $\dot{V}O_2$, $\dot{V}CO_2$, and REE were attributable to experimental error (eg, there may have been improper mixing of gases during measurements). However, we tested 2 other horses on the same day, both of which had measurements that were 2.5- to 3-fold lower than for that horse. Unfortunately, the owner would not allow us to use the horse for additional testing, so the experiment could not be repeated. It is of interest that this horse had the lowest body condition score (3 of a possible 9), and it may have had a high REE as a result of the combination of greater respiratory distress, small size, and cachexia potentially attributable to systemic inflammatory mediators.

Although the horses in 2 studies[23,24] were confined in metabolism stalls, even small movements can contribute to substantial changes in REE.[19,43] In contrast, horses were sedated in the study reported here to ensure more consistent data so that the effects of treatment could be more easily elucidated. The results for phase 2 support our use of indirect calorimetry in evaluation of the effects of pharmacologic intervention in horses with RAO in an effort to evaluate the effects of β_2-ARs on the work of breathing and overall REE.

Horses with RAO have an increased work of breathing,[44] and clinically normal horses have an increased total oxygen cost of ventilation when forced to breath against an imposed load.[45] This may be attributable to increases in work of diaphragmatic and extrathoracic muscles necessary to overcome increased airway resistance, as well as the consequences of breathing at a mechanical disadvantage engendered by overinflation. Similar to humans with COPD, horses with RAO are frequently treated with β_2-ARs to partially reverse airflow obstruction and, presumably, decrease the oxygen cost of ventilation. Stimulation of β_2-ARs activates adenylate cyclase, which catalyzes the conversion of ATP to cAMP by activating the Na^+–K^+-ATPase pumps that are linked to β_2-adrenoceptors on cells. Ultimately, this leads to relaxation of bronchial smooth muscles. As a second messenger, cAMP also sets into motion a cascade of events that leads to activation of glycogenolysis and lipolysis and a release of hormones that contribute to regulating REE, such as thyroid hormone and insulin.[46] Administration of aerosolized albuterol, a β_2-agonist, has little or no effect on oxygen consumption in clinically normal horses during exercise[10-12]; however, studies in humans with airway obstruction suggest 2 possible outcomes after administration of albuterol to horses with RAO: an increase in REE as a result of the thermogenic effects of β_2-ARs[6,a] or a decrease in REE as a result of a decrease in work of breathing consequent to bronchodilation.[47,48]

Horses in phase 3 of the study reported here had evidence of RAO. In addition to a history of clinical RAO, they had evidence of airway obstruction and airway inflammation.[2] Analysis of our results indicates that administration of the β_2-AR, albuterol, caused a small but significant decrease in RRS and REE in this population of horses with RAO. This suggests that bronchodilation sufficiently decreases the work of breathing to cause a decrease in REE, although lack of a linear correlation between REE and RRS or a change in these measures indicates that the degree of change in REE is not predicted by the degree of change in RRS. We wondered why horses with RAO had a decrease in REE when treated by aerosolized administration of albuterol when humans with airway obstruction attributable to COPD that are treated with albuterol tend to have an increase in REE. The finding that albuterol treatment results in greater improvement in pulmonary function and a smaller increase in REE in people with COPD with partially reversible, profound airway obstruction, compared with results in young healthy people,[2] suggests that when administration of albuterol does not result in bronchodilation, the thermogenic effects of β_2-adrenergic agonists predominate, whereas this response is attenuated when bronchodilation decreases the work of breathing. Horses with RAO are considered to be far more similar to humans with asthma than to humans with COPD[49] in that their airway obstruction is largely reversible,[50] thereby allowing the bronchodilatory effects of β_2-ARs to predominate. Thus, the effect of decreased work of breathing as a result of bronchodilation in the horses with RAO in phase 3 of the study reported here likely predominated over any thermogenic effects. However, it is important to mention that the degree of decreased work of breathing attributable to bronchodilation may have been obfuscated by thermogenic effects of treatment with a β_2-AR. The effect of increased resistance on work of breathing may be further elucidated by measuring REE in response to addition of variable external resistors or by measuring REE after administration of a nonthermogenic bronchodilator such as a parasympatholytic drug.

In phase 1 of the study reported here, we found that stable measurement of RRS in horses can be obtained at a frequency of 1 Hz during the period from 0 to 45 minutes after injection of xylazine and at a frequency of 2 Hz beginning 10 minutes after injection of xylazine. In phase 2, we found that the open-flow indirect calorimetry method can be used to obtain repeatable measurements of REE in horses sedated with xylazine throughout a 1-hour period. In phase 3, we found that albuterol administered via a metered-dose inhaler to horses with RAO resulted in a measurable, significant decrease in REE and RRS. Horses with chronic, advanced RAO may lose weight. Although this study did not investigate alternative explanations, such as the catabolic effects of systemic inflammatory mediators or decreased feed intake, we deduced that the increased work of breathing secondary to airway obstruction contributed to an increase in REE and, thus, potentially to cachexia in horses with RAO.

[a]Sridhar MK, Banham SW. Acute thermogenic response to inhaled salbutamol in patients with chronic obstructive pulmonary disease (abstr). *Am J Respir Crit Care Med* 1995;151:A466.
[b]5-gallon vacuum, Shop-Vac Corp, Williamsport, Pa.
[c]Flometer 70-670 L, Emerson Electric Co, Hatfield, Pa.
[d]R-1 flow control, AEI, Pittsburgh, Pa.
[e]Ametek model CD-3A, AEI, Pittsburgh, Pa.
[f]Ametek model S-3A, AEI, Pittsburgh, Pa.
[g]LabVIEW, National Instruments, Austin, Tex.
[h]On The Nose, Scientific Solutions, Eden Mills, ON, Canada.

[i]3-port proportional valve No. 602 00001, Joucomatic, Rueil, France.
[j]Fleisch No. 3, OEM Medical, Lenoir, NC.
[k]DP45-28, Validyne Engineering, Northridge, Calif.
[l]DP45-14, Validyne Engineering, Northridge, Calif.
[m]Proventil, Schering-Plough Corp, Kenilworth, NJ.
[n]Canadian Monaghan, Trudell Medical International, London, ON, Canada.
[o]Bivona tube, Bivona Medical Technologies, Gary, Ind.
[p]Rompun, Bayer Corp, Shawnee Mission, Kan.
[q]SPSS 10 for Windows, SPSS Inc, Chicago, Ill.

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