

Energetic cost of breathing, body composition, and pulmonary function in horses with recurrent airway obstruction

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Mazan, Melissa R., Edward F. Deveney, Shane DeWitt, Daniela Bedenice, and Andrew Hoffman. Energetic cost of breathing, body composition, and pulmonary function in horses with recurrent airway obstruction. *J Appl Physiol* 97: 91–97, 2004. First published February 13, 2004; 10.1152/jappphysiol.00629.2003.—This study was conducted to determine whether horses with naturally occurring, severe chronic recurrent airway obstruction (RAO) 1) have a greater resting energy expenditure (REE) than control horses, 2) suffer body mass depletion, and 3) have significantly decreased REE after bronchodilation and, therefore, also 4) whether increased work of breathing contributes to the cachexia seen in some horses with RAO. Six RAO horses and six control horses underwent indirect calorimetric measures of REE and pulmonary function testing using the esophageal balloon-pneumotachograph method before and after treatment with ipratropium bromide, a parasympatholytic bronchodilator agent, at 4-h intervals for a 24-h period. Body condition scoring was performed, and an estimate of fat mass was determined via B-mode ultrasonography. O₂ and CO₂ fractions, respiratory airflow, respiratory rate, and pleural pressure changes were recorded, and O₂ consumption, CO₂ production, REE, pulmonary resistance, dynamic elastance, and tidal volume were calculated. In addition, we performed lung function testing and calorimetry both before and after sedation in two control horses. RAO horses had significantly lower body condition scores (2.8 ± 1.0 vs. 6.4 ± 1.2) and significantly greater O₂ consumption than controls (4.93 ± 1.30 vs. 2.93 ± 0.70 ml·kg⁻¹·min⁻¹). After bronchodilation, there was no significant difference in O₂ consumption between RAO horses and controls, although there remained evidence of residual airway obstruction. There was a strong correlation between O₂ consumption and indexes of airway obstruction. Xylazine sedation was not associated with changes in pulmonary function but did result in markedly decreased REE in controls.

indirect calorimetry; naturally occurring disease; resting energy expenditure; oxygen consumption; ipratropium bromide

RECURRENT AIRWAY OBSTRUCTION (RAO), also known as heaves, is a common disease in horses that is characterized by reversible airway obstruction, bronchospasm, and airway hyperreactivity attributable to inflammation of the airways, mucus production, and thickening of airway walls (44). Putative causes include aeroallergens, especially the organic dusts, endotoxin, molds, and spores found in even clean stables (59, 34, 55). Even during clinical remission, horses with RAO have residual, subclinical airway inflammation, obstruction, and hyperresponsiveness (42). RAO is often compared with asthma (7); however, in its more chronic stages, it bears strong clinical resemblance to chronic obstructive lung diseases in the human: features shared by both RAO and chronic obstructive pulmo-

nary disease (COPD) include bronchiolitis with peribroncholar accumulation of neutrophils (9, 17), upregulation of both IL-8 (16, 50) and NF-κB, stimulating chronic inflammation, and airway reactivity (54), leading to limitations in airflow (2). Clinicians note that a subset of RAO horses suffers depletion of body mass; however, the physiology of cachectic RAO horses has not been described. Similarly, a subset of people with chronic obstructive lung diseases has been shown to experience tissue wasting; this is associated with both an increase in resting energy expenditure (REE) and the O₂ cost of ventilation (29, 49). Although the mechanical work of breathing in RAO horses has been demonstrated to be greater than in normal horses (36), whether this causes cachexia is unclear. Our laboratory previously reported that acute bronchodilation of mildly affected RAO horses with the β₂-adrenoceptor agonist albuterol sulfate results in small decreases in both respiratory resistance and REE, without any correlation between the degree of bronchodilation and the decrease in REE (32). To determine the contribution of pulmonary dysfunction to tissue wasting in RAO horses, we sought to measure pulmonary function, energy expenditure, body condition score, and body composition in a group of horses with naturally occurring, severe, and chronic RAO both compared with a group of control horses and after bronchodilation. To effect bronchodilation, we chose ipratropium bromide (IB), a parasympatholytic bronchodilator that has no demonstrable thermogenic effects in other species (6, 35, 37) and that has been shown to elicit bronchodilation in RAO horses (13, 43).

We hypothesized that horses with chronic, severe, naturally occurring RAO and loss of body condition would have elevated REE due to impaired pulmonary function and that bronchodilation would both improve pulmonary function and decrease REE. Conversely, we hypothesized that it was possible that underweight horses, like some underweight human COPD patients, might have a reduction in fat-free mass with subsequent decrease in REE, because of the fact that the majority of REE is derived from fat-free mass (51).

MATERIALS AND METHODS

All procedures described were approved by the Institutional Animal Care and Use Committee at Tufts University.

Animals

Six client-owned horses with spontaneously occurring RAO, including three mares and three geldings; and six healthy female horses were evaluated (Table 1). RAO horses were chosen on the basis that

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Table 1. *Physical characteristics of RAO and control horses*

	RAO	Control
Age	14.3 ± 3.2*	10.5 ± 4.7
Body condition score (1–9)	2.8 ± 1.0*	6.4 ± 1.2
Weight, kg	411.4 ± 63.8*	466.8 ± 55.0
Fat-free mass, kg	370.3 ± 50.9	377.2 ± 38.4
Percent fat, %	9.66 ± 2.99*	19.01 ± 3.33

Values are means ± SD for 6 horses in each group. RAO, recurrent airway obstruction. *Significantly different from controls, $P < 0.05$.

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, over a period of at least 2 yr, without evidence of infection or any other chronic systemic disease within the prior 4 mo. All RAO horses had an evident “heave” line. Control horses had no history or clinical evidence of respiratory, infectious, or chronic systemic disease. None of the horses had been treated with any corticosteroid for 4 wk or with any bronchodilator for 2 wk before the commencement of the study.

Study Design

All testing took place within a 1-yr period. Horses were admitted on the morning of *day 1* to the Lung Function Laboratory at the Hospital for Large Animals at Tufts University School of Veterinary Medicine, where they were housed in a clean hospital environment, bedded on shavings, and fed hay. On *day 1*, body condition scoring and physical examination were performed. Horses were fasted for 12 h before the commencement of physiological testing on the morning of *day 2*. Each horse was administered xylazine (0.5 mg/kg iv, Rompun, Bayer, Shawnee Mission, KS) and allowed to rest for 10 min (32). Then, O_2 consumption ($\dot{V}O_2$) and CO_2 production ($\dot{V}CO_2$) were measured, and respiratory quotient (RQ) and REE were calculated by use of indirect calorimetry during a 15- to 20-min period (10 min for equilibration and 5–10 min for data collection). Thirty minutes after administration of xylazine, lung mechanics were measured by use of the esophageal balloon-pneumotachograph method; data were recorded during a period of 3–5 min (33). Horses were then given aerosolized IB (180 µg, Atrovent, Boehringer Ingelheim, Ingelheim, Germany). The first horse did not show appreciable bronchodilation at 1 and 2 h [$<10\%$ decrease in pulmonary resistance (RL) or 10% increase in dynamic compliance (C_{dyn})]; thus the protocol was amended such that IB administration was repeated every 4 h for a 24-h period. On *day 3*, after 24 h of IB treatment, horses were again sedated with xylazine as above, and measurements of lung mechanics and indirect calorimetry were repeated between 30 and 60 min after administration of the final dose of IB.

In addition, to determine the effect of xylazine on baseline calorimetry and pulmonary function measurements, two control horses underwent baseline calorimetry and pulmonary function testing as described below, both unsedated and after sedation with xylazine (0.5 mg/kg body wt iv). In specific, each horse underwent calorimetry unsedated and was then instrumented as described below for pulmonary function measurements, and baseline measurements were made. The horse was then administered xylazine, and pulmonary function measurements were repeated. Immediately after pulmonary function testing, calorimetry testing was repeated. Once the horse was sedated, both pulmonary function testing and calorimetry were completed within a 30-min period (5 min for pulmonary function measurement and 15–25 min for calorimetry). The horses stood quite still but were extremely alert while calorimetry was performed.

Assessment of Sedation

During measurement of REE, sedation was judged to be adequate when there was a lack of voluntary movement (e.g., 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 (32).

Body Condition Scoring

The procedure was adapted from that described by Henneke et al. (18). 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.

Determination of Fat-Free Mass

Rump fat thickness was calculated by the method of Kane et al. (20). Briefly, the position of maximal fat thickness at a site ~5 cm lateral to the midline of the rump was determined by B-mode ultrasonography, and maximal fat thickness was measured at this site. Percent fat was estimated from the equations of Kearns et al. (21).

$$\% \text{Fat} = 2.47 + 5.47 [\text{rump fat (in cm)}]$$

$$\text{Fat mass} = (\text{total body mass in kg}) (\% \text{fat})$$

$$\text{FFM} = \text{total body mass} - \text{fat mass}$$

Measurement of Lung Mechanics

During all measurements of lung mechanics and calorimetry, the horses' heads were kept elevated at an angle at least 180° to the horizontal, to decrease the likelihood of upper airway obstruction (25). The horse wore an airtight Plexiglas mask, affixed above the nostrils with a latex shroud, which was attached to the pneumotachograph. The pneumotachograph (Fleisch no. 5, OEM Medical, Lenoir, NC) was calibrated by use of a precision syringe (3-liter volume syringe, Hans Rudolph, Kansas City, MO). The pneumotachograph was connected via tubing to a differential pressure transducer (DP45-14, Validyne Engineering). An esophageal balloon catheter was placed to the level of the midthorax and connected to a differential pressure transducer (DP45-28, Validyne Engineering) and amplified. The opposite pole of the pressure transducer was connected to a side port in the gas-collection mask to obtain transpulmonary pressure measurements. Ten-breath averages for respiratory rate (RR), tidal volume (V_T), RL, dynamic elastance (E_{dyn}), and change in pleural pressure (dP_{pl}) were recorded on a personal computer by using data acquisition software (XA Biosystems version 2.2, Buxco Electronics, Sharon, CT).

Indirect Calorimetry

The open-flow indirect calorimetry system used in the study has been previously described (32). Briefly, air was drawn through a rigid open face mask via flexible airway tubing (outside diameter, 7 cm), by using a vacuum (5-gallon vacuum, Shop-Vac, Williamsport, PA) located outside of the room. Flow was regulated with a rotameter equipped with a control valve (Flometer 70-670 L, Brooks Instrument, Emerson Electric, Hatfield, PA). 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-liter mixing chamber was interposed between the horse and gas analyzers. Air was drawn through the mixing chamber, traversing two plastic plates spaced 30 cm apart. Each plate contained nine 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 (R-1 Flow Control, AEI, Pittsburgh, PA), which was then split and metered to the CO_2 analyzer (Ametek model CD-3A, AEI) and O_2 analyzer (Ametek S-3A, AEI). Before entering the O_2 and CO_2 analyzers, the air sample traversed a drying column that consisted of anhydrous calcium sulfate. O_2 and CO_2 fractions were measured continuously. N_2 dilution and

Table 2. Calorimetry data for RAO and control horses both before and after treatment for 24 h with IB

	RAO Baseline	RAO Posttreatment	Control Baseline	Control Posttreatment
$\dot{V}O_2$, ml·kg ⁻¹ ·min ⁻¹	4.93±1.13†	3.46±0.80*†	2.93±0.70	3.00±0.62
$\dot{V}CO_2$, ml·kg ⁻¹ ·min ⁻¹	3.49±1.31†	2.52±1.67*	2.14±0.76	2.75±0.32
REE, Mcal/day	13.34±1.67†	9.70±3.12*	9.48±3.04	10.12±2.61
RQ ($\dot{V}CO_2/\dot{V}O_2$)	0.72±0.32	0.73±0.40	0.72±0.17	0.94±0.10

Values are means ± SD. IB, ipratropium bromide; $\dot{V}O_2$, O₂ consumption; $\dot{V}CO_2$, CO₂ production. *Significantly different from baseline value, $P < 0.05$. †Significantly different from control baseline, $P < 0.05$.

CO₂ infusion were used to calibrate the open-flow calorimetry system using the procedures of Fedak et al. (15). Before the experiment, N₂ and then CO₂ were bled into the system at 40 and 15 l/min (ATPD), respectively; total flow was held the same as during the experiment (for the average horse, 500 l/min) (15). Flow rates for N₂ and CO₂ were measured using a precision, custom-designed, dual N₂-CO₂ flowmeter (Brooks Instruments, accuracy ±1%). All volumes were corrected on the basis of standard temperature and pressure (dry). A minimum of 10 min was allowed for equilibration, at which time the system had reached a steady state, defined as a time when the horse stood quietly, with a relaxed posture, and without apparent movement, and with <20% deviation from the means for $\dot{V}O_2$. Once a steady state was achieved, data were collected for an additional 5–10 min for use in calculating mean $\dot{V}O_2$ and mean $\dot{V}CO_2$ for the period. Analog signals from the gas analyzers were digitized and processed by use of a computer and custom-written software (LabVIEW, National Instruments, Austin, TX). $\dot{V}O_2$ and $\dot{V}CO_2$ were subsequently expressed as milliliters per kilogram per minute. Values for REE were calculated from mean $\dot{V}O_2$ and $\dot{V}CO_2$ by use of the abbreviated Weir equation (58):

$$\text{REE (Mcal/day)} = (3.94\dot{V}O_2 + 1.1\dot{V}CO_2) \times 1.44$$

Administration of Aerosolized Drug

IB was administered by use of an aerosolizing face mask system. This system consisted of a well-fitting face mask with inhalation and exhalation valves and a holding chamber (Aeromask, Canadian Monaghan, Trudell Medical International, London, ON, Canada). Before administration of ipratropium (18 µg/actuation), the metered-dose inhaler was shaken for 1 min, followed by a single primer actuation. The metered-dose inhaler was then attached to the holding chamber and was actuated at end expiration. A total of 10 actuations were performed with 30-s intervals between each actuation (total of 180 µg of ipratropium delivered). This dose is in accordance with other doses that have been effective in eliciting bronchodilation in horses with RAO (14, 43).

Statistical Analysis

Data are reported as means ± SD. A general linear model, with heaves and treatment as main effects, was used to determine whether there were significant differences between and within the groups (heaves and control) before and after treatment with ipratropium.

Spearman's correlation coefficient was used to determine whether there were any associations among lung mechanics, gas analysis, and body composition.

RESULTS

Physical Characteristics

RAO horses were significantly thinner than control horses on the basis of body condition score, body weight (kg), and percent fat. There was no significant difference in fat-free mass between the two groups. RAO horses were significantly older than control horses (Table 1).

Baseline Calorimetry Data

RAO horses had higher REE, $\dot{V}O_2$, and $\dot{V}CO_2$ than control horses, but there was no significant difference in RQ between the two groups (Table 2). $\dot{V}O_2$ in control horses was in accord with resting values previously reported in the literature (3, 22, 19, 24).

Baseline Pulmonary Function Data

RAO horses had significantly higher RR, maximal dPpl, Edyn, and RL, and significantly lower V_T than control horses (Table 3).

Effect of Repeated Administration of IB Over 24 h

Calorimetry. All RAO horses had a decrease in REE, $\dot{V}O_2$, and $\dot{V}CO_2$ (Tables 2 and 4), with a mean decrease of 26.42 ± 21.2, 26.46 ± 20.99, and 23.68 ± 30.05%, respectively, after bronchodilation with IB, but control horses had no significant changes (Table 2). RQ was not significantly different after treatment. In contrast to the marked difference in $\dot{V}O_2$, $\dot{V}CO_2$, and REE between the two groups at baseline, posttreatment values were not different between the two groups (Table 2).

Pulmonary function. All RAO horses showed evidence of bronchodilation after treatment with IB, with mean decreases in RL, Edyn, dPpl, and RR of 35.95 ± 14.19, 38.28 ± 20.14,

Table 3. Pulmonary function data for RAO and control horses both before and after treatment for 24 h with IB

	RAO Baseline	RAO Posttreatment	Control	Control Posttreatment
RR, breaths/min	24.7±8.6†	15.5±3.6*†‡	9.3±2.0	10.2±3.5
V _T , liter	4.36±0.79†	5.52±0.70*†‡	6.87±0.83	6.50±1.03
dPpl, cmH ₂ O	27.49±11.67†	13.94±4.60*†‡	7.06±1.87	6.02±1.52
RL, cmH ₂ O·l ⁻¹ ·s	1.90±0.74†	1.19±0.42*†‡	0.71±0.19	0.58±0.12
Edyn, cmH ₂ O/l	2.20±1.59†	1.19±0.77*†‡	0.54±0.12	0.48±0.10

Values are means ± SD. RR, respiratory rate; V_T, tidal volume; dPpl, change in pulmonary pressure; RL, lung resistance; Edyn, dynamic elastance. *Significantly different from baseline value, $P < 0.05$. †Significantly different from control baseline, $P < 0.05$. ‡Significantly different from control posttreatment, $P < 0.05$.

47.42 ± 7.12, and 29.73 ± 28.48%, respectively. Control horses had no significant changes (Table 3).

Correlations

Calorimetry and pulmonary function. $\dot{V}O_2$ in RAO horses correlated strongly with Edyn (Fig. 1) and RL (Fig. 2).

Calorimetric and pulmonary function measurements in two control horses with and without xylazine. The data are not sufficient to support statistical analysis; nonetheless, $\dot{V}O_2$ (Fig. 3), $\dot{V}CO_2$, and REE were markedly lower in control horses after sedation with xylazine (Table 5). After sedation, control horses had only small changes in pulmonary function testing, including a mild decrease in Cdyn, and a moderate increase in RL in one horse. In addition, there were small changes in V_T , RR, and dPpl after sedation in the control horses (Table 5)

DISCUSSION

This study presents the novel finding that horses with marked, chronic, naturally occurring RAO have significantly greater $\dot{V}O_2$ and $\dot{V}CO_2$, owing to increased energy demands, than do control horses (Table 2). In addition, these RAO horses have less fat (with consequently lower body condition scores) than do controls (Table 1). Bronchodilation over a 24-h period resulted in significantly decreased REE (Tables 2 and 4).

Our findings concerning increased RL, Edyn, V_T , RR, and maximal dPpl in horses with RAO accord with previous reports (36, 44, 47). In our laboratory's previous study, although we observed a decrease in REE with bronchodilation, we were not able to correlate baseline measures of lung function in RAO horses with energy expenditure (32). In contrast, in our present study, we find strong correlation between baseline measures of energy consumption and pulmonary function in RAO horses (Figs. 1 and 2), similar to humans with chronic airflow obstruction (12). Likewise, our findings concerning the efficacy of IB in eliciting bronchodilation are consistent with prior reports on the effects of parasympatholytic agents in RAO horses (5, 14, 39, 43); namely, IB elicited significant bronchodilation without returning pulmonary function to control values. This lack of return to control values is likely due to the residual obstruction that remains in RAO horses during clinical remission, because of mucus plugging airways, inflammatory changes, and gas trapping, even after relief of acute bronchospasm (42). Contrary to earlier reports of maximum bronchodi-

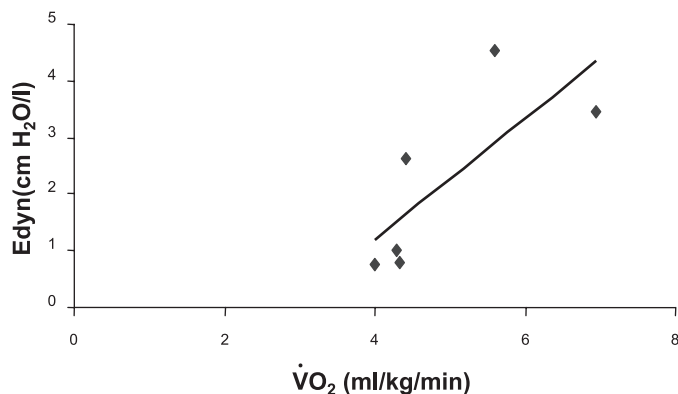


Fig. 1. Relationship between dynamic elastance (Edyn) and O_2 consumption ($\dot{V}O_2$). Spearman's rank correlation coefficient (R_s) = 0.886, P = 0.009. ♦, Horses with recurrent airway obstruction (RAO), no treatment.

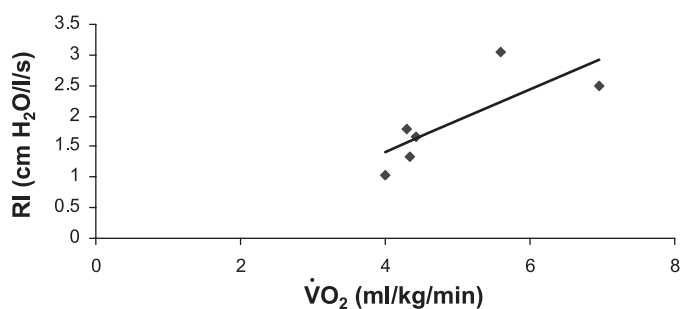


Fig. 2. Relationship between lung resistance (RL) and $\dot{V}O_2$. R_s = 0.771, P = 0.036. ♦, Horses with RAO, no treatment.

lation with aerosolized ipratropium within 1 h (14), we found that the first horse did not show appreciable bronchodilation at 1 and 2 h. However, there was a considerable increase in indexes of bronchodilation at 24 h. We theorize that the heterogeneous pathology of RAO (28) contributed to uneven ventilation with consequent uneven aerosol deposition and thus inadequate bronchodilation (57); this was likely partially amended by repeated administration of drug leading to increasing bronchodilation over a 24-h period. Consistent with our laboratory's previous report (32), we found that improved pulmonary function in RAO horses is accompanied by a decrease in REE (Tables 2–4). In contrast to our previous study in which albuterol, a thermogenic bronchodilator, resulted in a <10% decrease in $\dot{V}O_2$, $\dot{V}CO_2$, and REE, in our present study treatment with IB, a nonthermogenic bronchodilator (6, 35, 37), resulted in a far more striking reduction in REE, $\dot{V}O_2$, and $\dot{V}CO_2$, with all horses experiencing >10% change in $\dot{V}O_2$ and REE (Table 4).

Because horses breathe around, not at, functional residual capacity (23), we were not able to obtain measurements of end-expiratory lung volume and therefore were not able to document the presence of hyperinflation. However, horses with RAO have been shown to have increased an increased functional residual capacity due to hyperinflation (27), as well as

Table 4. Individual data for REE and $\dot{V}O_2$ in RAO horses before and after treatment with IB for 24 h

Horse	$\dot{V}O_2$, ml·kg ⁻¹ ·min ⁻¹	REE, Mcal/day	REE/day (NRC Recommendation)
Horse 1 pre	5.60 ± 0.12	14.89 ± 0.39	8.32
Horse 1 post IB	4.66 ± 1.16	12.44 ± 2.06	
Horse 2 pre	4.42 ± 1.14	11.25 ± 2.06	7.56
Horse 2 post IB	3.69 ± 0.95	8.78 ± 2.01	
Horse 3 pre	4.34 ± 0.56	14.06 ± 1063.34	10.19
Horse 3 post IB	3.16 ± 0.1	10.02 ± 2.36	
Horse 4 pre	6.95 ± 1.20	14.57 ± 2.56	6.70
Horse 4 post IB	2.21 ± 0.95	4.77 ± 1.79	
Horse 5 pre	4.29 ± 0.15	13.44 ± 0.69	9.39
Horse 5 post IB	3.72 ± 0.15	11.76 ± 0.69	
Horse 6 pre	3.99 ± 0.49	11.81 ± 1.27	9.64
Horse 6 post IB	3.32 ± 0.45	10.45 ± 1.27	
Mean ± SD baseline pre	4.93 ± 1.13	13.34 ± 1.49	8.63 ± 1.34
Mean ± SD baseline post	3.46 ± 0.80	9.70 ± 2.74	

Values are means ± SD. pre, Before treatment; post, after treatment. Resting energy expenditure (REE) is also calculated according to National Research Council (NRC) recommendations for REE in mature horses, according to each horse's weight. REE according to NRC recommendations: REE (Mcal/day) = [0.975 + (0.21 × body wt kg)] 1,000 (38).

Table 5. Calorimetry ($\dot{V}O_2$, $\dot{V}CO_2$, RQ, REE) and pulmonary function testing (R_L , C_{dyn} , RR, V_T , dP_{pl}) in control horses before and after sedation with xylazine

	$\dot{V}O_2$, ml·kg ⁻¹ ·min ⁻¹	$\dot{V}CO_2$, ml·kg ⁻¹ ·min ⁻¹	RQ $\dot{V}CO_2/\dot{V}O_2$	REE, Mcal/day	R_L , cmH ₂ O·l ⁻¹ ·s	E_{dyn} , cmH ₂ O/l	RR, breaths/min	V_T , liters	dP_{pl} , cmH ₂ O
Horse 1 NS	5.57±0.95	3.95±0.68	0.71±0.09	19.83±2.59	0.92±0.10	0.41±0.20	10±2.7	7.48±1.12	11.2±1.38
Horse 1 XS	3.93±0.28	2.95±0.26	0.75±0.06	14.15±0.91	0.91±0.16	52±0.66	8.0±1.9	8.22±1.42	10.02±4.8
Horse 2 NS	5.63±0.90	3.93±0.55	0.70±0.07	21.01±3.20	0.63±0.10	0.52±0.19	8.0±0.7	7.03±0.99	8.59±0.96
Horse 2 XS	4.01±0.21	3.05±0.15	0.76±0.03	15.19±0.78	0.90±0.11	0.61±0.56	8.0±2.5	7.54±2.39	8.69±2.05

Values are means ± SD for 2 horses. RQ, respiratory quotient; NS, no sedation; XS, sedation with xylazine.

increased RRs accompanied by increased peak inspiratory and expiratory flow rates (46). In human asthmatic subjects, hyperinflation has been shown to result in an unfavorable length-tension relationship of the respiratory muscles (31) as well as resulting in increased dead space, with accompanying increased ventilatory demands and work of breathing (8). Likewise, it is probable that these RAO horses have increased $\dot{V}O_2$ owing to increased resistance in both the large and small airways, as well as obstruction of small airways, resulting in hyperinflation with concomitant decreased vital capacity, increased work of gas compression, and possibly increased inspiratory load.

Although we were unable to completely bronchodilate the RAO horses in this study, nonetheless we conclude that the increased REE due to the energy cost of breathing associated with airway obstruction is a key contributor to the cachexia of RAO.

A potential source of error in this study is the use of xylazine hydrochloride as a sedative. We found sedation to be necessary to carry out testing, because we were using client-owned animals unaccustomed to the Lung Function Laboratory. Nonetheless, it is important to note that xylazine has been found to cause changes in lung mechanics in horses without history of respiratory disease (25, 26) and in ponies with preexistent bronchoconstriction (4). Lavoie and colleagues (25) showed that changes in esophageal balloon pressure and respiratory resistance were largely dependent on head position; that is, if the head was allowed to rest at an angle <180° to the horizontal, both esophageal balloon pressures and resistance tended to increase. To lessen this effect of sedation in our study, we took care that the head was never allowed to rest at an angle <180° to the horizontal. In a previous study (32), our laboratory had confronted the question of the stability of calorimetric measurements and measurements of respiratory system resistance using the forced oscillatory technique in horses sedated with xylazine during a 45-min testing period and found that $\dot{V}O_2$, $\dot{V}CO_2$, REE, and respiratory system resistance did not change significantly during this time. We did not, however, investigate the stability of lung mechanics using the esophageal balloon-pneumotachograph method. In our present study, we examined both lung mechanics and energy expenditure in control horses, both with and without sedation with xylazine, and found that whereas sedation was associated with a large decrease in $\dot{V}O_2$ and REE, lung mechanics were not so associated, with the exception of a moderate increase in R_L in horse 2 (Table 5). In both cases, energy expenditure decreased considerably after sedation (Fig. 3), without explanatory changes in lung mechanics. This is most likely explained by the extra energy expended by the horses in remaining alert; although their feet remained stationary, their heads were held

high, and their muscles appeared tense. This extra muscular effort without actual movement would not likely affect lung function. Had we not sedated these horses, we would have made an error in ascribing the “work of alertness” to the work of breathing.

Also prospectively troublesome was the potential for xylazine to cause bronchodilation, thus possibly obscuring the effect of treatment with IB (4). Broadstone and coworkers (4) examined the effect of xylazine in tracheostomized ponies with acutely induced bronchoconstriction and found significant decreases in R_L and increases in C_{dyn} . This reported finding has the potential to affect the measurements made in this study, because xylazine may bronchodilate such that further changes in airway caliber would be undetectable. However, in the RAO horses in our study, there were marked decreases in dP_{pl} , R_L , E_{dyn} , and RR, as well as an increase in V_T after treatment with IB (Table 3). Thus, although xylazine may indeed have induced some bronchodilation in these horses, we were still able to document significant changes in lung mechanics indicative of bronchodilation after treatment with IB. We believe that sedation is necessary to these measurements in client-owned, nonaccustomed animals to increase ease and safety and to achieve a truly resting measurement.

We used the abbreviated Weir equation to determine REE in accordance with the American Association for Respiratory Care (1) recommendations for indirect calorimetry. We recognize that there are assumptions that must be made in using this equation. Namely, in performing indirect calorimetry to determine REE, we are assuming that the substrates glycogen, lipid, and protein disappear by direct oxidation at a given rate that can be calculated, given measured $\dot{V}O_2$, measured $\dot{V}CO_2$, and knowledge of the standard amount of O_2 consumed and energy produced during oxidation of each particular substrate. In using the Weir equation, we assume that standard carbohydrate, fat,

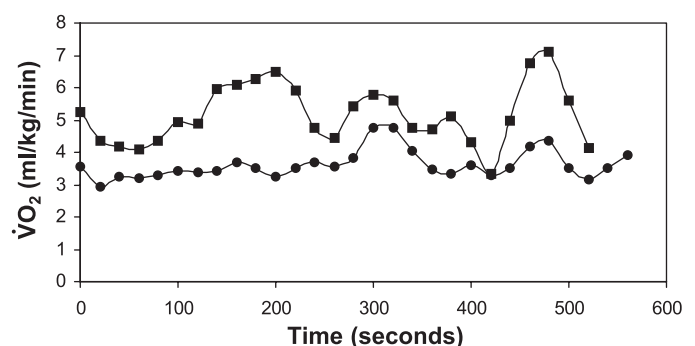


Fig. 3. $\dot{V}O_2$ in a control horse before and after sedation with xylazine (0.5 mg/kg iv). $\dot{V}O_2$ was markedly higher before sedation than after sedation. ■, No sedation; ●, after sedation.

and protein are being consumed (30). This may lead to errors if unusual substrates are being consumed or if gluconeogenesis, lipogenesis, or ketogenesis (as may be happening in the cachectic state) is increased. We try to avoid these errors by ensuring that the horse is eating a mixed carbohydrate-protein-lipid ration (good-quality hay) and by standardizing with a 12-h fast before indirect calorimetry testing. Moreover, unless unusual substrates are being consumed, these assumptions potentially lead to more errors in accurately determining substrate use, rather than overall energy production. We also assume that the animal is not losing excessive amounts of protein and that protein disappearance in horses, similar to the case in humans, has a negligible effect on energy production as measured by indirect calorimetry using the abbreviated Weir equation (52).

An additional potential problem with this study is that horses were not age matched. Basal metabolic rate decreases with age in humans (56). Nonetheless, if there were a decrease in REE in the older RAO horses, as is seen in humans, this should have the effect of bringing their REE closer to that of the controls, rather than having an elevated REE, as we observed. The difference between the two populations might have been more striking had they been age matched.

Unlike the loss of fat-free mass seen in humans with chronic airway obstructive diseases (48), absolute fat-free mass did not differ between control and RAO horses in this study. Rather, RAO horses had a significantly decreased percentage of fat, similar to humans and animals with chronic energy deficiency (Table 1) (40). In contrast to the hypometabolism seen in chronic energy deficiency, the RAO horses in this study were hypermetabolic before bronchodilation, whereas after bronchodilation REE in RAO horses was not significantly different from controls, although values in several individuals remained high (Table 4). Although treatment with IB resulted in measurable bronchodilation, there remained evidence of residual obstruction (RL, Cdyn, and dPpl above control levels), suggesting that the O₂ cost of breathing might still be elevated above normal (Table 3). There is evidence that humans with the similar disease, COPD, experience an increase in REE due to the biological effects of systemic inflammatory mediators (10, 11, 53); this may be a contributor to the increased REE in RAO horses, as well. Because we were unable to eliminate all airway obstruction in these horses, it is impossible for us to determine whether REE might actually be lower than control values, similar to chronic energy deficiency, or whether inflammation causes independent increases in REE.

Finally, it is important to note that indirect calorimetry has the potential to play an important role both in the clinical management of horses with cachexia associated with RAO and as a tool for determining whole body response to bronchodilator. The National Research Council (NRC) recommendation for REE needs is based on the formula

$$\text{REE (Mcal/day)} = [0.975 + (0.21 \times \text{body wt kg})]/1,000 \quad (38)$$

For control horses, mean measured REE (9.48 ± 3.04 Mcal/day) was not significantly different from NRC calculations (9.80 ± 1.15 Mcal/day). In RAO horses, however, REE measured by calorimetry (13.34 ± 1.49 Mcal/day) was signifi-

cantly higher than REE by NRC calculation (8.63 ± 1.34 , Table 4).

Thus simple caloric imbalance is likely an important contributor to body mass depletion in these horses.

Not only is indirect calorimetry a useful tool for clinical assessment of dietary needs, it is also potentially valuable as a method of assessing bronchodilation, because it is noninvasive and well tolerated in the sedated horse and correlates well with measurements of lung mechanics.

In conclusion, we find that the airway obstruction found in horses with naturally occurring, severe, chronic RAO results in increased energy expenditure and consequent weight loss, and bronchodilation results in a significant decrease in REE. Baseline pulmonary function is strongly correlated with energy expenditure. Thus weight loss in this population is likely due to an imbalance between increased energy expenditure due to increased work of breathing and caloric consumption.

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