Effects of aerosolized albuterol on physiologic responses to exercise in Standardbreds

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Objective—To examine the effects of an aerosolized β_2-adrenoreceptor agonist, albuterol, on performance during a standardized incremental exercise test in clinically normal horses.

Animals—8 Standardbred pacing mares.

Procedure—Clinically normal horses, as judged by use of physical examination, hematologic findings, serum biochemical analysis, and airway endoscopy, were randomly assigned to 2 groups and were given 900 µg of albuterol via a metered-dose inhaler 30 minutes before beginning a standardized incremental exercise test in a crossover design with a 7-day minimum washout. Further examination included measurement of baseline lung mechanics, response to histamine bronchoprovocation, and bronchoalveolar lavage.

Results—No significant differences (albuterol vs placebo) were seen for any incremental exercise test variables (ie, maximum oxygen consumption, maximum carbon dioxide consumption, respiratory quotient, treadmill speed at heart rate of 200 beats/min, or number of steps completed during an incremental exercise protocol). Mast cell percentage was significantly ($r = -0.84$) associated with the concentration of aerosolized histamine that evoked a 100% increase in total respiratory system resistance. No other direct correlations between bronchoalveolar lavage fluid cell types and any indices of exercise capacity or airway reactivity were found.

Conclusions and Clinical Relevance—Although no horse had exercise intolerance, 4 horses had airway hyperreactivity with bronchoalveolar lavage fluid mastocytosis; these horses may have been subclinically affected with inflammatory airway disease. In our study, albuterol did not enhance performance in 8 clinically normal racing-fit Standardbreds. (*Am J Vet Res* 2001;62:1812–1817)

Inflammatory airway disease (IAD) is a widespread problem in horses, manifesting as exercise intolerance, cough, and evidence of mucus in the airways.[1] A number of clinical investigations have made the association between airway inflammation and exercise intolerance,[2-5] lung dysfunction,[a] arterial hypoxemia during exercise,[6] and the severity of bronchiolitis seen histologically.[a] Horses with IAD have a propensity toward nonspecific histamine-induced airway hyperreactivity.[5,7] Despite the overwhelming evidence, it has not been proven that airway obstruction and inflammation cause exercise intolerance in these horses. There is a widespread clinical impression that bronchodilators such as albuterol and clenbuterol are effective in the temporary treatment of exercise intolerance caused by bronchoconstriction associated with IAD. It has yet to be demonstrated, however, that **β_2-adrenoreceptor agonists (β_2-AR)** can improve performance in horses with IAD.

The effect of aerosolized β_2-AR on performance in highly conditioned human athletes has been extensively examined. Although most studies[8-16] have revealed no significant improvement in speed or metabolic cost of exercise when athletes were given a β_2-AR before exercise challenge, there is some evidence that β_2-AR may improve exercise endurance[17] and may result in improvement during short bursts of exercise.[18] In contrast, little is known about the effects of β_2-AR on performance in clinically normal or IAD-affected horses; evidence supports[19] and refutes a performance-enhancing effect in clinically normal horses.[20] It is important, in order to fully explore the role that β_2-AR treatment may play in the treatment of IAD in horses, to further elucidate the effect that β_2-AR treatment has on performance in clinically normal horses.

Albuterol is a β_2-selective adrenoreceptor agonist that acts on bronchial and vascular smooth muscle receptors.[21] The purpose of the study presented here was to investigate the effects of aerosolized albuterol on performance in 8 clinically normal Standardbred mares.

Methods and Materials

Animals—The Institutional Animal Care and Use Committee at Tufts University School of Veterinary Medicine approved all procedures. Entrance criteria for our study were designed to incorporate those indices of health that would typically be available to a racetrack practitioner. Thus, we used 8 Standardbred pacing mares in race training that were 3 to 6 years old (mean ± SD, 4.3 ± 0.4 years), weighed 368 kg to 447 kg (mean ± SD, 407 ± 11 kg), and had no clinically important abnormalities on hematologic evaluation or serum biochemical analysis, no abnormalities on physical examination or lameness examination in hand, no history of cough or nasal discharge, and no upper airway abnormalities or evidence of excessive mucus in the trachea on resting endoscopic airway evaluation. Bronchoalveolar lavage, baseline lung function testing, using the forced oscillatory mechanics technique, and histamine bronchoprovocation were performed on all horses 2 weeks prior to incremental exercise testing to characterize the group in greater detail. Horses purchased from the racetrack were maintained in training by use of a treadmill during the 2 months preceding incremental exercise testing. This training included 30 to 40 minutes of treadmill training 3 to 4 times/wk and 1 exercise test/wk during the final month, which was considered equivalent exercise to racing. This training sufficed to acclimate horses to the treadmill and to the open respirometry system apparatus.[22] Horses were housed in box stalls and turned out daily. Horses were bedded on wood shavings and fed good-

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quality dry timothy hay ad libitum (except immediately before treadmill exercise) and 2 to 3 kg of sweet feed twice daily. Feed was withheld 2 hours before routine treadmill exercise and 4 hours before the incremental exercise test. All experiments took place in January and February of 1998 during a 4-week period. The room temperature and barometric pressure were similar for all tests (19.0 to 21.5 C and 744 to 762 mm Hg, respectively).

Bronchoalveolar lavage—Xylazine hydrochloride[b] (0.5 mg/kg of body weight, IV) was administered. A sterile bronchoalveolar lavage tube[c] was used as described.[5] Fifty milliliters of sterile saline (0.9% NaCl) solution was instilled in two 250-ml aliquots and suctioned (–10 cm H_2O). Samples were transferred to EDTA tubes and prepared for cytologic examination (centrifugation of 600 µl at 600 × g for 5 minutes). The 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 and were expressed as percentages of the total count.

Measurement of lung mechanics and airway reactivity—Horses were sedated with xylazine[b] (0.5 mg/kg, IV), and **total respiratory system resistance (R_{RS})** and **compliance (C_{RS})** were measured as described.[5,12,23-24] In brief, a sinusoidal flow was generated, using a proportional pneumatic valve[d] and compressed air (75 psi) source, and applied to the horse's respiratory system via a rigid low dead-space shrouded facemask. Flow at the mask opening was measured, using a pneumotachograph[e] attached to a differential pressure transducer.[f] A differential pressure transducer[g] was used to measure the pressure generated at the airway opening, and the difference between mask and atmospheric pressures was measured. **Total respiratory impedance (Z_{RS})** was calculated as the ratio of instantaneous pressure at the airway opening (mask pressure) to flow, averaged over 10-second periods. The respiratory system was oscillated over a range of frequencies: 1 to 7 Hz for baseline measurements and 1 to 3 Hz during histamine challenge. The Z_{RS} was calculated by use of a microcomputer and commercial software.[24,h] Values for R_{RS} were used to monitor the effects of histamine aerosol challenge.

Prior to histamine challenge, baseline measurements of R_{RS} and C_{RS} were made (1 to 7 Hz). Then, bronchoprovocation was performed, using a modification from human studies, as described.[5,23] Physiologic saline solution was first nebulized directly into the facemask, using a fine-particle (mean median diameter, 1.6 µm) jet nebuliser[i] and a high-pressure (30 psi) high-flow (10 L/min) air compressor[j] for 2 minutes. Twenty seconds after the nebulisation was complete, a series of forced oscillatory measurements were made at 1 to 3 Hz input frequencies until values at each frequency were observed to be returning toward baseline. A succession of doubling concentrations of histamine[k] (1, 2, 4, 8, 16, and 32 mg/ml) were then nebulised (2 minutes each, with at least 5 minutes between doses) until R_{RS} at 1 Hz doubled from the mean after-saline value for R_{RS}, a plateau (change in R_{RS} < 10% after 3 successive doses) was reached, or the maximal aerosol dose was delivered. The **concentration of histamine aerosol that evoked a 100% increase in R_{RS} ($PC_{100}R_{RS}$)** was determined by interpolation of the histamine dose-response curve, using a statistical function of commercial software.[l] The baseline used for this purpose was the y-intercept of the line generated by linear regression, incorporating all data points including baseline, after saline, and all after histamine points.[5,7]

Standardized incremental exercise test—The standardized incremental exercise test has been well described by Seeherman and Morris.[25] Briefly, the exercise test measured **oxygen consumption ($\dot{V}O_2$)**, **carbon dioxide production ($\dot{V}CO_2$)**, **respiratory quotient (RQ)**, **heart rate (HR)**, and the **number of steps completed ($STEP_{max}$;** decimal equivalents) during an incremental exercise protocol, using a high velocity motorized treadmill.[m] The warm-up consisted of 1 mile of pacing at a speed of 4.5 m/s with no incline. The treadmill was then inclined to 3° and the exercise test was begun at a walk (1.8 m/s). The velocity was increased at 1-minute intervals to 2.7, 3.4, 4.5, 5.4, 6.8, 9.0, 10.8, and 12.0 m/s. After completion of the exercise test, the treadmill was lowered to the level position, and horses were walked for 15 minutes.

Respiratory gas analysis and HR—An open flow respirometry system calibrated at the end of each exercise test was used to measure $\dot{V}O_2$ and $\dot{V}CO_2$ continuously.[25] Briefly, air was drawn through a rigid open facemask through flexible airway tubing, using a high velocity centrifugal blower[n] located outside the building. Flow through the system was between 10,000 and 13,000 L/min, depending on the size of the horse, allowing all of the expired air to be drawn into the mask at peak exercise. Flow was measured with a venturi tube flow indicator[o] coupled to a differential pressure transducer.[f] Adequate flows were ensured by noting identical V_{O_2} at flow rates 25% higher and lower than the flow rate used during the exercise test.[25] The system was calibrated after each test, using the nitrogen dilution technique.[25,26] Oxygen[p] and carbon dioxide[q] fractions were measured continuously. Analogue signals from the gas analyzers and pressure transducers were digitized and processed by use of a computer[r] and custom-written software.[s] Values of $\dot{V}O_2$ and $\dot{V}CO_2$ were determined for each step in the incremental exercise test, using the mean values durng the 5-second period before and after each change. All volumes were corrected to standard temperature and pressure (dry). Heart rate was measured by use of a telemetered HR monitor[t] and was recorded during the last 5 seconds of each step.

Delivery of aerosolized drug—Albuterol[u] was administered by use of a well-fitting mask[v] with inhalation and exhalation valves and a holding chamber. Before delivering albuterol, the metered-dose inhaler (90 µg of albuterol/actuation) was shaken for 1 minute, followed by a single primer actuation. The metered-dose inhaler was then attached to the holding chamber and was actuated at end-expiration. Ten actuations were performed, with 30 seconds between each actuation, for

Table 1—Baseline lung mechanic values, indices of airway reactivity, and bronchoalveolar lavage (BAL) fluid cytologic findings in the study horses (n = 8)

	Lung mechanic values			BAL cytologic findings (% cells)			
Variables	**R_{RS}**	**C_{RS}**	**$PC_{100}R_{RS}$**	**Lymph**	**Mac**	**PMN**	**Mast**
Mean	0.68	0.37	6.0	45.2	49.2	3.7	2.8
SD	0.13	0.04	4.3	12.7	11.0	3.7	2.0

R_{RS} = Baseline respiratory system resistance (cm H_2O /L per second). C_{RS} = Baseline respiratory system compliance (L/cm H_2O). $PC_{100}R_{RS}$ = Histamine aerosol concentration evoking 100% increase in R_{RS}. Lymph = Lymphocytes.; Mac = Aalveolar macrophages. PMN = Polymorphonuclear neutrophilic leukocytes. Mast = Mast cells.

900 µg of albuterol delivered. Placebo consisted of placement of the mask, holding chamber, and inhaler on the horse for the same amount of time, without actuation of the inhaler.

Experimental protocol—A crossover design was used. Horses (n = 8) were randomly allocated into 2 groups receiving treatment or placebo in opposite order. Two incremental exercise tests were performed on each horse, with a 1-week interval between testing. Horses treated with albuterol (n = 4) during the first week were treated with placebo during the second week and vice versa. The investigator (MRM) performing the exercise testing and acquiring and analyzing the data was unaware of assignment to treatment groups. Horses were given 900 µg of albuterol[u] via metered-dose inhaler by use of a mask[v] 30 minutes before beginning the exercise test. This dose causes bronchodilatation in horses with severe heaves.[27] Horses were acclimated to the mask at least 1 week prior to the exercise test.

Statistical analysis—Data are presented as mean ± SD throughout. Repeated-measures (2-way) ANOVA was used for the independent variables treatment (albuterol vs no albuterol) and treadmill speeds (test steps) and their interaction. Group means (treated vs control) for $\dot{V}O_{2max}$, $\dot{V}CO_{2max}$, $STEP_{max}$, **treadmill speed at heart rate of 200 beats/ min** (V_{200}), and RQ were compared, using a 2-tailed paired *t*-test. The Spearman rank correlation coefficient was used to test the association between bronchoalveolar lavage fluid cell percentages and baseline R_{RS} and C_{RS} values, as well as between bronchoalveolar lavage fluid cell percentages and $\dot{V}O_{2max}$, $\dot{V}CO_{2max}$, $STEP_{max}$, V_{200}, and RQ. Commercial software[l] was used for all calculations.

Results

Bronchoalveolar lavage fluid neutrophils (range, 0.6 to 12.0%) and mast cells (range, 0.6 to 6.0%) were found in low numbers; no eosinophils were seen in bronchoalveolar lavage fluid of any horse. In all horses, the predominant cell types were lymphocytes (range, 24.0 to 64.4%) and alveolar macrophages (range, 33.0 to 68.0%; **Table 1**). Baseline lung function had a wide range (R_{RS}, 0.49 to 0.91 cm H_2O/L per second; C_{RS}, 0.32 to 0.44 L/cm H_2O), as did reactivity to histamine, with $PC_{100}R_{RS}$ ranging from 1.3 to 12.8 mg/ml. The percentage of mast cells was significantly (r_s = –0.84; P = 0.032) associated with $PC_{100}R_{RS}$. There were no direct correlations between the percentages of lymphocytes or alveolar macrophages and $PC_{100}R_{RS}$. Percentages of cells in bronchoalveolar lavage fluid did not correlate independently with any indices of exercise capacity (ie, $\dot{V}O_{2max}$, $\dot{V}CO_{2max}$, $STEP_{max}$, V_{200}, and RQ). Neither R_{RS} nor C_{RS} correlated independently with percentages of cells in bronchoalveolar lavage fluid or with any indices of exercise capacity.

The effect of albuterol pretreatment and placebo on selected incremental exercise test-derived variables was determined (**Table 2**). All horses were able to complete the eighth step (10.8 m/s) and perform at least a fraction of the ninth step (12.0 m/s) when treated with placebo. When treated with albuterol, horse 2 was unable to complete the eighth step; all others were able to perform at least a fraction of the ninth step. The mean $\dot{V}O_2$ and

Table 2—Exercise test measurements in 8 horses after treatment with placebo or albuterol

	Placebo		Albuterol	
Variable	**Mean**	**SD**	**Mean**	**SD**
$\dot{V}O_{2max}$	131.5	11.7	128.1	10.3
$\dot{V}CO_{2max}$	150.6	17.5	158.7	21.2
RQ	1.2	0.2	1.3	0.2
$STEP_{max}$	8.7	0.3	8.6	0.6
V_{200}	3.9	1.0	4.0	1.3

$\dot{V}O_{2max}$ = Maximum oxygen consumption (ml/kg per minute).; $\dot{V}CO_{2max}$ = Maximum carbon dioxide consumption (ml/kg per minute). RQ = Respiratory quotient. ($\dot{V}CO_2$ /$\dot{V}O_2$)$STEP_{max}$ = Maximum No. of steps completed during the exercise test. V_{200} = Treadmill speed (m/s) at heart rate of 200 beats/min.

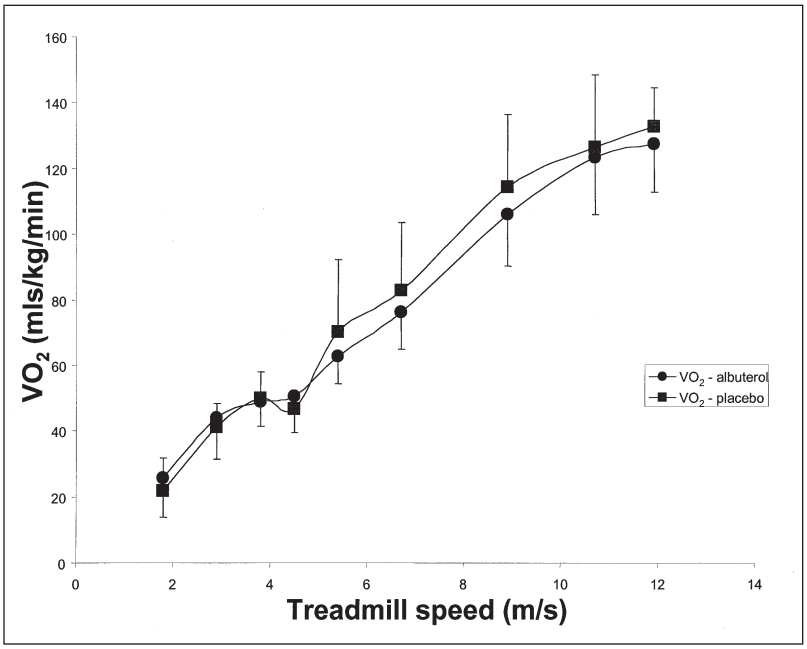

Figure 1—Oxygen consumption ($\dot{V}O_2$) in 8 horses (mean ± SD) during an incremental exercise test when treated with aerosolized albuterol versus placebo. There are no significant differences between groups.

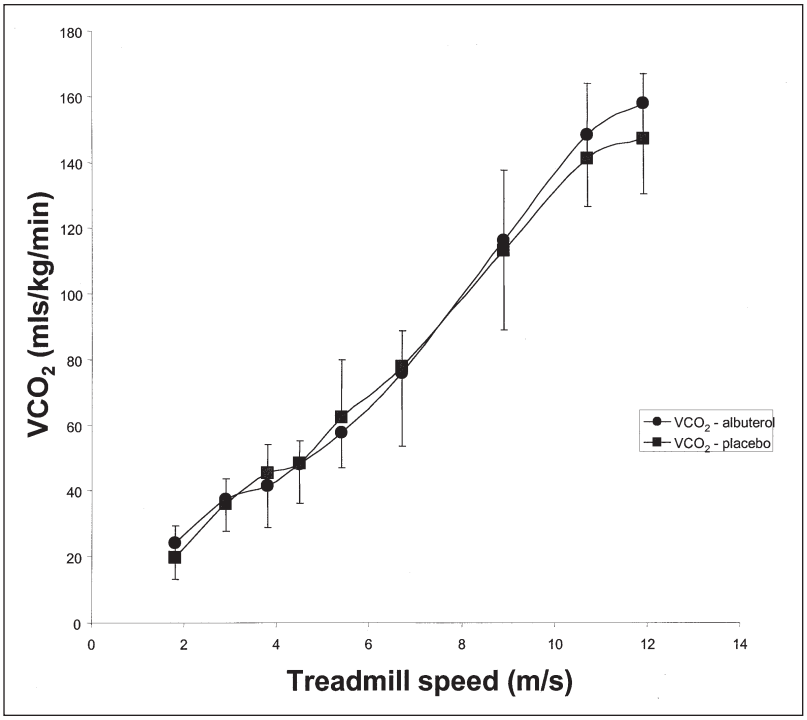

Figure 2—Carbon dioxide production ($\dot{V}CO_2$) in 8 horses (mean ± SD) during an incremental exercise test when treated with aerosolized albuterol versus placebo. There are no significant differences between groups.

$\dot{V}CO_2$ versus treadmill speed curves, respectively, for albuterol- versus placebo-treated groups were determined (**Fig 1 and 2**). There was no significant effect of treatment for any variable in any group.

Discussion

In the present study, we found that administration of a therapeutic dose of albuterol[27,28] given via metered-dose inhaler had no significant effect on $\dot{V}O_{2max}$, $\dot{V}CO_{2max}$, V_{200}, RQ, or $STEP_{max}$ in 8 clinically normal Standardbreds. Although the question must always arise regarding whether an incremental exercise test adequately approximates the demands of racing, previous studies[29,30] have demonstrated that physiologic variables such as $\dot{V}O_{2max}$ can be correlated with superior performance. A second important finding was that 5 of these clinically normal horses without signs of IAD such as externally or endoscopically visible mucus, exercise intolerance, or cough nonetheless had evidence of airway inflammation, that is, high percentages (> 2%)[5] of mast cells in the bronchoalveolar lavage fluid, and 1 horse had a high percentage (> 10%) of neutrophils in the bronchoalveolar lavage fluid (Table 1).[1] Four horses had strikingly reactive airways ($PC_{100}R_{RS} < 6$ mg/ml).[5,7] Three horses had mildly high ($0.6 < R_{RS} < 1.0$) baseline R_{RS} values (Table 2).[5] Moreover, there was a strong correlation between the percentage of mast cells in bronchoalveolar lavage fluid and airway hyperreactivity ($PC_{100}R_{RS}$), which agrees with results of a previous study.[5] Thus, it should be questioned whether this experimental group, purchased directly from the racetrack with no known respiratory disease, represented a completely clinically normal population. These mares may represent a subset of horses that, despite lack of clinical evidence for airway disease, performed below expectation because of subclinical airway disease and were thus available for purchase for research. Nonetheless, this group does represent a typical population as investigated in the field; that is, these horses were without endoscopically visible airway abnormalities, had no history of respiratory tract disease, and had no important findings on physical examination, hematologic evaluation, or serum biochemical analysis suggestive of airway or other disease.

Histologic changes compatible with mild to moderate bronchiolitis and IAD in horses without overt signs of airway disease have been reported in the equine veterinary literature,[31,32] suggesting that these horses may be affected by clinically undetectable but important small airway disease. Recently, Standardbreds with clinically evident mild bronchiolitis have been shown to have adequate gas exchange during exercise testing, in comparison with horses with no evidence of respiratory tract disease; however, no measurements of respiratory resistance were made, and it may be that the work of breathing is increased in such horses.[33] If, as results of recent studies suggest, even clinically normal horses experience flow limitations during strenuous exercise, then bronchodilation may be expected to enhance performance in horses with possible preexistent airway obstruction.[34] We hypothesize that our study population of asymptomatic horses may have had subclinical IAD and thus theoretically would have had a greater likelihood of experiencing a performance-enhancing effect of aerosolized β_2-AR, contrary to our actual findings. Our results suggest that β_2-AR do not enhance performance in horses with airway hyperreactivity and possible underlying low-grade airway disease.

The β_2-AR are postulated to have exercise-enhancing effects because of their ability to cause bronchodilatation and other physiologic effects. Airway resistance may require as much as 8% of total O_2 uptake; thus, the bronchodilatory effect of β_2-agonists may enhance performance by decreasing the oxygen cost of respiratory work.[8] Other potentially exercise-enhancing effects of β_2-AR include changes in the rate and contractility of the heart, systolic blood pressure, and metabolism, as well as increased blood glucose concentrations.[21,35-37] Epinephrine increases the peak force production and rate of force production when bound to skeletal muscle.[38] Systemic administration of β_2-AR also has anabolic effects on skeletal muscle, with increases in muscle size and strength in many species.[39,40] The β_2-AR clenbuterol is used as a repartitioning agent in cattle[41] and has been investigated as a potential aid in rehabilitating muscular atrophy.[42]

Despite these potential benefits, most studies investigating the effects of β_2 agonists on clinically normal human athletes performing incremental exercise tests have revealed either no improvement[10-11,13-16,39] or a decrease in performance and measures of aerobic ability (eg, $\dot{V}O_{2max}$, $\dot{V}CO_{2max}$, RQ, and run time).[8,11,13,w] In contrast, Bedi et al[18] and Signorile et al[43] noted improvements in short bursts of high-intensity work in clinically normal athletes following administration of β_2-AR. More recently, short-term oral administration of the β_2-AR salbutamol increased the time to exhaustion during intense submaximal exercise (cycling).[17] Surprisingly, despite improvements in ventilatory variables such as forced expiratory volume in 1 second, β_2-AR also have minimal or no beneficial effects on aerobic performance in athletes with exercise-induced bronchoconstriction,[9,44-49] and chronic (4 times/d for 1 week) administration of albuterol actually worsened performance in 1 group of athletes with reversible bronchoconstriction attributable to asthma.[50] A similarly comprehensive body of knowledge concerning the effects of selective β_2-AR in clinically normal horses and those with IAD is lacking. Clenbuterol delivered IV to clinically normal Thoroughbreds performing an incremental exercise test elicited no significant change in any exercise test variable[51] or respiratory mechanics[20] but did result in a higher HR during exercise and a higher arterial oxygen concentration and lower arterial carbon dioxide concentration after exercise.[52] More recently, albuterol given via aerosol to clinically normal horses resulted in a small but significant increase in run time and peak $\dot{V}O_2$.[19] Although the horses involved in the studies cited were all free of clinical signs of respiratory disease, lung function testing, bronchoalveolar lavage, and histamine bronchoprovocation testing were not performed. Thus it is impossible to know what percentage, if any, of these horses may have had subclinical airway disease. These various findings support the importance of further investigation into the effects of β_2-AR on performance in clinically normal horses and those affected with IAD.

The question remains as to why no performance-enhancing effects of β_2-AR treatment were seen in our study horses. Many theories have been offered in studies on humans, including increased deleterious β_2-stimulation of skeletal muscle,[11] increased myocardial demand for oxygen,[36] and a hypoxic effect of β_2-stimulation.[47] Horses have ventilation and perfusion mismatching during exercise: it is possible that this is exacerbated by albuterol as a result of greater ventilation of areas that are underperfused.[53] Results of studies[15,50] also indicate that there is a down-regulation of β_2 receptors in athletes undergoing long-term endurance training as a result of the increased blood catecholamines present during exercise. This may result in the inability of the pulmonary system to respond to β_2-stimulation, particularly in well-conditioned athletes. However, the results of our study may also reflect failure of a physiologic response to treatment or a dose-related effect that we have not explored. It also may be that the statistical power of our study was inadequate to detect changes that may have been physiologically important, because of the effect of treatment. Moreover, the effect of albuterol on ventilation in exercising horses remains largely unknown. If a subgroup of our horses did have low-grade underlying airway obstruction, then it may be that this airway obstruction is not fully reversible, thus rendering bronchodilatory agents ineffective. It may also be that the high pleural pressures generated during strenuous exercise result in flow limitations caused by collapse of noncartilaginous airways[34]; conceivably this dynamic airway closure would not be reversed by albuterol administration. These questions could be answered in part by examining the bronchodilatory effect of a range of albuterol doses on clinically normal horses before, during, and after exercise.

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[c]Bivona Medical Technologies, Gary, Ind.
[d]Three-port proportional valve No. 602 00001, Joucomatic, Rueil, France.
[e]Fleisch No. 4, OEM Medical, Lenoir, NC.
[f]DP45 to 28, Validyne Engineering, Northridge, Calif.
[g]DP45 to 14, Validyne Engineering, Northridge, Calif.
[h]On the Nose, Eden, ON, Canada.
[i]Pari LC JET, Pari Corp, Paris, France.
[j]Compair, Model NE-C08, Omron HealthCare Inc, Vernon Hills, Ill.
[k]Sigma Chemical Co, St Louis, Mo.
[l]Statistix, version 4.1, Analytic Software, Tallahassee, Fla.
[m]Sato Treadmill, AB, Sweden.
[n]Dayton Electric Manufacturing Co, Dayton, Ill.
[o]BIF Industries, Providence, RI.
[p]S-3A, Ametek, Pittsburgh, Pa.
[q]CD-3A, Ametek, Pittsburgh, Pa.
[r]MacIntosh Quatra, Apple, city, state abbrev.
[s]LabVIEW, National Instruments, Austin, Tex.
[t]Hippocard, Speedtest Thoroughbreds Inc, Red Lake, ON, Canada.
[u]Proventil, Schering-Plough Corp, Kenilworth, NJ.
[v]Canadian Monaghan, Trudell Medical International, London, ON, Canada.
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