

**Comparison of Two Aerosolized Bronchodilators  
in the Treatment of  
Severe Equine Chronic Obstructive Pulmonary Disease**

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# Comparison of Two Aerosolized Bronchodilators in the Treatment of Severe Equine Chronic Obstructive Pulmonary Disease

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(ABSTRACT)

Aerosolized bronchodilator drugs are commonly used for treatment of horses with chronic obstructive pulmonary disease. Relative efficacy of sympathomimetic and parasympatholytic bronchodilators for relief of acute airway obstruction and improvement of pulmonary gas exchange was compared in 6 horses with COPD. Physical examination, arterial and venous blood gas analysis and measurement of end-tidal CO<sub>2</sub> tension were performed at time zero, 30 minutes, 1, 2, 3 and 4 hours after administration of aerosol ipratropium (0.35 ug/kg), albuterol (1 ug/kg) or placebo via an equine Aeromask and metered dose inhaler. Physiologic shunt fraction ( $Q_S/Q_T$ ), alveolar dead space fraction ( $V_D/V_T$ ) and alveolar to arterial oxygen tension difference ( $p_{(A-a)}O_2$ ) were calculated using standard formulas. At time zero, horses demonstrated severe respiratory compromise and marked alterations in pulmonary gas exchange, indicative of alveolar hypoventilation,  $V_A/Q$  mismatching and diffusion impairment.

Ipratropium treatment significantly ( $p < 0.05$ ) reduced arterial CO<sub>2</sub> tension and end-tidal CO<sub>2</sub> tension toward normal, but significantly increased  $p_{(A-a)}O_2$  from baseline. The change in  $paCO_2$  after ipratropium treatment was significantly different from albuterol and placebo treatment groups. There were no significant changes in response variables after albuterol and there were no treatment by time interactions. These results indicate that, under the conditions of this study, ipratropium (0.35 ug/kg) improved alveolar ventilation and had superior bronchodilator efficacy than albuterol (1 ug/kg) in horses with severe COPD. Marked impairment of pulmonary gas exchange persisted after bronchodilators, emphasizing that anti-inflammatory therapy and environmental control are also necessary for effective treatment of severe equine COPD.

## **DEDICATION**

This work is dedicated to my parents, Rosalind and John,  
and my sisters, Georgie and Cindy,  
in thanks for their constant love, understanding, generosity and friendship.

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## CONTENTS

ACKNOWLEDGEMENTS .....	iv
TABLE OF CONTENTS.....	v
LIST OF FIGURES .....	vi
LIST OF TABLES.....	vii
LIST OF ABBREVIATIONS.....	viii
CHAPTER 1: INTRODUCTION .....	1
1) Normal Respiratory Physiology	
Lower respiratory tract anatomy and ultrastructure.....	2
Mucociliary function.....	3
Autonomic regulation of pulmonary function .....	4
Pulmonary ventilation and perfusion .....	14
2) Chronic Obstructive Pulmonary Disease	
Etiology.....	18
Ultrastructural changes and histology.....	19
Pulmonary function and gas exchange .....	20
Inflammation.....	23
Bronchospasm.....	27
Management.....	32
Therapy .....	33
CHAPTER 2: MATERIALS AND METHODS .....	41
CHAPTER 3: RESULTS .....	44
CHAPTER 4: DISCUSSION.....	56
CHAPTER 5: CONCLUSIONS .....	63
BIBLIOGRAPHY .....	64
APPENDIX A.....	75
VITA.....	76

## FIGURES

Figure 1. Sensory and autonomic efferent nerve supply to equine airway smooth muscle .....	6
Figure 2. Receptor-mediated mechanisms regulating airway smooth muscle contraction .....	8
Figure 3. Alveolar dead space fraction equation .....	14
Figure 4. Alveolar gas equation .....	16
Figure 5. Physiologic shunt fraction equation .....	17
Figure 6. Oxygen content equation .....	17
Figure 7. Mean difference in heart rate from baseline of horses with COPD after treatment with aerosolized albuterol, ipratropium or placebo .....	47
Figure 8. Mean difference in respiratory rate from baseline of horses with COPD after treatment with aerosolized albuterol, ipratropium or placebo .....	48
Figure 9. Mean difference in body temperature from baseline of horses with COPD after treatment with aerosolized albuterol, ipratropium or placebo .....	49
Figure 10. Mean difference in arterial O <sub>2</sub> tension from baseline of horses with COPD after treatment with aerosolized albuterol, ipratropium or placebo .....	50
Figure 11. Mean difference in arterial CO <sub>2</sub> tension from baseline of horses with COPD after treatment with aerosolized albuterol, ipratropium or placebo .....	51
Figure 12. Mean difference in ETCO <sub>2</sub> tension from baseline of horses with COPD after treatment with aerosolized albuterol, ipratropium or placebo .....	52
Figure 13. Mean difference in Q <sub>S</sub> /Q <sub>T</sub> from baseline of horses with COPD after treatment with aerosolized albuterol, ipratropium or placebo .....	53
Figure 14. Mean difference in V <sub>D</sub> /V <sub>T</sub> from baseline of horses with COPD after treatment with aerosolized albuterol, ipratropium or placebo .....	54
Figure 15. Mean difference in p <sub>(A-a)</sub> O <sub>2</sub> from baseline of horses with COPD after treatment with aerosolized albuterol, ipratropium or placebo .....	55

## TABLES

Table 1. Mean responses at baseline before treatment of COPD-affected horses with aerosolized albuterol, ipratropium or placebo .....	45
Table 2. Differences (d) in response variables, subtracted from baseline, after treatment of COPD-affected horses with aerosolized albuterol, ipratropium and placebo .....	46

## ABBREVIATIONS

(as they appear in text)

COPD	chronic obstructive pulmonary disease
ACH	acetylcholine
LT	leukotrienes
M	muscarinic
N	nicotinic
NANC	nonadrenergic noncholinergic
$\beta$	beta adrenergic
$\alpha$	alpha adrenergic
NE	norepinephrine
EPI	epinephrine
SP	substance P
NO	nitric oxide
VIP	vasoactive intestinal peptide
GTP	guanosine triphosphate
cAMP	cyclic adenosine monophosphate
PLC	phospholipase C
AC	adenylyl cyclase
PIP <sub>2</sub>	phosphoinositol
IP <sub>3</sub>	inositol 1,4,5-triphosphate
DAG	diacyl glycerol
EFS	electrical field stimulation
PG	prostaglandin
V <sub>T</sub>	tidal volume
f	respiratory frequency
V <sub>D</sub> /V <sub>T</sub>	alveolar dead space fraction
paCO <sub>2</sub>	arterial carbon dioxide tension
ETCO <sub>2</sub>	end-tidal carbon dioxide tension
$\Delta P_{pL_{max}}$	maximum pleural pressure difference between start and end inhalation
C <sub>dyn</sub>	dynamic compliance

$R_L$	airway resistance
$V_A/Q$	alveolar ventilation to perfusion ratio
$P_AO_2$	alveolar oxygen tension
BP	barometric pressure
$P_{H_2O}$	partial pressure of water vapor
$FiO_2$	fraction inspired oxygen
R	respiratory exchange ratio
$Q_S/Q_T$	physiologic shunt fraction
$Cc'O_2$	alveolar oxygen content
$CaO_2$	arterial oxygen content
$CvO_2$	venous oxygen content
[Hb]	hemoglobin concentration
%Sat	percent saturation oxyhemoglobin
$p_{(A-a)O_2}$	alveolar to arterial oxygen tension difference
BALF	bronchoalveolar lavage fluid
Wb	work of breathing
$E_T:I_T$	expiratory to inspiratory time ratio
TX	thromboxane
HETE	hydroxyeicosatetraenoic acid
PAF	platelet activating factor
HT	hydroxytryptamine
MCH	methacholine
$ED_{65}C_{dyn}$	dose of histamine or MCH that reduces $C_{dyn}$ to 65% of baseline
$PC_{35}C_{dyn}$	dose of histamine or MCH required to decrease $C_{dyn}$ by 35% from baseline
$EC_{50}$	concentration required for 50% effect in dose/response study
DPI	dry powder inhalant
MDI	metered dose inhalant

## **CHAPTER 1: INTRODUCTION**

Aerosolized bronchodilator drugs are commonly used in the treatment and prevention of equine chronic obstructive pulmonary disease (COPD). A wide variety of pharmacologic agents that relax airway smooth muscle are available to equine clinicians. However, there is insufficient documentation of the comparative therapeutic efficacy of different types of bronchodilators in horses with COPD. Albuterol, a  $\beta_2$  adrenergic receptor agonist, and ipratropium, a muscarinic receptor antagonist, are popular aerosol bronchodilator drugs that were chosen for comparison in treatment of acute airway obstruction in COPD-affected horses.

A variety of methods are used to quantitatively assess pulmonary function and efficiency of gas exchange in horses with COPD. Measurement of indices of pulmonary gas exchange is simpler and more convenient than traditional pulmonary function testing. In horses with COPD, physiologic shunt fraction and alveolar dead space fraction have been reported, but have not been previously used to monitor and compare response to bronchodilator treatment. Arterial blood gas analysis and indices of pulmonary gas exchange were chosen to monitor response to albuterol and ipratropium in horses with COPD.

To understand how bronchodilators work and interpret possible effects on pulmonary gas exchange efficiency in horses with COPD, a review is provided of normal pulmonary physiology and equine chronic obstructive pulmonary disease, focusing on autonomic airway regulation, alterations in pulmonary function and therapy in affected horses.

## **1) Normal Respiratory Physiology**

### **Lower respiratory tract anatomy and ultrastructure**

The thoracic cavity of the horse is lined by two pleural sacs which contact each other in the median plane to form the mediastinum.<sup>1</sup> The mediastinum contains the heart and all other thoracic organs except the lungs, and is usually complete. In regions where the mediastinum contains no organs it is relatively thin and may be fenestrated, commonly caudal to the heart. The lungs are long, have indistinct lobulation and are laterally compressed cranially. The root of the lung lies opposite the 6<sup>th</sup> rib and includes the principal bronchus and the pulmonary artery and veins. The right lung is larger than the left and carries the centrally located accessory lobe. Inter- and intra-lobar fissures are absent so that cranial and caudal lobes are separated only by the cardiac notch. Part of the mediastinal surface of both lungs fuses caudal to the hilus.<sup>1</sup> The incomplete division of the horse lung into secondary lobules by connective tissue septa allows gas to pass between adjacent regions of lung via collateral pathways, but collateral time constants are long. The fraction of ventilation traversing collateral pathways decreases as respiratory frequency increases.<sup>2, 3</sup>

The tracheobronchial tree has up to 24 branches. The larger, cartilagenous airways are intrapulmonary from the mid mainstem bronchi caudally, and lie within a loose connective tissue sheath, the bronchovascular bundle. Bronchioles lack cartilage and a connective tissue sheath.<sup>4</sup> The total cross-sectional area of the tracheobronchial tree increases only slightly between the trachea and the first four generations of bronchi, but increases dramatically toward the periphery of the lung.<sup>4</sup> Horse lungs have a thick pleura supplied by the bronchial artery and poorly developed respiratory bronchioles. The bronchial artery terminates in the distal airways and alveoli. The pulmonary vein follows the bronchus and artery in the periphery, but departs from these structures as it approaches the hilum.<sup>2, 3</sup> Submucosa of larger airways contains a plexus of blood vessels derived from the bronchial circulation, allowing warming of inhaled air but also causing airway wall edema when inflamed.<sup>4</sup>

In normal horses, dorsal trachea to caudal lobar bronchus is lined with pseudostratified ciliated columnar epithelium with numerous mucus cells and occasional patches of non-ciliated microvillus cells.<sup>5</sup> Small bronchi have predominantly ciliated cells, numerous non-ciliated mucus cells, and duct openings from submucosal glands. Larger bronchioles have decreasing numbers of ciliated cells with increasing sparsity of cilia and progressively more numerous non-ciliated bronchiolar epithelial cells that become dominant in the terminal bronchioles. Cuboidal bronchiolar epithelium contains predominantly non-ciliated, domeshaped cells (Clara cells) distinguished by abundant agranular endoplasmic reticulum and numerous cytoplasmic granules, but also small numbers of ciliated cells up to the alveolar duct junction. Respiratory bronchioles are poorly developed and the junction of the terminal bronchioles and alveolar ducts is abrupt. Type I pneumocytes cover most of the alveolar surface with Type II pneumocytes occurring singly or in groups. Inter-alveolar pores are more numerous in older animals and often contain migrating alveolar macrophages.<sup>5</sup> Alveolar macrophages are the most prominent phagocytic cell in the lung, but neutrophils, eosinophils and lymphocytes are also present and increase dramatically with inflammation in response to chemotaxis. Secretory Immunoglobulin A, IgG, IgG<sub>t</sub> and IgE are all found in BAL fluid, but not IgM.<sup>6</sup>

### **Mucociliary function**

Mucociliary clearance rates from the trachea have been determined in normal horses after an injection of technetium-99m sulphide colloid into the tracheal lumen, indicating a tracheal mucus velocity of 20mm/min.<sup>7</sup> Both adrenergic and cholinergic receptors are involved in the regulation of mucociliary clearance, as suggested by the increase in ciliary beat frequency induced by beta ( $\beta$ )<sub>2</sub> adrenergic and cholinergic agonists, and the inhibitory effects induced by  $\beta$  blockers and anticholinergic drugs.<sup>8, 9</sup>

Mucus performs multiple important functions including trapping of inhaled particles for transportation to the pharynx, humidification of inspired air, hydration of the underlying mucosa and immunological defense via IgG<sub>b</sub>, lactoferrin and lysozyme.<sup>10</sup> Airway secretions are composed of 2 layers, the sol layer of periciliary fluid which is 5  $\mu$ m deep

and forms via transepithelial osmosis, and an overlying gel layer which is 5-10  $\mu\text{m}$  thick and composed of mucus strands. Alveolar fluid, surfactant and alveolar cells, including macrophages, are also present in airway secretions.<sup>10</sup> Serous secretion predominates in terminal bronchioles and alveoli, produced by Clara cells. Mucus rafts float on the sol layer and are secreted in response to mechanical stimulation, then carried away with the aid of myoepithelial cells. Goblet cells, present in large numbers throughout the bronchi and bronchioles, contribute a viscous secretion and play the major role in mucus production in horses.<sup>5, 11</sup> Submucosal glands in the bronchi contain both goblet and serous cells.<sup>6</sup> Airway mucus contains 95% water and 5% combination of glycoproteins (eg. mucin), proteoglycans, lipids, carbohydrates and minerals. The rheological properties of mucus depend largely on viscosity, elasticity and spinnability, which are essential for effective coupling of mucus and cilia.<sup>10</sup>

Ciliated cells predominate in the larger bronchi and trachea, but as horses lack respiratory bronchioles, ciliated epithelium can extend to the bronchiolar-alveolar duct junction.<sup>5, 12</sup> Each ciliated cell is 5  $\mu\text{m}$  and has 200 cilia 5 to 6  $\mu\text{m}$  in length with 3 to 7 short claws at the tip. Cilia propel fluid with asymmetric cyclic movement and ciliary action of adjacent cells appears coordinated.<sup>6</sup> During the effective stroke, the ciliary tips contact the gel layer, then the cilia retract back to the cell surface by bending at the base. Marked ciliary ultrastructural changes and malformations have been observed in both COPD affected and normal horses.<sup>11</sup> Cooling slows ciliary activity, while  $\beta$  adrenoceptor agonists and methylxanthine derivatives increase the frequency and amplitude of ciliary beats and improve mucokinesis in humans.<sup>10</sup> Xylazine and detomidine administration to normal horses decreased tracheal clearance rates significantly by 18 to 54%.<sup>7</sup>

### **Autonomic Regulation of Pulmonary function**

Airway smooth muscle is the main regulator of airway diameter throughout the tracheobronchial tree, and is regulated primarily by the autonomic nervous system with some epithelial interactions. Nervous system control occurs via sensory receptors, upper centers and autonomic efferent motor innervation. The parasympathetic pathway acts via cholinergic nerves and receptors, the sympathetic pathway acts via adrenergic receptors,

nerves and circulating catecholamines, while the nonadrenergic noncholinergic pathway acts mainly via neuropeptide transmitters (Figure 1).<sup>4, 8, 13</sup>

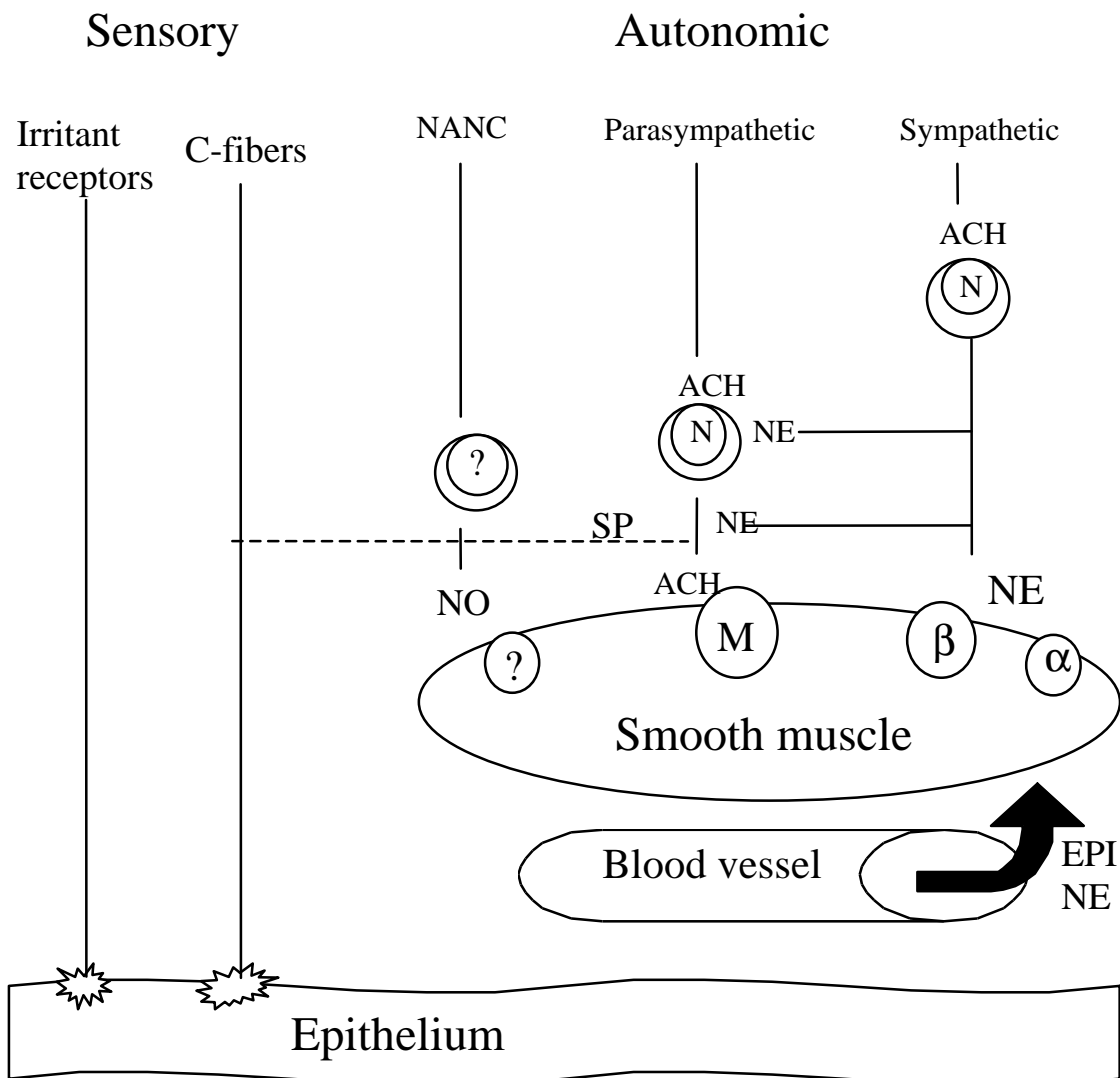
### *Sensory Receptor types*

There are three types of receptors with vagal afferences located in the lungs that may influence respiration. Pulmonary stretch receptors within tracheal and bronchial smooth muscle have afferent myelinated nerve fibres in the vagus nerve. These receptors respond to lung inflation, inhibiting further inspiratory activity and slowing respiratory frequency by prolonging expiratory time.<sup>6, 8</sup> The reverse occurs in response to lung deflation, constituting the Hering-Breuer reflexes.<sup>14</sup> Irritant receptors and their myelinated nerve fibres respond to noxious stimuli with reflex responses of bronchoconstriction, tachypnea, coughing and mucus secretion. They are located beneath the epithelium of conducting airways and may be exposed in allergic or infectious conditions. Pulmonary C fiber receptors and their unmyelinated fibers are located in the parenchyma, conducting airways and blood vessels. They may be stimulated by pulmonary capillary engorgement, edema and chemical mediators in disease, but their importance in horses is unknown (Figure 1).<sup>6, 8</sup>

Efferent pathways regulate airway smooth muscle and pulmonary function via autonomic receptors.<sup>8</sup> Smooth muscle contraction is initiated by increased intracellular calcium concentration which can occur via entry of extracellular calcium through voltage-dependent or receptor-operated calcium channels, or via intracellular release from calcium stores in the sarcoplasmic reticulum.<sup>15</sup> Receptor operated channels are opened by agonists such as histamine, ACH and LT's, but the importance of these channels is questionable as contraction appears to be initiated via calcium release from intracellular stores.<sup>15</sup> The most important functional class of receptors in regulation of the tracheobronchial tree are those coupled to guanosine triphosphate (GTP) binding proteins (Figure 2).<sup>15</sup> Receptor activation results in binding of GTP to a G protein located on the inner membrane, regulating the activity of enzymes such as adenylyl cyclase, ion channels or transport proteins. The effect of receptors on cellular function may involve generation of second messengers, importantly cyclic adenosine monophosphate (cAMP)

Figure 1. Sensory and autonomic efferent nerve supply to equine airway smooth muscle.

ACH released from parasympathetic nerves activates muscarinic (M) or nicotinic (N) receptors; nitric oxide from iNANC nerves activates unknown (?) receptor types; norepinephrine (NE) from sympathetic nerves activates beta ( $\beta$ ) and alpha ( $\alpha$ ) adrenoceptors, in addition to circulating NE and epinephrine (EPI). Substance P (SP) may mediate the eNANC system.



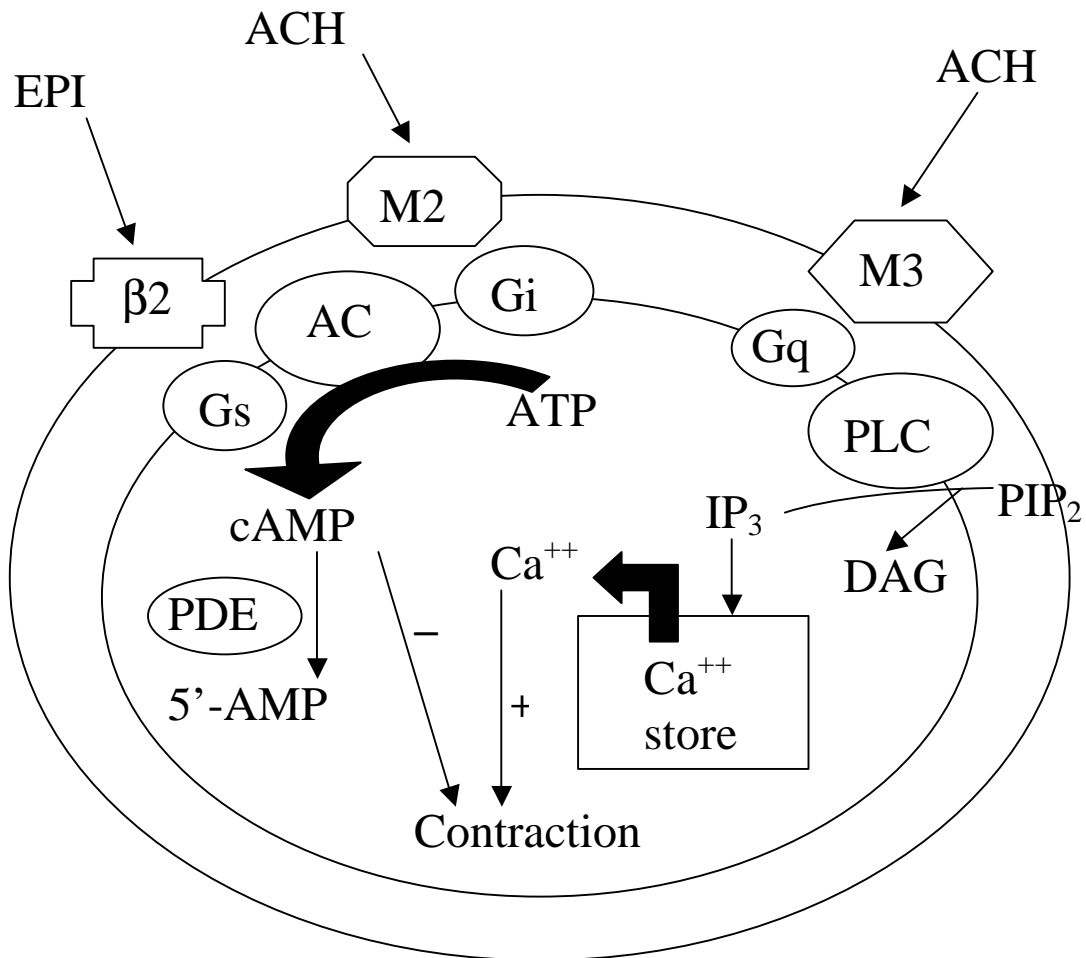
and calcium ( $\text{Ca}^{2+}$ ). Stimulatory and inhibitory G proteins modulate adenylyl cyclase which catalyzes formation of cAMP from adenosine triphosphate.<sup>15</sup> Beta ( $\beta$ ) adrenergic agonists increase cAMP via the stimulatory G protein, activating protein kinases, catalyzing phosphorylation of intracellular proteins and inhibiting smooth muscle contraction. Muscarinic subtype 2 (M2) receptor activation decreases intracellular cAMP via the inhibitory G protein, opposing smooth muscle relaxation. Phosphodiesterase enzymes catalyze breakdown of cAMP to 5'-AMP.<sup>15</sup> The opening of  $\text{Ca}^{2+}$  channels is controlled by electrical depolarization, G proteins, protein kinases and intracellular  $\text{K}^+$  or  $\text{Ca}^{2+}$  concentrations. Acetylcholine causes bronchospasm by muscarinic subtype 3 (M3) receptor-activated G protein stimulation of phospholipase C, which catalyzes formation of inositol 1,4,5-triphosphate ( $\text{IP}_3$ ) and diacylglycerol. These products initiate release of  $\text{Ca}^{2+}$  from intracellular stores and activate protein kinase C, allowing sustained smooth muscle contraction.<sup>15</sup> Down-regulation of a particular receptor after continuous activation can result in decreased response to agonist administration.<sup>15</sup>

### ***Cholinergic regulation of airways***

The parasympathetic system provides the only excitatory innervation to smooth muscle of the equine trachea and bronchi. There is minimal resting bronchomotor tone in normal horses.<sup>16-18</sup> Parasympathetic motor efferents travel in the vagus nerve to parasympathetic ganglia in the airway walls and release acetylcholine (ACH) which activates nicotinic receptors. Short postganglionic parasympathetic fibres also release ACH which stimulates muscarinic receptors types 1, 2 and 3 on smooth muscle.<sup>8, 19</sup> Smooth muscle M3 receptors are the primary type responsible for contraction and M2 receptors inhibit relaxation.<sup>15</sup> Smooth muscle of equine small bronchi, third generation bronchi and trachea contracted in response to ACH in a concentration dependent manner, indicating minimal variation in tracheobronchial sensitivity of airway smooth muscle to ACH.<sup>16, 20</sup> All ACH-induced smooth muscle contractions were blocked by atropine, a nonspecific muscarinic receptor antagonist, but not by tetrodotoxin. Tetrodotoxin is a sodium channel blocker that prevents action potential propagation in nerves and blocks release of ACH from cholinergic nerves, but has no effect on smooth muscle membranes or the ability of receptors on membranes to respond to ACH.<sup>20</sup> A diminishing contractile

Figure 2. Receptor-mediated mechanisms regulating airway smooth muscle contraction.

Binding of ACH to M3 receptors activates a G protein and phospholipase C (PLC) which converts phosphoinositol ( $PIP_2$ ) to  $IP_3$  and diacylglycerol (DAG).  $IP_3$  releases  $Ca^{++}$  from intracellular stores causing smooth muscle contraction. This is inhibited by increases in cAMP via  $\beta$  adrenoceptor activation by catecholamines such as epinephrine (EPI), which activate adenylyl cyclase (AC) via a stimulatory G protein ( $G_s$ ), converting ATP to cAMP. ACH inhibits AC and decreases cAMP via the M2 receptor and inhibitory G protein ( $G_i$ ). cAMP is degraded by phosphodiesterase (PDE) to 5'-AMP.



response to EFS in comparison to ACH was observed from the trachea (89%) to 3<sup>rd</sup> generation airways (68%) to the small airways (65%). Contractile responses of small bronchi, trachea and third generation airways to EFS were abolished by atropine or tetrodotoxin,<sup>16, 20</sup> and were therefore attributable to the release of ACH from nerves in muscle strips.<sup>20</sup> Diminishing contractile response to EFS may be caused by decreased cholinergic innervation in the peripheral airways, differences in cell-to-cell contact in airway smooth muscles, coactivation of inhibitory nerves, noninnervated receptors or endogenous inhibitory mediators.<sup>16, 20</sup>

Functional parasympathetic innervation and muscarinic receptors were demonstrated in equine small (1mm), peripheral (greater than 15<sup>th</sup> generation) airways and, to a lesser extent, in lung parenchyma. Responses of small peripheral airways to EFS, in the presence and absence of atropine or tetrodotoxin, confirmed that ACH released from cholinergic nerves is the predominant excitatory input for airway contraction.<sup>21</sup> Decreasing efficacy of cholinergic agonists and EFS responses towards peripheral airways may be caused by decreasing receptor density from trachea to the bronchi or predominance of other M receptor subtypes.<sup>21</sup> Changes in sensitivity to cholinergic agonists and histamine shift in opposite directions along the respiratory tract. While the muscarinic response decreases dramatically toward alveoli, airways become more sensitive to histamine, presumably via smooth muscle H1 receptors, with 4.6 times more potency in lung parenchyma than in small airways. Histamine also increases EFS responses, possibly due to synergism, facilitation of ACH release from cholinergic nerves, activation of sympathetic alpha ( $\alpha$ ) adrenergic responses or facilitation of excitation spread through smooth muscle cells via gap junctions.<sup>21</sup>

Post-ganglionic fibers of parasympathetic nerves possess muscarinic autoreceptors which provide negative feedback on ACH release from airway cholinergic nerves. These prejunctional inhibitory receptors have been identified as M2 subtype in other species.<sup>8, 22</sup> The auto-inhibitory effect on ACH release is blocked by the nonselective muscarinic receptor antagonist, atropine.<sup>23</sup> The M1 receptor antagonist, pirenzepine, and the M3 receptor antagonist, 4-diphenylacetoxy-N-methylpiperidine, dose-dependently inhibited

the contractile responses of equine trachealis strips to EFS and exogenous ACH. The M2 receptor antagonist gallamine did not affect the response of trachealis to exogenous ACH and low-frequency EFS (0.1-2 Hz), but decreased the responses to high-frequency EFS (4-16 Hz). Therefore muscarinic receptors mediating contractions induced by ACH in equine tracheal smooth muscle are primarily of the M3 subtype and functional prejunctional inhibitory M2 receptors are not present on the cholinergic nerves innervating equine tracheal smooth muscle (Figure 1).<sup>19</sup>

In vitro studies revealed that  $\alpha_2$  adrenoceptor agonists concentration-dependently inhibited ACH release and the contractile response of equine airway smooth muscle to EFS, but had no effect on contractile response of distal airway segments to exogenous ACH.<sup>24, 25</sup> Lack of effect of  $\alpha_2$  agonists on contractile potential of airway smooth muscle to barium chloride or exogenous ACH, indicates that these agents do not alter contractile potential of smooth muscle or have significant post-junctional effects.<sup>24</sup> Therefore, the inhibitory effects of  $\alpha_2$  agonists must be due to prejunctional inhibition of cholinergic neurotransmission, most likely due to inhibition of ACH release from cholinergic terminals. The inhibitory effect on EFS response was abolished by  $\alpha_2$ , but not  $\alpha_1$ , receptor antagonists indicating that  $\alpha_2$  adrenoceptors exist on cholinergic nerves innervating equine airway smooth muscle, and activation of these receptors inhibits cholinergic neurotransmission.<sup>24, 25</sup> Prejunctional inhibition of ACH release from equine airway cholinergic nerves by the  $\alpha_2$  adrenoceptor is activated in normal horses by high levels of circulating catecholamines during exercise.<sup>26</sup>

The prostanoid PGE<sub>2</sub>, a major product of equine airway epithelium, inhibits the contractile response of equine airway smooth muscle to EFS, suggesting an inhibitory effect of exogenous PGE<sub>2</sub> on equine tracheal smooth muscle.<sup>27</sup> Exogenous PGE<sub>2</sub> had no effect on EFS-induced ACH release in equine bronchi, but at high doses (10<sup>-7</sup> M) augmented ACH release in the trachea, suggesting an excitatory role of PGE<sub>2</sub> on ACH release from parasympathetic nerves.<sup>28</sup> Cyclooxygenase inhibition by indomethacin or meclofenamate did not influence EFS-induced contractile response or ACH release in normal equine bronchi or tracheal smooth muscle, indicating lack of significant effect of

endogenous prostanoids on cholinergic responses of equine airway smooth muscle.<sup>27, 28</sup> Cyclooxygenase inhibition had no effect on EFS-induced ACH release nor the magnitude of excitatory prejunctional  $\beta$ 2 adrenoceptor induced augmentation of ACH release from airway tissues throughout the equine tracheobronchial tree, suggesting that prostanoids do not play a major inhibitory role in cholinergic neurotransmission. Epithelium removal potentiated EFS-induced ACH release, suggesting that an epithelium derived relaxing factor (EDRF) inhibits ACH release.<sup>29</sup> Decreasing magnitude of cholinergic contraction in response to EFS and increasing sensitivity to histamine occurred in small, peripheral (>15<sup>th</sup> generation) airways. Inhibition of cyclooxygenase potentiated the contractile response to EFS, suggesting that endogenous inhibitory prostanoids modulate responses to cholinergic nerve stimulation in small, peripheral airways.<sup>21</sup>

Airway epithelial cells and submucosal glands express muscarinic receptors, and vagal activation increases the rate of mucus secretion from goblet cells and glands and water transport toward the airway lumen. Atropine, but not ipratropium bromide, depresses mucociliary function by an unknown mechanism.<sup>15</sup>

### ***Adrenergic regulation of airways***

Lung adrenergic receptors are minimally innervated by sympathetic fibres but may be stimulated by circulating catecholamines or specific adrenergic agonists. Preganglionic sympathetic nerves may release ACH, stimulating ganglionic nicotinic receptors. Postganglionic sympathetic fibres release norepinephrine (NE) which may inhibit parasympathetic neurotransmission or activate adrenergic receptors (Figure 1). Epinephrine, released from the adrenal medulla, also activates adrenoceptors in the lungs.<sup>8, 15</sup> Adrenergic nerves, immunoreactive for sympathetic markers, are found throughout healthy equine lung,<sup>30</sup> but only have significant effects on airway smooth muscle in the cranial trachea, where sympathetic activation causes relaxation.<sup>16, 17</sup> Sympathetic nerve fibres were numerous in pulmonary and bronchial blood vessels, suggesting a major role in regulation of mucosal blood flow and control of airway vascular resistance.<sup>30</sup> The equine trachea has both a sympathetic inhibitory and a nonadrenergic inhibitory system, whereas third generation bronchi have only

nonadrenergic inhibition.<sup>17</sup> Small bronchi precontracted with histamine failed to relax in response to EFS, confirming a pattern of decreasing inhibitory innervation from the central to peripheral airways.<sup>16, 31</sup> Adrenergic receptors, however, are widespread throughout the lung and can be activated by circulating epinephrine or adrenergic agonists.<sup>32</sup>

Alpha-1 adrenergic receptors are not activated in healthy ponies, but may have a minimal excitatory role in bronchoconstriction in ponies with recurrent airway obstruction.<sup>33</sup> Prejunctional  $\alpha_2$  adrenoceptors inhibit release of ACH from parasympathetic nerves and are activated by circulating catecholamines during exercise.<sup>25, 26</sup> Epinephrine has a slightly higher affinity for  $\alpha$  receptors than norepinephrine. Alpha receptor activation increases mucosal mucus production and rate of water transport toward the airway lumen and cause vasoconstriction of pulmonary vasculature.<sup>8, 15</sup> Smooth muscle  $\beta_1$  and  $\beta_2$  adrenoceptors exist in horse airways, but are only activated in acute airway obstruction. Equine small bronchi and tracheal strips, precontracted with histamine and indomethacin, relaxed in a dose-dependent response to isoproterenol, confirming existence of  $\beta$  adrenoceptors.<sup>16</sup> Bronchodilation in response to  $\beta$  receptor agonists is mediated primarily by  $\beta_2$  adrenergic receptors,<sup>34</sup> which have a high affinity for EPI and a lesser affinity for NE. In disease,  $\beta_2$  receptor activation by endogenous catecholamines may moderate bronchoconstriction.<sup>35</sup> Other  $\beta_2$  functions include stimulation of mucus secretion and ciliary function, inhibition of mediator release from mast cells and vasodilation of pulmonary vasculature.<sup>8, 32</sup> Beta 1 receptors may contribute to smooth muscle relaxation by modulation of cholinergic transmission.<sup>34</sup> Activation of cardiac  $\beta_1$  adrenoceptors, which have a high affinity for NE and a low affinity for EPI, results in an increase in heart rate.<sup>15</sup>

There is evidence that horse airway cholinergic nerves are modulated by both  $\alpha_2$  inhibitory and  $\beta_2$  excitatory adrenoceptors, with the former predominating.<sup>26</sup> Equine tracheal smooth muscle strips incubated in atropine (to block muscarinic autoreceptors), neostigmine (cholinesterase inhibitor to prevent breakdown of ACH) and guanethidine

(to block sympathetic nerve function) were stimulated with EFS. Epinephrine and norepinephrine inhibited ACH release, but this effect was attenuated by  $\alpha$  adrenoceptor antagonism. Epinephrine, but not NE, augmented ACH release after  $\alpha_2$  adrenoceptor blockade which may be due to  $\beta_2$  receptor mediated facilitation of ACH release. This is supported by augmentation of ACH release by  $\beta$  adrenoceptor agonist, isoproterenol, which was subsequently blocked by  $\beta_2$  adrenoceptor antagonism.<sup>26</sup> Beta 2 adrenoceptor mediated augmentation of ACH is independent of inhibitory nerve function<sup>36</sup> and persists in the presence of cyclooxygenase inhibition and absence of epithelium.<sup>29</sup> Excitatory  $\beta_2$  adrenoceptors are distributed throughout the tracheobronchial tree (trachea to 5mm bronchi branch) and are located prejunctionally on parasympathetic nerves.<sup>29</sup> Activation of this receptor by  $\beta_2$  agonists may prejunctionally augment ACH release and limit their post-junctional effectiveness as bronchodilators.<sup>29</sup>

Augmentation of ACH release induced by activation of  $\beta_2$  adrenoceptors is mediated by cAMP dependent intracellular pathways, but the mechanism whereby cAMP increases ACH release is unknown. Therefore, bronchodilators that increase cAMP may paradoxically augment ACH release while relaxing smooth muscle.<sup>37</sup> The  $\beta_2$  agonists used as bronchodilators are racemic mixtures of R- and S- enantiomers. R-enantiomers of albuterol and formoterol can augment ACH release from equine tracheal parasympathetic nerves, but also inhibit tracheal smooth muscle contraction in response to ACH. The latter postjunctional spasmolytic effect predominates in producing bronchodilation. However, the S-enantiomers do not inhibit equine tracheal smooth muscle contraction in response to ACH, but facilitate ACH release when prejunctional muscarinic autoreceptors are dysfunctional.<sup>38</sup>

### ***Noncholinergic Nonadrenergic innervation of airways***

A nonadrenergic noncholinergic excitatory system (eNANC) with transmitter Substance P causes bronchoconstriction in some species.<sup>6</sup> Nerves immunoreactive for Substance P and tachykinins (neurokinin A), are abundant around blood vessels in the lamina propria and epithelium of equine airways, and probably regulate bronchial circulation.<sup>39</sup>

Substance P binding sites were very dense over small bronchial vessels, tracheobronchial glands and airway epithelium in equine lung.<sup>40</sup> Activation of afferent nerves containing substance P may lead to increased mucus secretion and decreased airway diameter due to vascular congestion.<sup>40</sup> A nonadrenergic noncholinergic inhibitory system (iNANC) has been demonstrated in healthy horses.<sup>31</sup> The magnitude of relaxation induced by iNANC nerve stimulation decreased from the trachea to central bronchi and was absent in peripheral airways. Nitric oxide is the mediator of iNANC function in equine airways.<sup>31</sup>

### **Pulmonary Ventilation and Perfusion**

The volume of air exhaled is the tidal volume, reported to be 12ml/kg in horses, and the remaining lung volume is the functional residual capacity.<sup>8</sup> Minute ventilation equals the product of tidal volume ( $V_T$ ) and respiratory frequency ( $f$ ) and consists of dead space ventilation of conducting airways plus alveolar ventilation where gas exchange occurs.<sup>6, 8</sup> The physiological dead space ventilation describes alveolar dead space and is a functional measurement of the amount of ventilation to lung units with abnormally high  $V_A/Q$  ratios. Alveolar dead space gas does not eliminate carbon dioxide ( $CO_2$ ) and tends toward the composition of inspired gas. The ratio of this dead space to the tidal volume, the alveolar dead space fraction ( $V_D/V_T$ ), may be markedly increased in lung disease and can be calculated via the Bohr equation (Figure 3).<sup>8, 14</sup>

Figure 3. Alveolar Dead Space Fraction

$$V_D/V_T = \frac{(p_aCO_2 - ETCO_2)}{p_aCO_2}$$

( $p_aCO_2$ : arterial carbon dioxide tension;  $ETCO_2$ : end-tidal  $CO_2$  tension of expired gas)

The distribution of ventilation is not uniform throughout the lung due to intrapleural pressure changes, gravity and inequalities in regional airway resistance and alveolar compliance.<sup>8</sup> A vertical pleural pressure gradient in the horse causes preferential ventilation of the ventral lung regions.<sup>6, 41</sup> During inhalation, the diaphragm and intercostal muscles contract, increasing the maximum pressure difference ( $\Delta P_{pL_{max}}$ ) between the atmosphere and the pleural cavity to overcome the elastic recoil of the lung

and frictional resistance of the airways, allowing the lung to inflate.<sup>4</sup> Exhalation is passive until functional residual capacity is reached and expiratory muscles are recruited. The slope of the curve constructed by plotting driving pressure versus lung volume, is a measurement of lung compliance.<sup>6, 42</sup> Dynamic compliance ( $C_{\text{dyn}}$ ) is measured during breathing and equals  $V_T$  divided by  $\Delta P_{\text{PL}_{\text{max}}}$  between the start and end of inhalation.  $C_{\text{dyn}}$  is influenced by changes in airway resistance, lung elasticity and surface tension forces which are controlled by surfactant.<sup>4, 6</sup>

Airway resistance ( $R_L$ ) opposes the flow of air and is equal to  $\Delta P_{\text{PL}_{\text{max}}}$  divided by the change in air flow rate.<sup>6, 42</sup> Total cross-sectional area increases dramatically toward the periphery of the lung so that  $R_L$  is much greater in the trachea and bronchi than in the bronchioles, but can be affected by lung volume, inflammation and smooth muscle tone. As lung volume decreases during tidal breathing, inflamed bronchioles close prematurely and gas is trapped behind closed airways, necessitating recruitment of expiratory muscles at end-exhalation.<sup>6</sup> In disease, non-homogenous pulmonary compliance and airway resistance alter time constants of lung units, causing changes in inspired lung volume and uneven distribution of ventilation. Gas transfer can be further limited by alterations in pulmonary perfusion and diffusion.<sup>6, 14</sup>

The pulmonary circulation is characterized by an extensive capillary network providing surface area for gas exchange and low pulmonary vascular resistance to accommodate cardiac output. Circulation to the conducting airways, interlobular septa and pleura is supplied by the bronchial artery.<sup>6, 8</sup> The vertical gradient of pulmonary perfusion in the horse is related to the balance between pulmonary arterial, venous, alveolar and interstitial pressures, creating preferential perfusion and decreased  $V_A/Q$  ratios in the basal portion of the lung and increased  $V_A/Q$  ratio in apical lung regions.<sup>43</sup> Alveolar vessels, including capillaries and slightly larger vessels in the alveolar walls, are exposed to alveolar pressure which is close to atmospheric pressure. When alveolar pressure rises, increased transmural pressure may cause capillary collapse. Extra-alveolar vessels, including arteries and veins of lung parenchyma, are affected by lung volume via radial traction of elastic parenchyma, while very large hilar vessels are exposed to intrapleural

pressure.<sup>14</sup> Pulmonary vascular resistance is altered by lung volume, exercise, alveolar hypoxia, emboli, hypotension, vasoactive agents and autonomic nervous system effects.<sup>6, 8</sup> As pulmonary perfusion pressure decreases when the pulmonary circulation is obstructed, blood is diverted from the bronchial circulation through anastomoses into the pulmonary circulation. Proliferation of the bronchial circulation with subsequent pulmonary infiltration follows lung injury and has been demonstrated in the dorsal lung region of horses with EIPH.<sup>6</sup>

Efficient gas exchange occurs in lung regions where alveolar ventilation to perfusion ( $V_A/Q$ ) ratio equals 0.8 to 1.<sup>8</sup> Uniformity of regional  $V_A/Q$  has been demonstrated in healthy standing horses.<sup>41, 43</sup> Decreased  $V_A/Q$  is caused by increased shunting of blood from non-ventilated alveoli, while increased  $V_A/Q$  ratio correlates with increased alveolar dead space in poorly perfused regions. The balance between alveolar oxygen removal by pulmonary capillaries and replenishment by alveolar ventilation can be represented by the alveolar gas ( $p_AO_2$ ) equation (Figure 4).<sup>8, 14</sup>

Figure 4. Alveolar gas equation

$$p_AO_2 = (BP - P_{H_2O}) \times F_iO_2 - \frac{p_aCO_2}{R}$$

( $p_AO_2$ : Alveolar oxygen tension; BP: Barometric pressure;  $P_{H_2O}$ : partial pressure of water vapour at body temperature;  $F_iO_2$ : fraction inspired  $O_2$ : 0.2093; R: respiratory exchange ratio<sup>46</sup>)

The physiologic shunt fraction ( $Q_S/Q_T$ ) represents the proportion of pulmonary venous return not participating in gas exchange due to shunting of mixed venous blood and can be calculated using previously determined equations and coefficients for humans and horses (Figures 5 & 6).<sup>14, 44, 45</sup>

Figure 5. Physiologic shunt fraction

$$Q_s / Q_T = \frac{(Cc'O_2 - CaO_2)}{(Cc'O_2 - CvO_2)}$$

(Cc'O<sub>2</sub>: alveolar oxygen content (C<sub>A</sub>O<sub>2</sub>); CaO<sub>2</sub>: arterial blood oxygen content; CvO<sub>2</sub>: venous blood oxygen content)

Figure 6. Oxygen content equation

$$CxO_2 = 1.39 \times [Hb_x] \times \%Sat_x + 0.003 \times p_x O_2$$

(1 gram of hemoglobin carries 1.39ml oxygen when fully saturated; the solubility constant of oxygen in aqueous solution is 0.003 (ml O<sub>2</sub>/mmHg pO<sub>2</sub>/dl blood); [Hb]: hemoglobin concentration g/dl: 0.34 (mean corpuscular Hb concentration) x hematocrit; percentage oxygen saturation of hemoglobin is derived from the equine oxygen equilibrium curve; and pxO<sub>2</sub>: measured oxygen tension of the blood sample<sup>14, 44, 45</sup>)

Another measurement of pulmonary gas exchange is the (ideal) alveolar to arterial oxygen tension difference (p<sub>(A-a)</sub>O<sub>2</sub>), which is generally increased with decreased V<sub>A</sub>/Q ratio.<sup>14, 47, 48</sup> Using the alveolar gas equation at sea level, p<sub>A</sub>O<sub>2</sub> should equal 100 mmHg and normal p<sub>a</sub>O<sub>2</sub> should be greater than 90 mmHg, therefore normal p<sub>(A-a)</sub>O<sub>2</sub> is between 0 and 10 torr.<sup>6</sup> Significant elevations in p<sub>(A-a)</sub>O<sub>2</sub> have been demonstrated in healthy geriatric horses compared to young horses, in association with decreased p<sub>a</sub>O<sub>2</sub> values.<sup>48</sup> In humans, the p<sub>(A-a)</sub>O<sub>2</sub> was demonstrated to be an unreliable index of gas exchange in mechanically ventilated patients with true intrapulmonary shunting, increasing progressively with increasing F<sub>i</sub>O<sub>2</sub> despite minimal variation in the physiologic shunt fraction.<sup>49</sup> As F<sub>i</sub>O<sub>2</sub> was increased sequentially, 55% of the changes in p<sub>(A-a)</sub>O<sub>2</sub> were opposite in direction to the corresponding changes in Q<sub>S</sub>/Q<sub>T</sub>.<sup>49</sup>

## 1) Chronic Obstructive Pulmonary Disease

### Etiology

Equine COPD has a multifactorial etiology, including genetic susceptibility and hypersensitivity to dusts rich in organic material.<sup>50</sup> Acute airway obstruction can be reliably induced in COPD-susceptible, but not control, horses by stabling and feeding poor quality hay containing dusts and molds.<sup>51, 52</sup> Clinical remission from airway inflammation and obstruction occurs with pasture turnout, low dust environment and prevention of access to moldy hay,<sup>52-54</sup> indicating that the pathophysiologic mechanisms occurring in COPD are reversible.<sup>55</sup> Spores of molds and actinomycetes are abundant in poorly cured herbage and can be inhaled deep into the respiratory system.

*Micropolyspora faeni* aerosol challenge caused neutrophilic inflammation in bronchoalveolar lavage fluid (BALF) of previously sensitized and control horses, while increasing minute ventilation and respiratory frequency only in previously sensitized ponies. Concurrent hyper-responsiveness to aerosol histamine was not observed.<sup>56</sup> Natural challenge of COPD horses with moldy hay and inhaled extracts of *Micropolyspora faeni* and *Aspergillus fumigatus* increased respiratory frequency and minute ventilation and decreased arterial oxygen tension, while controls were unaffected. Inhaled *Thermoactinomyces vulgaris* induced disease in both COPD horses and controls.<sup>51, 57</sup> Results indicate that COPD is a pulmonary hypersensitivity to specific antigens, but that duration of exposure or antigen combination may contribute to increased severity of the naturally occurring disease.<sup>50, 51</sup> Horses with COPD or urticaria had greater total percentage of allergen extract reactions after intradermal testing than clinically normal horses. Significant differences between normal and COPD horses was evident for only 3% of extract reactions.<sup>58</sup>

Clinical signs include coughing, tachypnea, dyspnea, mucopurulent nasal discharge, exercise intolerance and biphasic accentuated expiratory effort.<sup>59</sup> Thoracic auscultation may reveal wheezes and expiratory crackles. Horses are usually afebrile. Weight loss, cachexia and hypertrophy of the external abdominal oblique muscles may occur in severe cases.<sup>55, 59</sup> Clinical classification of COPD separates affected horses into stages one to five. Stage 1 includes horses with a history of COPD, but no clinical signs; Stage 2 horses

have an occasional cough and seasonality of clinical signs; Stage 3 horses exhibit coughing and exercise intolerance with some seasonal improvement of clinical signs; Stage 4 horses have persistent respiratory compromise and effort visible at rest; Stage 5 horses have severe respiratory compromise, tachycardia and poor body condition.

### **Ultrastructural changes and histology**

Descriptions of lung pathology in COPD include bronchiolitis characterized by peribronchial mononuclear inflammation and intraluminal neutrophils, gas trapping in obstructed airways, loss of ciliated epithelium, granulation of clara cells, goblet cell metaplasia, mucus accumulation, alveolar fibrosis, Type I pneumocyte necrosis with replacement by Type II alveolar epithelium and increased pores of Kohn.<sup>50</sup>

Pathology in large conducting airways correlated with the degree of clinical severity in only 50% horses with COPD.<sup>11</sup> Hyperplasia and desquamation of the bronchial epithelium, intraepithelial mucosal cysts and dilation of intercellular clefts filled with mast cells at various stages of degranulation occur, without changes in tight junctions or subepithelial basement membranes.<sup>11</sup> Loss of ciliated cells from the segmental bronchi distally and predominance of undifferentiated cells with apical microvilli indicate increased mucosal turnover rate and impaired mucociliary clearance mechanisms.<sup>11</sup> Goblet cell hyperplasia is primarily responsible for excess intraluminal secretions, as peribronchial glands in the horse contribute less to mucus production.<sup>10, 12</sup> Changes in mucus quality in respiratory disease include increased viscosity and reduced elasticity, due to leukocyte and epithelial cell destruction with release of DNA, increase in glycoprotein content, purulent secretions and leakage of serum proteins.<sup>10</sup> Ciliary changes include compound cilia, irregularly arranged, swollen cilia, extra tubules, absence of microtubules and lack of ciliary orientation.<sup>11</sup>

There was no significant difference between tracheal clearance rates of normal horses and those with COPD before and after exercise, as determined scintigraphically after an injection of technetium-99m sulphide colloid into the tracheal lumen.<sup>7</sup> The alveolar-capillary membrane and intercellular tight junctions control passive diffusion of fluid and

solutes, allow gradients created by active transcellular mechanisms and maintain fluid free alveoli and efficient gas exchange.<sup>60</sup> Alveolar epithelium membrane permeability can be assessed via scintigraphical clearance of nebulized 99m-Techneium from the lung into the circulation.<sup>60</sup> COPD horses demonstrated a significantly higher alveolar clearance rate of 99m-Techneium (4.17%/min) than normal horses (1.8%/min).<sup>61</sup> BAL results suggested that increased alveolar epithelium membrane permeability in COPD horses could result from lung inflammatory responses.<sup>60</sup> COPD horses had significantly faster alveolar clearance rates during crisis than remission at pasture, but an intermediate value was found when stabled in a controlled environment.<sup>61</sup>

In horses suffering from COPD, there was good correlation between clinical severity and morphological changes in terminal airways and alveoli, indicating that the smaller airways are the primary site of COPD pathology.<sup>12</sup> Clara cells appear to be targeted by inflammatory mediators, undergoing morphologic changes and replacement by metaplastic goblet and vacuolated cells.<sup>12</sup> Alveolar light microscopy revealed emphysema, atelectasis, fibrosis, inflammatory cell infiltrate and dilatation of alveolar septa with edema, collagen, elastic fibres and connective tissue cells.<sup>12</sup> Electron microscopy revealed necrosis of Type I alveolar epithelium replaced by cuboidal Type II alveolar epithelium lining fibrotic septa, increased pores of Kohn's and lamellated structures resembling tubular myelin. Type II alveolar cells showed crater-like secretory orifices, fatty change, necrosis and increased lamellar bodies. Abnormal Type II alveolar cells, lamellated structures and peribronchial cysts are morphological evidence of functional impairment of the surfactant system.<sup>12</sup>

### **Pulmonary function and gas exchange**

Compared to controls, horses with clinical COPD have increased pulmonary resistance ( $R_L$ ), respiratory frequency ( $f$ ), maximum transpulmonary pressure difference ( $\Delta P_{pL_{max}}$ ), work of breathing ( $W_b$ ), minute ventilation, maximum inspiratory and expiratory flow rates and expiratory to inspiratory time ratio ( $E_T:I_T$ ), with decreased dynamic compliance ( $C_{dyn}$ ) and arterial oxygen tension ( $paO_2$ )<sup>52, 55, 62, 63</sup>. Tidal volume ( $V_T$ ) is normal<sup>55, 62, 63</sup> or decreased<sup>52</sup> in COPD-affected horses. Administration of bronchodilators decreases  $R_L$

in COPD horses due to smooth muscle relaxation,<sup>18, 64, 65</sup> but an inconsistent effect on  $C_{dyn}$  suggests persisting peripheral airway obstruction or interstitial disease. Time to remission after environmental change positively correlates with age, duration of clinical signs,  $\Delta P_{pl_{max}}$  and  $W_b$ , and negatively correlates with  $C_{dyn}$ .<sup>55</sup>

Closure of inflamed airways and alveolar gas trapping towards end exhalation can increase functional residual capacity (FRC) and decrease  $C_{dyn}$  in COPD-affected horses.<sup>50, 66</sup> Minute ventilation increases due to increased respiratory rate induced by hypoxemia and vagal mechanisms.<sup>47, 66, 67</sup> To maintain tidal volume despite increased end-inspiratory volume and respiratory frequency, increased pulmonary elastic recoil creates high peak expiratory flow rates. Flow rates are highest when lung volume is greatest and airway lumens have the greatest diameter. Low flow and increased  $R_L$  at end expiration reflects dynamic airway compression during forced abdominal effort.<sup>50, 66</sup> Frequency dependence of  $C_{dyn}$  indicates peripheral airway obstruction as it reflects inequality of time constants in peripheral parallel time units.<sup>67</sup>

Traditional pulmonary function testing is insensitive in detecting subclinical airway disease during remission.<sup>61</sup> COPD-affected horses had airway hyper-reactivity to methacholine and significantly faster scintigraphical alveolar clearance rates of 99m-technetium than during remission at pasture, but intermediate values were found when stabled in a controlled environment. However, clinical examination and pulmonary function testing (ie. RR,  $R_L$ ,  $C_{dyn}$ ,  $\Delta P_{pl_{max}}$ ,  $V_T$ ,  $paO_2$  and  $paCO_2$ ) could not differentiate between remission at pasture or in a controlled stabled environment.<sup>61</sup>

Hypoxemia in horses with COPD results from increased venous admixture, ventilation to perfusion ( $V_A/Q$ ) mismatch and diffusion impairment due to airway obstruction, shunting of blood through non-ventilated alveoli, thickening and fibrosis of the alveolocapillary membrane, and alveolar destruction in severe cases. Multiple inert gas elimination studies in COPD-affected horses revealed increased scatter of  $V_A/Q$  ratios and magnitude of  $V_A/Q$  mismatch, correlating with severity of disease.<sup>47</sup> Mean  $V_A/Q$  ratios were higher than normal horses, with significantly increased dead space ventilation. Increased minute

ventilation causes acinar hyperinflation, collateral ventilation through increased pores of Kohn and compression of the pulmonary vascular bed, further increasing  $V_A/Q$  ratio.<sup>12, 47, 50</sup> Hypoxic pulmonary arteriole vasoconstriction, inflammatory mediator induced vasospasm and capillary destruction due to emphysema can cause pulmonary arterial hypertension and contribute to  $V_A/Q$  mismatching in horses with severe COPD.<sup>50</sup> Carbon dioxide retention and increased  $paCO_2$  is not a common feature of COPD,<sup>18, 47, 52, 55, 68</sup> but has been reported in COPD affected horses.<sup>63, 69, 70</sup> Hypercapnia can occur with hypoventilation,  $V_A/Q$  mismatching or severe diffusion impairment.<sup>14, 44, 71</sup> The most common cause of increased  $paCO_2$  in humans with chronic lung disease is  $V_A/Q$  mismatch and relative alveolar hypoventilation.<sup>72</sup> Scintigraphical lung images of COPD-affected horses revealed increased heterogeneity of  $V_A/Q$  ratios and decreased ratio of inhalation to perfusion after administration of inhaled and intravenous 99m-Technetium.<sup>73</sup>

In COPD-affected horses, alveolar-arterial oxygen tension difference ( $p_{(A-a)}O_2$ ) and physiologic shunt fraction ( $Q_S/Q_T$ ) are increased.<sup>47, 73, 74</sup> The  $p_{(A-a)}O_2$  is a measure of direct anatomical and physiologic shunts due to areas of low  $V_A/Q$  ratios or impairment of alveolar gas diffusion,<sup>14, 74</sup> but may vary from 4 to 25mmHg in healthy horses.<sup>48, 75</sup> The physiologic shunt fraction ( $Q_S/Q_T$ ) is a convenient index of the relative amount of right to left shunting of mixed venous blood through non-ventilated alveoli and lung units with decreased  $V_A/Q$  ratios.<sup>44, 47, 49, 74</sup> However, increased  $Q_S/Q_T$  has been reported with increased  $V_A/Q$  ratios in horses with COPD.<sup>47</sup> Unlike  $p_{(A-a)}O_2$ , calculation of  $Q_S/Q_T$  (Figures 5 & 6) considers the effects of nonlinear oxyhemoglobin dissociation.<sup>44, 49</sup> Normal  $Q_S/Q_T$  values in horses are variable (mean +/- s.d., 0.7 % +/- 0.31, 8.8% +/- 3.9) and increases are observed in horses with mild to moderate signs of lower respiratory tract disease or COPD, and in normal horses after aerosolized albuterol.<sup>44, 74, 76</sup>

Alveolar dead space fraction ( $V_D/V_T$ ) represents the amount of ventilation to lung units with abnormally high  $V_A/Q$  ratios. This fraction of alveolar gas in unperfused alveoli cannot participate in  $CO_2$  elimination, so has a decreased  $pCO_2$  more similar to that of inspired air.<sup>14, 72</sup>  $V_D/V_T$  increases as a result of increased arterial  $CO_2$  tension and

decreased end-tidal CO<sub>2</sub>.<sup>14, 44, 69, 71</sup> Decreased end-tidal CO<sub>2</sub> may be caused by increased V<sub>A</sub>/Q ratio, rapid flow rates or asynchronous time constants, but values reported in COPD-affected horses are not different from normal horses.<sup>69</sup> In normal horses, reported ETCO<sub>2</sub> (mean +/- s.d., 43.7 +/- 2.99 mmHg) was not significantly different from paCO<sub>2</sub> (mean +/- s.d., 44.9 +/- 2.47 mmHg).<sup>77</sup> Increased V<sub>D</sub>/V<sub>T</sub> has been reported in horses with COPD (mean 19.98%) in association with hypercapnia, and in horses with moderate lower airway disease (median -5.09%, range -17.5 to 57.04) in association with normal paCO<sub>2</sub>.<sup>44, 69</sup> A wide range of reference values have been reported in normal horses (means +/- s.d., 0.49% +/- 0.05, -18.2% +/- 3.1 and 6.1%).<sup>44, 46, 69</sup>

### **Inflammation**

During remission of COPD-susceptible horses, bronchoalveolar lavage fluid (BALF) cell populations do not differ from control horses, consisting primarily of macrophages and lymphocytes.<sup>51, 78</sup> Following natural challenge, neutrophils accumulate in the lung between 3 and 5 hours, and predominate in BALF within 5 hours.<sup>51, 78</sup> During acute exacerbations of COPD, inflammation is prominent while obstruction and altered gas exchange are characteristic clinically, yet correlation between severe dyspnea and inflammation is inconsistent.<sup>50, 79</sup> The immediate airway response, after exposure of COPD-susceptible horses to etiological antigens, is bronchospasm. This may represent a Type I hypersensitivity response involving IgE and histamine.<sup>50</sup> Airway hyperresponsiveness to histamine, methacholine and citric acid, characterized by excessive airway narrowing and decreased ED<sub>65</sub>C<sub>dyn</sub> (dose of histamine to reduce C<sub>dyn</sub> to 65% of baseline), occurs during acute heaves and wanes during remission.<sup>52, 80</sup> Delayed airway responses at 3 to 5 hours include neutrophilic infiltration, bronchospasm and mucus secretion.<sup>50, 51</sup>

In COPD-susceptible ponies, BALF neutrophil numbers were increased during acute airway obstruction (mean 184/ul), but total BALF cell count was not significantly increased.<sup>78</sup> There was concomitant and variable decrease in BALF macrophage and lymphocyte counts of ponies with acute COPD. No changes were observed in control ponies. After 1 week recovery of COPD-affected ponies on pasture, BALF inflammation

was detected by increased mean IgG/albumin ratio and 50% of ponies had BAL eosinophilia.<sup>78</sup> Airway hyperreactivity has previously been shown to disappear by 1 week post recovery on pasture.<sup>52</sup> Aerosol *Micropolyspora faeni* challenge in experimentally sensitized ponies caused increased total nucleated cell count and neutrophilia in BALF with decreased large mononuclear (macrophage) cell numbers, without increasing airway responsiveness to aerosol histamine.<sup>56</sup> These results support the hypothesis that airway inflammation may be present without causing airway hyperresponsiveness.<sup>56</sup>

Investigation of the mechanisms leading to infiltration of neutrophils into the horse lung revealed that strong chemotactic activity in BALF is associated with high levels of dust exposure.<sup>81</sup> In addition, in vitro stimulated alveolar macrophages have impaired phagocytosis efficiency and secrete 2 chemo-attractants specific for neutrophils, interleukin-8 and macrophage inflammatory protein-2, associated with chemotactic activity in the cell free culture supernatant. Excessive stimulation of lung macrophages in susceptible horses exposed to high levels of dust, may cause a functional change from phagocytic to immunomodulatory macrophage activity, with subsequent secretion of cytokines chemotactic for neutrophils.<sup>81</sup>

In acute exacerbation of COPD, airway mucosal prostaglandin (PG)E<sub>2</sub> production is reduced while tracheal epithelial 15-hydroxyeicosatetraenoic acid (15-HETE) production and plasma thromboxane (TXA<sub>2</sub>) are increased.<sup>53, 82</sup> COPD-affected (principal) ponies demonstrated hypoxemia, tachypnea, decreased C<sub>dyn</sub>, increased R<sub>L</sub>, and increased airway reactivity to aerosol histamine in comparison to controls. Plasma and BAL concentrations of TXA<sub>2</sub>, PGI<sub>2</sub> and PGD<sub>2</sub> were measured, but only a significant increase in plasma TXB<sub>2</sub> was detected in principal ponies. Cyclooxygenase inhibition with flunixin meglumine prevented the increase in TXB<sub>2</sub>, but did not affect the alterations in pulmonary function and airway reactivity. These results suggest that TXA<sub>2</sub> and cyclooxygenase products of arachidonic acid metabolism are not responsible for airway hyperreactivity and alterations in pulmonary function in COPD-affected ponies.<sup>53</sup> Increased airway epithelial production of 15-HETE in COPD-affected horses may increase mucus secretion and smooth muscle contraction, but can also inhibit 5-lipoxygenase, decrease LT production

and block LTB<sub>4</sub>-mediated neutrophil chemotaxis.<sup>50, 82</sup> Equine tracheal epithelium is not a significant source of 15-HETE, but does produce PGE<sub>2</sub>, an epithelial derived relaxing factor.<sup>82</sup> Endogenous airway mucosal PGE<sub>2</sub> production is reduced in COPD-affected horses.<sup>82</sup> Tracheal epithelial strips from affected horses tended to produce less PGE<sub>2</sub> after stimulation with histamine, bradykinin and A23187 than did strips from control horses, and there was a significant correlation between epithelial PGE<sub>2</sub> production and the time taken for affected animals to develop airway obstruction. These results suggest that a relative decrease in the endogenous bronchorelaxant substance, PGE<sub>2</sub>, may be a factor in the pathogenesis of COPD.<sup>82</sup>

Furosemide reduced  $\Delta PpL_{max}$  and R<sub>L</sub>, and increased C<sub>dyn</sub> in COPD-affected horses for 5 hours. Cyclooxygenase inhibition abolished the effect of furosemide on airway calibre, but did not prevent diuresis, indicating that the reversal of airway obstruction may be mediated by endogenous prostanoids.<sup>83</sup> Cyclooxygenase inhibitor, indomethacin, exerts excitatory effects on equine airway smooth muscle that are almost fully reversible with exogenous PGE<sub>2</sub>. Possible mechanisms are increased cholinergic neurotransmission or direct effects on smooth muscle via decreased cyclooxygenase products and increased lipoxygenase metabolism.<sup>84</sup> Exogenous PGE<sub>2</sub> inhibits the contractile response of airway smooth muscle to EFS,<sup>27</sup> and cyclooxygenase inhibition can potentiate EFS-induced contractile response of small peripheral airways.<sup>21</sup> However EFS-induced ACH release was not affected by cyclooxygenase inhibition throughout the tracheobronchial tree, but was potentiated by epithelium removal, suggesting direct effects of PGE<sub>2</sub> on smooth muscle or an alternative epithelial derived relaxing factor (EDRF) that is decreased in COPD.<sup>29</sup>

Inhaled LTD<sub>4</sub> caused a dose-dependent increase in  $\Delta PpL_{max}$  in normal horses within 3-5 minutes due to bronchoconstriction. LTB<sub>4</sub> induced early recruitment of radiolabelled neutrophils to the lungs in normal horses, persisting for more than 5 hours.<sup>85</sup> Antigen challenge of COPD-affected horses and subsequent development of clinical signs and abnormal pulmonary function was not affected by pre-treatment with fenleuton, a 5-lipoxygenase inhibitor. However, in horses with marked increases in  $\Delta PpL_{max}$  (>20 cm

H2O), fenleuton did attenuate this response by 63-64%, indicating that leukotrienes are involved in the pathogenesis of obstructive pulmonary disease in severely affected horses.<sup>86</sup>

The most predominant inflammatory response in COPD-affected horses occurs in peripheral airways.<sup>21</sup> In peripheral small airways of COPD-affected horses, EFS-induced cholinergic contractions are increased by cyclooxygenase blockade and histamine, implicating involvement of increased histamine release and altered prostanoid profile in peripheral bronchospasm. Zymosan-activated neutrophils did not significantly effect EFS (cholinergic) responses of small airways in COPD or control horses.<sup>21</sup> Histamine, serotonin (5-hydroxytryptamine, 5-HT) and LTD<sub>4</sub> had a synergistic effect on cholinergic EFS responses in equine small airways.<sup>87</sup> Mast cell derived mediators, histamine, LTD<sub>4</sub> and 5-HT, usually associated with a Type I allergic reaction, probably contribute to increased cholinergic small airway tone in COPD.<sup>87</sup>

The role of mast cell degranulation and histamine in COPD and airway hyperresponsiveness has been further investigated.<sup>88</sup> During remission, COPD-susceptible horses were significantly more reactive to histamine exposure than control horses. After environmental challenge, PC<sub>35</sub>C<sub>dyn</sub> decreased fourfold in principals, significantly different from controls where no alteration was observed.<sup>88</sup> Airway reactivity that persists during remission has been associated with underlying inflammation, demonstrated by higher numbers of nucleated cells, macrophages and neutrophils in BALF of principals than controls.<sup>61, 88, 89</sup> Environmental challenge caused a significant increase in neutrophils and a decrease in macrophages and mast cells in BAL fluid of principal horses.<sup>88</sup> Percentage release of histamine from pulmonary mast cells was significantly increased in response to *A.fumigatus*, *Alternaria tenuis*, *M.faeni* and calcium ionophore A23187. This increase was significantly higher for principals in response to *A.fumigatus* during remission and exacerbation, and *M.faeni* during exacerbation of COPD. Results suggest that Type I hypersensitivity and increased histamine release from mast cells in COPD-susceptible horses may contribute to airway hyperresponsiveness after antigenic exposure.<sup>88</sup>

The significance of pulmonary inflammation in the pathogenesis of COPD is further emphasized by the finding that pulmonary function after atropine administration in COPD affected horses, did not improve to that achieved after maintenance on pasture.<sup>62</sup> Atropine relieves vagus-mediated airway obstruction and secretion, whereas maintenance at pasture prevents exposure to allergens responsible for inflammation of small airways and alveoli.<sup>62</sup>

## **Bronchospasm**

### *Excitatory activity and Airway hyperresponsiveness*

Contraction of airway smooth muscle is a major cause of airway obstruction in horses with COPD and may be caused by direct stimulation of smooth muscle by inflammatory mediators, autonomic nervous system effects and nonspecific airway hyperresponsiveness.<sup>18, 52, 64, 65, 80</sup> Muscarinic receptor antagonist atropine reduced  $R_L$  and attenuated the rise in  $R_L$  in response to 0.1mg/ml histamine in COPD-affected ponies, but had no effect on  $C_{dyn}$  or  $ED_{65}C_{dyn}$ , indicating a major role for muscarinic receptors in modulating larger airway calibre. Studies have demonstrated a small decrease in  $R_L$  at FRC following vagal cooling in normal ponies.<sup>18</sup> Bronchoconstriction alleviated by vagal blockade may be induced by a vagal reflex originating in pulmonary sensory receptors, a centrally mediated increase in efferent vagal activity, facilitation of reflexes at the ganglia or postganglionic fibres, or increased receptor response on smooth muscle.<sup>50, 90</sup>

In COPD-affected horses, responses of isolated tracheal strips, third generation bronchi and distal airway to ACH are decreased and in vitro ACH release from airway parasympathetic nerves is not elevated.<sup>16, 17, 22</sup> Contractile responses to EFS are increased in tracheal smooth muscle strips and third generation bronchi,<sup>17</sup> but decreased in distal airway segments in horses with COPD.<sup>16</sup> These results suggest hyporesponsiveness to ACH in diseased airways and provide no evidence for up-regulation of muscarinic receptors, increased sensitivity of airway smooth muscle to vagal influences or decreased ACH breakdown. Enhanced contractile response to EFS

could result from lack of inhibitory innervation, increased excitatory innervation or a lack of presynaptic inhibition by mediators such as PGE<sub>2</sub> in diseased equine airways.<sup>17</sup>

Tachypnea and increased R<sub>L</sub> induced by ovalbumin aerosol challenge to sensitized ponies, were reversed by vagal blockade.<sup>91</sup> Tachypnea induced by unilateral ovalbumin challenge was reversed by bilateral vagal section and was most likely caused by increased activity of pulmonary receptors with vagal afferent fibres.<sup>91</sup> Inflammation affects various components of the reflex arc so that an augmented response to activation of airway sensory receptors by inflammatory mediators could be important in the pathogenesis of COPD.<sup>50</sup>

Furosemide (1mg/kg) administered by aerosol or intravenously significantly decreased pulmonary R<sub>L</sub> and increased C<sub>dyn</sub> in horses with acute airway obstruction, but had no effect on control horses or principals in clinical remission.<sup>92</sup> A major component of airway obstruction is vagally mediated,<sup>18</sup> but other factors such as inflammation, mucus plugging and airway wall edema are involved. Furosemide causes a greater decrease in R<sub>L</sub> than atropine, but its effect is inhibited by cyclooxygenase blockade.<sup>92</sup> Furosemide may stimulate PGE<sub>2</sub> release from airway epithelium causing smooth muscle relaxation, dampen vagal reflexes by altering the ionic and osmotic environment of sensory epithelial receptors, and decrease airway edema, fluid or mucus.<sup>92</sup>

Bronchial hyperreactivity can be defined as increased sensitivity of bronchi and an abnormal response of bronchial smooth muscle to non-specific stimuli. COPD-susceptible horses exhibit airway hyperresponsiveness, demonstrated by histamine challenge, during acute exacerbations of airway obstruction induced by stabling and exposure to hay dust.<sup>93</sup> In COPD-affected ponies housed in stables, the ED<sub>65</sub>C<sub>dyn</sub> (dose of histamine to reduce C<sub>dyn</sub> to 65% of baseline) decreased by 2.5log doses of histamine, as compared to principals in remission at pasture and controls. Lack of correlation between ED<sub>65</sub>C<sub>dyn</sub> and R<sub>L</sub> suggests that changes in airway calibre are unlikely to be solely responsible for hyperreactivity.<sup>52</sup> Airway hyperresponsiveness cannot be explained by an exaggerated smooth muscle response to cholinergic stimulation, as hyperreactivity to

histamine is not ameliorated by atropine.<sup>18</sup> In addition, hyperresponsiveness to aerosol histamine in COPD-affected ponies was not altered by  $\beta$ -adrenergic blockade<sup>35</sup> or cyclooxygenase inhibition.<sup>53</sup> Bronchial hyperresponsiveness is most likely caused by changes in local airway structure or environment and is exaggerated by mucosal thickening.<sup>50</sup>

Airway hyperresponsiveness to histamine or methacholine (MCH), a muscarinic receptor agonist, was not present in COPD horses in remission at pasture or in stabled healthy horses.<sup>80, 93</sup> However, intermediate bronchial hyperreactivity to MCH has been demonstrated in COPD-susceptible horses maintained in clinical remission in a controlled barn environment, fed grass silage and bedded on wood shavings.<sup>89</sup> After 6 weeks in a controlled environment,  $PC_{35}C_{dyn}$  (dose of MCH required to decrease  $C_{dyn}$  by 35% from its basal value) was significantly lower than horses in remission at pasture, but significantly higher than during obstructive crisis.<sup>89</sup> Airway response to histamine is a composite of direct receptor-mediated actions with smooth muscle and stimulation of central and local neural reflexes.<sup>89</sup> Histamine-induced augmentation of EFS (cholinergic) responses in equine small airways, suggests that increased histamine release contributes to peripheral bronchospasm in COPD.<sup>21</sup> Direct contractile effects and augmentation of endogenous cholinergic responses by histamine are both mediated via H1 receptors, whereas inhibitory H3 receptors partially oppose the direct contractile effect.<sup>87</sup>

Inhibitory muscarinic autoreceptors on cholinergic nerves of large airways are functionally normal in COPD-affected horses.<sup>22</sup> Gallamine, a selective M2 muscarinic receptor antagonist, does not potentiate EFS-induced contraction in horses, suggesting there are no functional inhibitory M2 autoreceptors on horse airway cholinergic nerve terminals.<sup>19</sup> The SS- distomer of the  $\beta$ 2 adrenoceptor agonist formoterol does not inhibit smooth muscle contraction, but facilitates ACH release from airways of COPD-affected horses in the absence of atropine, similar to that in control horses in the presence of atropine. This could be caused by dysfunctional prejunctional muscarinic autoreceptors in horses with COPD.<sup>94</sup> RR- and RR/SS- formoterol, enantiomers which inhibit smooth muscle contraction, increased ACH release from tracheal parasympathetic nerves of

COPD-affected horses to a similar extent as that in control horses, indicating that neuronal  $\beta_2$  excitatory adrenoceptor function is not up-regulated in horses with COPD.<sup>94</sup>

The role of eNANC has not been investigated in COPD<sup>50</sup>. However, distribution of eNANC innervation and substance P binding sites in horses,<sup>39, 40, 50</sup> suggest that activation of afferent nerves containing substance P may lead to increased mucus secretion and decreased airway diameter due to vascular congestion.<sup>40</sup> Sensory neuropeptides such as Substance P and neurokinin A, released from non-myelinated sensory C fibers, are not inactivated when epithelial cells are damaged. These neuropeptides can cause smooth muscle contraction, increased vascular permeability and an influx of inflammatory cells.<sup>15</sup>

### ***Inhibitory activity***

Adrenergic regulation of airway smooth muscle may be altered in airway disease.<sup>24</sup> Phenylephrine, an  $\alpha_1$  adrenoceptor agonist, increases pulmonary resistance in COPD-affected horses, but not controls, indicating that airway  $\alpha_1$  adrenergic receptors may be upregulated in disease.<sup>33</sup> Pretreatment of COPD-affected ponies with atropine and propranolol, to decrease cholinergic and  $\beta$  adrenergic receptor influences, followed by treatment with aerosol phenylephrine resulted in decreased  $C_{dyn}$  and increased  $R_L$  during remission and exacerbation of airway obstruction.<sup>33</sup> Subsequent aerosol of prazosin, an  $\alpha_1$  receptor antagonist, did not result in bronchodilation. These results indicate an increase in the number or activity of alpha-1 receptors in horses with COPD, but that the role of these receptors in bronchospasm is minimal.<sup>33</sup>

Xylazine HCl, an  $\alpha_2$  adrenoceptor agonist, administered intravenously (0.5 mg/kg) to COPD-affected horses significantly decreased  $R_L$  and increased  $C_{dyn}$  within 2 minutes, without affecting  $paO_2$  or  $paCO_2$ . Xylazine may activate inhibitory prejunctional  $\alpha_2$  adrenoceptors located on cholinergic neurons, directly relax smooth muscle, inhibit release of inflammatory mediators by leukocytes or inhibit release of neuropeptides by afferent C fibers.<sup>68</sup> Dysfunction of prejunctional inhibitory alpha-2 adrenoceptors or autoinhibitory muscarinic receptors may result in exaggerated release of ACH and

contribute to bronchospasm and airway hyperresponsiveness in horses with COPD. Clonidine, an  $\alpha_2$  adrenoceptor agonist, failed to inhibit EFS-induced contraction of isolated trachealis muscle in COPD-affected horses as compared to controls. EFS-induced ACH release from equine tracheal cholinergic nerves was not different in COPD-affected horses, but inhibition of ACH release by clonidine was reduced in the trachea and absent in bronchi, as compared to normal horses.<sup>22</sup> These results suggest that inhibitory prejunctional  $\alpha_2$  adrenoceptors are dysfunctional in the trachea and bronchi of COPD-affected horses.<sup>22</sup> Physiological  $\alpha$  adrenoceptor agonists, epinephrine and norepinephrine, caused less inhibition of ACH release in COPD-affected horses than controls. This indicates dysfunction of prejunctional  $\alpha_2$  adrenoceptors in response to circulating catecholamines in COPD.<sup>94</sup>

A large proportion of airway obstruction in COPD is reversible by administration of atropine,<sup>18</sup> but  $\beta$  adrenergic receptor blockade causes further obstruction.<sup>35</sup> Propranolol, a  $\beta_1$  and  $\beta_2$  adrenoceptor antagonist, significantly increased  $R_L$  in COPD-affected horses, without affecting  $C_{dyn}$  or airway responsiveness to histamine, suggesting that  $\beta$  adrenergic activation modulates central airway caliber in COPD.<sup>35</sup> Atropine prevents propranolol-induced bronchoconstriction in COPD-affected ponies, indicating that increased  $R_L$  with  $\beta$  adrenoceptor blockade may result from unopposed cholinergic activity.<sup>33</sup> Aerosol  $\beta$  antagonists (propranolol, atenolol  $\beta_1$ , butoxamine  $\beta_2$ ) decreased  $C_{dyn}$  and increased  $R_L$  in COPD-affected ponies without affecting clinically normal ponies, indicating activation of  $\beta$  adrenoceptors in COPD.<sup>34</sup> Atropine reversed the effect of atenolol, indicating that  $\beta_1$  adrenoceptor activation inhibits parasympathetic tone. Changes in  $C_{dyn}$  in COPD-affected horses were most significant with combined  $\beta_1$  and  $\beta_2$  blockade, but changes in  $R_L$  were greatest after  $\beta_2$  blockade.<sup>34</sup> These results suggest increased activity of  $\beta_2$  receptors in central airways in COPD.

Dose-response curves of histamine pre-contracted bronchial segments to isoproterenol, a  $\beta_1$  and  $\beta_2$  adrenoceptor agonist, indicated similar  $\beta$  adrenergic receptor function in COPD-affected horses and controls.<sup>16</sup> These observations do not support up-regulation of

$\beta$  adrenoceptor function in COPD. Inhibition of smooth muscle contraction produced by  $\beta$  agonists has been demonstrated to be identical in COPD-affected and control horses at all levels of the airways.<sup>16, 17</sup> However, EFS-induced relaxation of precontracted 3<sup>rd</sup> generation bronchi was absent in COPD-affected horses as compared to 21% relaxation in controls, suggesting lack of inhibitory innervation in diseased bronchi.<sup>17</sup> Aerosol isoproterenol-induced bronchodilation was not attenuated by atenolol, suggesting it is mediated primarily by  $\beta_2$  adrenergic receptors.<sup>34</sup> Removal of airway epithelium increases sensitivity of smooth muscle to spasmogens and decreases  $\beta$  agonist induced relaxation of airway smooth muscle in horses. Epithelium has a high density of  $\beta$  adrenoreceptors, making  $\beta$  agonists more effective when administered via aerosol.<sup>34</sup>

Consistent lack of iNANC function has been demonstrated in the larger bronchi of COPD-affected horses.<sup>17, 95</sup> Nitric oxide, the mediator of iNANC function in horses is rapidly inactivated by reactive oxygen species liberated during acute inflammation.<sup>50</sup> Loss of this smooth muscle inhibitory function could contribute to bronchoconstriction and airway hyperresponsiveness.

## **Management**

Control of equine chronic obstructive pulmonary disease entails minimizing exposure to etiological antigens, thermophilic moulds and actinomycetes in hay and straw, and environmental dust.<sup>54, 55, 96</sup> Airborne dust concentrations and level of aero-allergens were measured in the stall and breathing zone of horses housed in either an uncontrolled environment with straw bedding and hay diet, or in a controlled environment utilizing wood shaving bedding and a complete pelleted diet.<sup>97</sup> Air dust concentrations were significantly higher in the uncontrolled environment, and there was a marked increase in the level of airborne dust from the stall to the breathing zone of these horses, as compared to horses in a controlled environment. In addition, major aeroallergens, *Micropolyspora faeni*, *Aspergillus fumigatus* and mite allergens, were significantly higher in the uncontrolled environment.<sup>97</sup> There was no significant difference in pulmonary function and arterial blood gas analysis in COPD-affected horses maintained in clinical remission at pasture or in a stabled controlled environment with wood shavings or wheat straw

bedding and grass silage diet.<sup>54</sup> These horses were then fed hay in the same stabled environment and developed clinical signs of COPD and significant alterations in pulmonary function within 8 +/- 3 (mean +/- s.d.) days after exposure.<sup>54</sup> These results emphasize the necessity of environmental control for treatment and prevention of COPD.

## **Therapy**

Treatment of acute episodes of airway obstruction in affected horses includes parenteral or inhaled corticosteroids, to reduce the allergic response and airway inflammation, and bronchodilators.<sup>96, 98-100</sup> Bronchodilators relieve obstruction due to bronchospasm by relaxing airway smooth muscle, and may also improve mucociliary clearance.

Bronchodilator therapy is useful for acute respiratory distress and maintenance bronchodilation in COPD, but can only partially improve pulmonary airflow and gas exchange due to concurrent inflammation.<sup>98, 99</sup> Exudative bronchiolar inflammation, characterized by hyperresponsiveness to inhaled stimuli, mucus hypersecretion and airway wall thickening, can be suppressed by glucocorticoids.<sup>101, 102</sup> Corticosteroids also increase  $\beta$  adrenoceptor numbers and their coupling to adenylyl cyclase, causing increased cAMP-mediated response of airway smooth muscle to  $\beta$  agonists.<sup>103</sup> Tapering dosages of oral prednisone or parenteral dexamethasone, depot triamcinolone and twice daily inhaled beclomethasone dipropionate have all been used for treatment of horses with COPD.<sup>15, 104-106</sup>

Inhaled sodium cromoglycate is believed to stabilize sensitized membranes of pulmonary mast cells, preventing degranulation, and has been effective for prophylaxis in asymptomatic COPD-affected horses with inadequate environmental control.<sup>98, 107</sup> Protease inhibitors, acetylcysteine, pentamidine and diminazene, inhibit proteolytic activity in tracheal aspirates of COPD-affected horses *in vitro*, and may protect the lungs from proteolytic damage *in vivo*.<sup>108</sup>

Bronchodilators should be administered by inhalation to deliver high concentrations locally without significant systemic side effects.<sup>96</sup> Several devices have been developed for aerosol delivery to horses including the Aero-mask with attached delivery device and

metered-dose canister; a handheld, metered dose, aerosol delivery device that fits into the left nostril; and a dry-powder inhaler with face mask.<sup>15, 109-111</sup> Nebulization, or inhalation of liquid droplets, was the first method adapted for horses, but requires more cumbersome equipment, prolonged drug delivery time and possibly higher doses to account for losses of drug in the delivery tubing.<sup>65, 112, 113</sup> The three basic types of bronchodilator agents are  $\beta_2$  adrenoceptor agonists, anticholinergics and phosphodiesterase inhibitors.<sup>98, 99</sup> Methylxanthines, aminophylline and theophylline, are nonspecific phosphodiesterase inhibitors used in horses, but have a very narrow therapeutic margin and require higher therapeutic concentrations than normally achieved in vivo.<sup>15, 99</sup>

### ***Sympathomimetic bronchodilators***

Beta 2 adrenergic agonists available to humans and horses are divided into those with an intermediate duration of action (3-6hrs), including clenbuterol, albuterol, terbutaline, pirbuterol, fenoterol and salbutamol, and those that are longer acting (>12 hr), including salmeterol and formoterol.<sup>99, 114</sup> Combined  $\beta_1$  and  $\beta_2$  agonist, isoproterenol, has also been used for diagnosis and short term clinical improvement in COPD horses.<sup>99</sup>

Desensitization of  $\beta_2$  adrenergic receptors after high dose or repeated exposure to the agonist occurs via receptor phosphorylation and internalization below the cell surface, and decreased number of receptors due to decreased production of messenger RNA. Up-regulation of the receptor via increased production of mRNA, is stimulated by glucocorticoid and thyroid hormone.<sup>114</sup>

Beta 2 agonists are reported to have important actions in addition to bronchodilation.<sup>114</sup> Clenbuterol increased mucus secretion and had a positive effect on tracheal mucociliary transport rate, visualised by an Indian ink marker deposited in the tracheal mucus layer, in both healthy and COPD-affected horses.<sup>115</sup> Increased mucociliary clearance induced by  $\beta_2$  agonists may be due to increased blood flow and mucus production, enhanced vascular integrity, altered sol:gel ratio as a result of bronchodilation, direct cilio-excitation mediated via cAMP, active ion transport across ciliated epithelial cells with passive water transport via osmosis, and secretion from submucosal gland cells.<sup>114, 115</sup>

Aerosolized fenoterol significantly increased ciliary beat frequency (by five fold) in dogs over a forty five minute period.<sup>9</sup> Beta 2 agonists increase the movement of chloride ions and water into the bronchial lumen in animals and humans.<sup>114</sup> In vitro,  $\beta_2$  agonists inhibit the release of histamine, leukotrienes and prostaglandins from human lung mast cells and may inhibit release of mediators from basophils, eosinophils and alveolar macrophages.<sup>114</sup>

Clenbuterol, a partial  $\beta_2$  agonist, administered at 0.8 ug/kg orally twice daily, caused clinical improvement in 24% of horses affected with COPD.<sup>116</sup> Increasing the dosage of clenbuterol incrementally over the range of 1.6, 2.4, and 3.2 ug/kg produced clinical improvement in 75% of all horses treated, without a concomitant increase in severity or incidence of side effects. This incremental dosing regimen aims at development of selective tolerance to inhibit the side effects of sweating, muscle tremor and nervousness that were observed in < 7% of all horses in the study.<sup>116</sup> Clenbuterol hydrochloride (0.8 ug/kg IV) administered to anesthetized horses in lateral recumbency induced decreased  $paO_2$ , increased  $Q_S/Q_T$  caused by right to left shunting of pulmonary blood, and increased dead space ventilation (regions with high  $V_A/Q$  ratios).<sup>117</sup> Clenbuterol administration was also associated with increased cardiac index due to increased heart rate, and decreased total peripheral and pulmonary vascular resistance due to vasodilation of systemic and pulmonary vessels.<sup>117</sup> A transient decrease in  $paO_2$  (> 5mmHg) can occur in human asthma patients after  $\beta_2$  agonist administration, probably due to relaxation of compensatory vasoconstriction in areas of decreased ventilation, combined with increased pulmonary blood flow due to increased cardiac output.<sup>114</sup> Tachycardia, sweating, muscular tremor, hyperglycemia, hypokalemia and hypomagnesemia have been observed as adverse effects of  $\beta_2$  sympathomimetic drugs in humans and horses.<sup>100, 114,</sup>

116

Clenbuterol, terbutaline and salbutamol are equally potent in their ability to relax equine tracheal muscle strips precontracted with carbachol.<sup>118</sup> However, the affinity of clenbuterol for  $\beta$  adrenoceptors of equine tracheal muscle is much higher than the affinity of salbutamol and terbutaline, indicating a lower intrinsic efficacy of clenbuterol than the

other 2 drugs.<sup>118</sup> The concentration of clenbuterol required for 50% effect in dose/response studies ( $EC_{50}$  value) corresponded to plasma concentrations obtained after a recommended dosage of 0.8 ug/kg (1.6-2.7 nM) to horses. However, the level of response to clenbuterol, a partial  $\beta_2$  agonist, will vary with receptor density, level of functional antagonism and other interindividual differences.<sup>118</sup>

Nebulized isoprenaline ( $\beta_1$  and  $\beta_2$  agonist) and terbutaline at 0.02mg/kg decreased  $\Delta PpL_{max}$  in COPD-affected horses by 72 and 63% respectively. These bronchodilator drugs caused a decrease in respiratory rate and an initial decrease in  $paO_2$  followed by a significant increase in  $paO_2$  to above resting levels in COPD-affected horses.<sup>100</sup> Values of  $\Delta PpL_{max}$  and  $paO_2$  after treatment still remained significantly different from those of control horses indicating residual airway obstruction.<sup>100</sup> Isoprenaline increased heart rate in COPD and control horses, presumably due to  $\beta_1$  effects.<sup>100</sup> Administration of aerosolized fenoterol (1-2mg) via Aeromask and metered-dose inhaler to COPD-affected horses caused a significant improvement in  $\Delta PpL_{max}$ ,  $C_{dyn}$ ,  $R_L$ ,  $W_b$  and lung auscultation, without any observed side effects. This emphasizes the advantages of local delivery of the  $\beta_2$  agonists versus parenteral administration in avoiding adverse effects.<sup>109</sup>

Aerosolized albuterol (360 and 720ug) delivered to COPD-affected horses via a metered dose inhaler (MDI) with ozone-friendly hydrofluoroalkaline-based propellant and delivery device inserted into the left nostril, significantly decreased  $\Delta PpL_{max}$  at 5, 10 and 30 minutes and 1 hour after administration as compared to the vehicle placebo.<sup>112</sup> Pulmonary  $R_L$  was decreased at 5 and 10 minutes after administration of aerosolized albuterol (120, 360 and 720 ug), but effects on  $C_{dyn}$  were variable, suggesting that exudate and bronchospasm occluding small airways may prevent peripheral drug deposition in COPD-affected horses.<sup>112</sup> Bronchodilation using aerosolized albuterol has a rapid onset, lasts 30 minutes to 1 hour, is optimally achieved at a dosage of 360ug and does not cause clinically relevant adverse effects.<sup>112</sup>

Aerosolized therapeutic agents (0.5-2um) are suspended in the airflow stream in large airways and deposited by sedimentation in small airways and alveoli.<sup>119</sup> Pulmonary

scintigraphic images of horses with COPD indicate patchy distribution of aerosolized technetium (Tc) 99m pentetate in peripheral lung fields and particle impaction in central airways due to airway turbulence. Bronchoconstriction and small airway inflammation, including mucus plugging, mural edema and cellular infiltration, prevent uniform distribution of aerosolized drugs to peripheral airways. Aerosolized albuterol sulfate (360ug) administered with the equine-adapted MDI improved pulmonary distribution of aerosolized radiolabelled pentetate suspension and decreased  $\Delta P_{pL_{max}}$  in COPD-affected but not normal horses.<sup>119</sup> Failure of scintigraphic images and pulmonary function to return to control values indicate persistent small airway obstruction and inflammation, emphasizing the importance of concurrent anti-inflammatory therapy. Corticosteroids can reduce pulmonary inflammation and may potentiate efficacy of  $\beta$  adrenergic agonists via induction of new  $\beta$  adrenoceptors and prevention of down-regulation of these receptors in the lung. Precedent bronchodilator therapy may improve pulmonary distribution of aerosolized anti-inflammatory preparations.<sup>119</sup>

Aerosolized albuterol (1 ug/kg), administered via Aero-mask and metered dose inhaler to horses with inflammatory airway disease or exercise-induced pulmonary hemorrhage, decreased physiologic shunt fraction ( $Q_S/Q_T$ ) for 240 minutes after administration in horses with elevated baseline (time 0)  $Q_S/Q_T$  (>1.5%), but increased  $Q_S/Q_T$  for 30 minutes after administration in horses with normal baseline  $Q_S/Q_T$  (<1.5%).<sup>76</sup> These results indicate that albuterol administration is beneficial when respiratory function is compromised, but can adversely alter gas exchange in horses with normal  $Q_S/Q_T$ , possibly due to changes in pulmonary circulation.<sup>76</sup>

Aerosolized pirbuterol (3200 ug), a selective  $\beta_2$  agonist, administered via MDI through a tracheostoma reduced  $R_L$  and increased  $C_{dyn}$  and minute ventilation in COPD-affected ponies (average weight 192kg).<sup>64</sup> Onset of bronchodilation was 5 minutes after administration, significant effects on pulmonary function lasted 30 minutes, and adverse effect of mild sweating was observed. More severe side effects of sweating, trembling, excitement and tachycardia were observed after cumulative pirbuterol administration at 30 minute intervals of 1600, 2400, and 3200 ug.<sup>64</sup> Pirbuterol has also been administered

via the equine Aerosol Delivery System into the left nostril, at lower dosages (400 to 1600 ug) that did not produce clinical adverse effects in average weight adult horses.<sup>110</sup> Significant decreases in  $\Delta PpL_{max}$  and  $R_L$  were observed at 5, 10 and 30 minutes and 1 hour after administration of pirbuterol compared to vehicle. However, vehicle plus pirbuterol produced improvement in pulmonary function for the 7 hour duration of the experiment. Optimal effect on lung function with a high therapeutic index was attained with pirbuterol aerosol dosage of 600 ug, and higher doses did not further increase effect or duration.<sup>110</sup>

### ***Parasympatholytic bronchodilators***

Atropine, a nonspecific muscarinic receptor antagonist, administered via nebulization and intravenous injection at 0.02 mg/kg, significantly decreased  $\Delta PpL_{max}$  (by 68%), decreased respiratory rate and increased heart rate and  $paO_2$  after transient exacerbation of hypoxemia, in COPD-affected horses. Values of  $paO_2$  and  $\Delta PpL_{max}$  after treatment remained significantly different from control horses.<sup>100</sup> Atropine (0.01mg/kg IV) is effective in reducing signs of respiratory distress and reducing  $\Delta PpL_{max}$  in horses with COPD.<sup>99</sup> Atropine also causes tachycardia, increased viscosity of bronchial secretions, reduced bowel motility and mydriasis in horses<sup>98, 100</sup> and cannot be used routinely for bronchodilation because of its side effects on the gastrointestinal and central nervous systems even when administered via aerosol.<sup>120</sup>

Ipratropium is a quaternary ammonium derivative of atropine which is not significantly absorbed from the respiratory tract when administered by aerosol and does not dry secretions or inhibit mucociliary clearance.<sup>65, 121, 122</sup> Atropine and ipratropium are nonspecific muscarinic receptor antagonists, so they block both M3 and M2 receptors on airway smooth muscle and the inhibitory neuronal prejunctional muscarinic autoreceptors.<sup>15</sup> Optimal bronchodilation is achieved with ipratropium (2-3 ug/kg) administered by nebulization, but the duration of action is only 4 hours.<sup>65</sup> Ipratropium bromide was administered via nebulization of 25, 50 or 75 ug/ml to horses with COPD, providing a total dose of 500 to 1500 ug (1-3ug/kg).<sup>65</sup> Significant improvement in  $\Delta PpL_{max}$ ,  $R_L$  and  $C_{dyn}$  were observed at one hour after administration of all three doses of

ipratropium, but dose dependency was observed in  $R_L$  and  $C_{dyn}$ . Significant effects of ipratropium persisted to 6 hours for  $\Delta PpL_{max}$  and to 4 hours for  $R_L$ , but significant differences in these values compared to vehicle were only present up to 4 hours and 1 hour after ipratropium, respectively.<sup>65</sup> Dose dependence of the airway response to ipratropium is indicated by changes in  $R_L$ , suggesting that the drug has a major effect on large airways where parasympathetic innervation is greater and response to neural activation is more pronounced in horses with COPD.<sup>65</sup>

Ipratropium bromide dry powder inhalation (DPI) has been successfully administered to horses using a dry powder delivery device connected to an adapted facemask.<sup>111</sup> In control horses, ipratropium DPI (2400 ug) had no effect on pulmonary function. In horses suffering from COPD, ipratropium DPI (1200ug) caused significant improvement in  $R_L$  and  $C_{dyn}$  at 15 minutes after administration, persisting for the 1 hour duration of the experiment.<sup>123</sup> The effects on  $C_{dyn}$  may indicate penetration of ipratropium DPI to peripheral airways and subsequent alleviation of bronchospasm, although cholinergic innervation is decreased in smaller airways.<sup>20</sup> Doubling the dose caused no significant improvement of pulmonary function from the 1200ug dose and systemic side effects were not observed.<sup>123</sup>

In another study, significant improvements in  $\Delta PpL_{max}$ ,  $C_{dyn}$  and  $R_L$  were measured 15 minutes after administration of ipratropium DPI (2400 ug) to horses with COPD.<sup>113</sup> Ipratropium bromide DPI (2400 ug) was administered to horses with COPD followed by strenuous treadmill exercise and measurement of pulmonary function and blood gas analysis in the initial 10 minute recovery period.<sup>113</sup> Alveolar oxygen tension ( $p_{AO_2}$ ) was the only parameter significantly improved during recovery following ipratropium DPI, causing a non-significant increase in calculated alveolar-arterial oxygen tension difference ( $p_{(A-a)O_2}$ ). The minimal difference between treated and control horses is probably due to bronchodilation associated with the sympathetic drive of exercise being equal to or greater than that induced by ipratropium DPI.<sup>113</sup>

Lower doses of aerosol ipratropium, 180-360 ug (0.35-0.7 ug/kg) delivered via metered dose inhaler, can produce maximal bronchodilation in horses with tracheobronchial disease.<sup>15</sup> However, administration at least four times daily may be necessary for maintenance bronchodilation. In humans, 0.27 to 1.07 ug/kg of aerosol ipratropium causes bronchodilation.<sup>121</sup> A new compound, tiotropium bromide, is being used in clinical trials in humans and has a duration of action greater than 12 hours in horses.<sup>15</sup>

## **CHAPTER 2: MATERIALS AND METHODS**

### **Horses**

Six horses, 5 geldings and 1 mare, with a history of COPD were selected from the research herd at Virginia Tech. Ages ranged from 8 to 20 years old, and breeds included 3 American Quarter Horses, 1 American Saddle Horse and 2 Thoroughbreds. Weights ranged from 420 to 590 kilograms. Diagnosis of COPD had previously been obtained by results of bronchoalveolar lavage and favourable response to atropine administration (0.015 mg/kg IV) during acute clinical exacerbation. Physical examination, weight estimation by tape measure, arterial and venous blood gas analyses and measurement of hematocrit and total plasma protein were performed on each horse prior to exposure to hay. Mean physical parameters and blood gas values before induction of acute airway obstruction were within normal ranges, except for elevated mean respiratory rate (22 breaths per minute +/- s.e. 2.25) and decreased mean arterial oxygen tension (77 mmHg +/- s.e. 2.34). Horses were exposed to moldy, round bales of hay on bare pasture for 60 hours prior to the start and for the 5 day duration of the experiments.

### **Treatments**

Treatments were arranged in a 3 treatment, 3 period crossover design with a 48 hour washout interval between periods. Horses were randomly allocated to treatment sequences, with 3 different horses per 4 hour morning and afternoon session for each treatment day. Horses were housed in a barn with access to hay and water during the treatment sessions, but were turned out on bare pasture with moldy, round bales of hay for the remainder of the study period. Treatments were aerosolized and administered via a well-fitted medium or large size Equine AeroMask<sup>a</sup> with aerosol holding chamber and metered dose canister. Treatment groups included (1) Albuterol<sup>b</sup> 1 ug/kg (5-7 x 90ug MDI), (2) Ipratropium bromide<sup>c</sup> 0.35 ug/kg (8-12 x 18ug MDI) and (3) Placebo<sup>d</sup> vehicle aerosol containing dichlorodifluoromethane, dichlorotetrafluoroethane and trichloromonofluoromethane propellants (8-12 MDI).

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<sup>a</sup> Equine AeroMask<sup>TM</sup> with Aerovet<sup>TM</sup> Holding Chamber, Trudell Medical International, Ontario Canada.

<sup>b</sup> Albuterol Inhalation Aerosol, Glaxo Wellcome Inc., Research Triangle Park, NC USA.

<sup>c</sup> Atrovent<sup>®</sup> Inhalation Aerosol, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT USA

<sup>d</sup> Placebo Inhalation Aerosol, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT USA

## Samples

Measurement of heart rate (HR), respiratory rate (RR), rectal temperature (Temp) and end-tidal CO<sub>2</sub> (ETCO<sub>2</sub>), and collection of arterial and venous blood were performed at 0, 30, 60, 120, 180 and 240 minutes after each treatment. End-tidal carbon dioxide tension was determined using an infrared capnograph<sup>e</sup> from a sample continuously aspirated from the trachea via an 18 gauge 1.5 inch needle placed through a clipped, aseptically prepared area of skin into the trachea at midcervical level. Maximum end-tidal CO<sub>2</sub> of each breath waveform was recorded for 30 seconds and a mean value calculated for each sample time. Arterial blood was collected anaerobically from the carotid or transverse facial arteries using a 22 gauge 1.5 inch or 25 gauge 5/8 inch needle respectively and a heparinized 3 cc syringe. Venous blood was collected anaerobically from the jugular vein using a 20 gauge 1.5 inch needle and heparinized 3 cc syringe. All blood samples were capped with a rubber stopper and placed on ice prior to immediate processing using a portable blood gas analyzer<sup>f</sup>. Measurements of arterial and venous pO<sub>2</sub>, pCO<sub>2</sub>, pH, hematocrit and barometric pressure were recorded from the blood gas analyzer at each sample time.

## Calculations

Alveolar oxygen tension (p<sub>A</sub>O<sub>2</sub>) was calculated using the alveolar gas equation (Figure 4). The partial pressure of water vapour was corrected for temperature (Appendix A). The inspired oxygen fraction was assumed to be 0.2093 and the respiratory exchange ratio was assumed to be 0.85.<sup>46</sup> The percentage saturation of hemoglobin with oxygen (%Sat) for arterial, venous and pulmonary end-capillary blood was calculated using an algorithm describing the equine oxygen equilibrium curve.<sup>45, 124</sup> The measured arterial and venous oxygen tensions (p<sub>a</sub>O<sub>2</sub>, p<sub>v</sub>O<sub>2</sub>) were adjusted to correct for displacements of the curve with changes in temperature, pCO<sub>2</sub> and pH, using the equation and coefficients specifically derived for the horse.<sup>45</sup> The total oxygen content was calculated for alveolar gas, and arterial and venous blood (Figure 6), using the measured p<sub>a</sub>O<sub>2</sub> and p<sub>v</sub>O<sub>2</sub>, and the

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<sup>e</sup> Ohmeda 4700 Oxicap<sup>®</sup> Monitor, Louisville, CO USA

<sup>f</sup> IRMA SL Series 2000 Blood Analysis System, Diametrics Medical Inc., St Paul, MN USA.

calculated  $p_AO_2$  and %Sat for these samples. The hemoglobin concentration ([Hb]) was calculated as the product of mean corpuscular hemoglobin concentration (0.34) and mean arterial hematocrit per horse per day. The alveolar dead space fraction ( $V_D/V_T$ ) (Figure 3), physiologic shunt fraction ( $Q_S/Q_T$ ) (Figure 5) and the difference between calculated  $p_AO_2$  and measured  $p_aO_2$  ( $p_{(A-a)}O_2$ ) were calculated.

### **Statistical Analysis**

Repeated measures Analysis of Variance was performed using The Mixed Procedure of the SAS System<sup>§</sup>. Baseline means for each response variable were tested for differences between treatment groups. The differences from baseline for each response variable were tested for treatment effects, time effects and treatment by time interactions. Post-hoc pairwise comparisons and Bonferroni correction were performed when significant treatment or time effects were detected. Differences in treatments from baseline were also evaluated using Bonferroni correction for multiple comparisons. All comparisons were declared significant at  $\alpha \leq 0.05$ .

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<sup>§</sup> The SAS System ver. 7.01, SAS Institute Inc., Cary, NC USA.

### CHAPTER 3: RESULTS

Before treatment, all horses exhibited clinical signs of COPD including coughing, dyspnea, exaggerated biphasic expiration, nostril flaring and auscultatory wheezes with or without rebreathing. Mean baseline responses +/- standard errors were RR 28 bpm +/- 2.43, HR 59 bpm +/- 3.65, rectal temperature 38.11°C +/- 0.12,  $p\text{aO}_2$  59.7 mmHg +/- 2.06,  $p\text{aCO}_2$  51.38 mmHg +/- 1.66,  $\text{ETCO}_2$  48.06 mmHg +/- 1.17, physiologic shunt fraction ( $Q_S/Q_T$ ) 35.15% +/- 3.56, alveolar dead space fraction ( $V_D/V_T$ ) 5.65% +/- 2.16, and alveolar to arterial oxygen tension difference ( $p_{(A-a)}\text{O}_2$ ) 42.88mmHg +/- 1.04. None of these response variables were significantly different between treatment groups at baseline (Table 1). Statistical analyses of differences (d) in response variables from baseline revealed significant treatment effects for  $d\text{p}\text{aCO}_2$  ( $p=0.0030$ ) and  $d\text{p}_{(A-a)}\text{O}_2$  ( $p=0.0323$ ), significant time effects for  $d\text{HR}$  ( $p=0.0448$ ) and  $d\text{Temp}$  ( $p=0.0434$ ), but no significant treatment by time interactions.

Ipratropium significantly decreased  $p\text{aCO}_2$  ( $p=0.0005$ ) and  $\text{ETCO}_2$  ( $p=0.007$ ), but increased  $p_{(A-a)}\text{O}_2$  ( $p= 0.0096$ ) for 4 hours after treatment (Table 2). Mean decrease in  $p\text{aCO}_2$  after ipratropium was 5.47 mmHg, significantly different from albuterol ( $p=0.0012$ ) and placebo ( $p=0.0057$ ) treatment which did not improve  $p\text{aCO}_2$ . However, mean  $p\text{aCO}_2$  remained elevated after ipratropium (48.7 mmHg). Mean  $\text{ETCO}_2$  decreased by 3.87 mmHg to within normal range (44.3 mmHg) after treatment with ipratropium. Mean increase in  $p_{(A-a)}\text{O}_2$  was 5.48 mmHg after treatment with ipratropium. There were no other significant treatment responses (Table 2) and no adverse effects were observed. Treatment means across time for differences in each response variable from baseline are presented with standard error bars (Figures 7-15). Although non-significant, numerical trends were observed toward a decrease in  $p\text{aO}_2$  at 1 hour after albuterol treatment and a decrease in  $Q_S/Q_T$  1hour after ipratropium treatment. Across all treatments, mean heart rate significantly decreased from time zero at 2 hours ( $p=0.0063$ ) and 3 hours ( $p=0.0025$ ). Mean body temperature decreased significantly from time zero across all times and treatments.

Table 1. Mean responses at baseline before treatment of COPD-affected horses with aerosolized albuterol, ipratropium or placebo<sup>Y</sup>.

Treatment	RR bpm	HR bpm	Temp °C	p <sub>a</sub> O <sub>2</sub> mmHg	p <sub>a</sub> CO <sub>2</sub> mmHg	ETCO <sub>2</sub> mmHg	Q <sub>S</sub> /Q <sub>T</sub> %	V <sub>D</sub> /V <sub>T</sub> %	p <sub>(A-a)</sub> O <sub>2</sub> mmHg
Albuterol	26.0	53.0	37.9	64.3	48.2	47.5	30.5	0.28	42.2
Ipratropium	32.3	67.0	38.4	57.1	54.2	48.2	36.6	10.62	42.0
Placebo	25.7	56.3	38.0	57.8	51.8	48.5	38.3	6.05	44.4

<sup>Y</sup>Each value is the mean of 6 horses at time zero. No values were significantly different between treatment groups.

Table 2. Differences (d) in response variables, subtracted from baseline, after treatment of COPD-affected horses with aerosolized albuterol, ipratropium or placebo<sup>Y</sup>.

Treatment	RR	HR	Temp	p <sub>a</sub> O <sub>2</sub>	p <sub>a</sub> CO <sub>2</sub>	ETCO <sub>2</sub>	Q <sub>S</sub> /Q <sub>T</sub>	V <sub>D</sub> /V <sub>T</sub>	p <sub>(A-a)</sub> O <sub>2</sub>
	bpm	bpm	°C	mmHg	mmHg	mmHg	%	%	mmHg
Albuterol	1.87	2.27	0.04	0.61	-1.21a	0.73	0.35	-4.41	1.15
Ipratropium	1.83	8.67	0.23	-0.81	5.47*b	3.87*	2.67	1.72	-5.48*
Placebo	-1.27	5.13	0.05	-1.25	0.33a	1.10	4.86	-1.96	1.16

<sup>Y</sup>Each value is the mean of 6 horses averaged across time.

\*Means are significantly different from baseline. Means followed by different letters are significantly different at  $\alpha = 0.05$  according to Bonferroni-corrected multiple comparisons.

Figure 7. Mean difference in heart rate from baseline of horses with COPD after treatment with aerosolized albuterol, ipratropium or placebo.

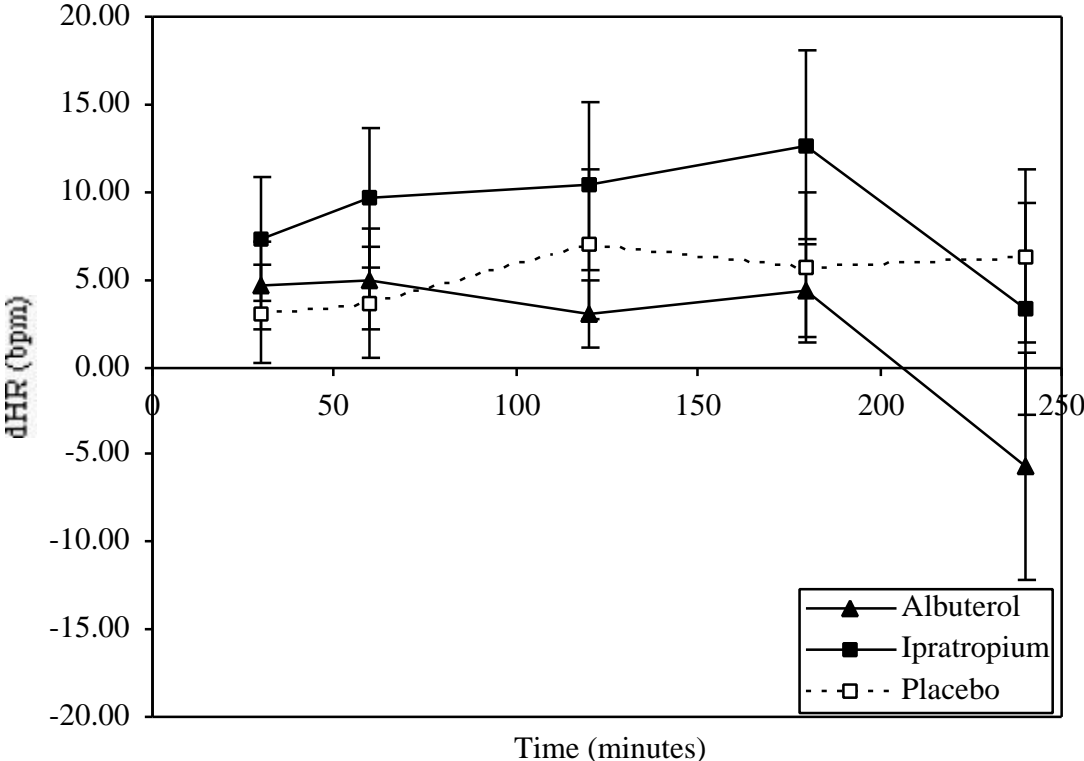


Figure 8. Mean difference in respiratory rate from baseline of horses with COPD after treatment with aerosolized albuterol, ipratropium or placebo.

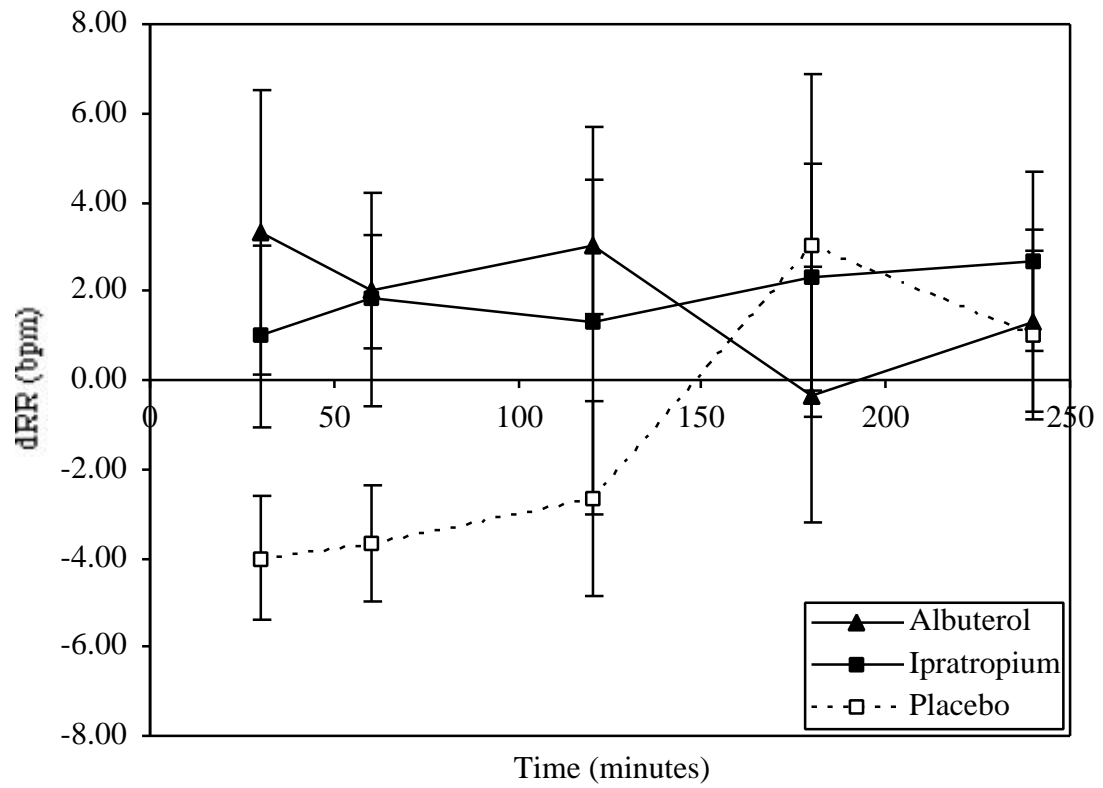


Figure 9. Mean difference in body temperature of horses with COPD after treatment with aerosolized albuterol, ipratropium and placebo.

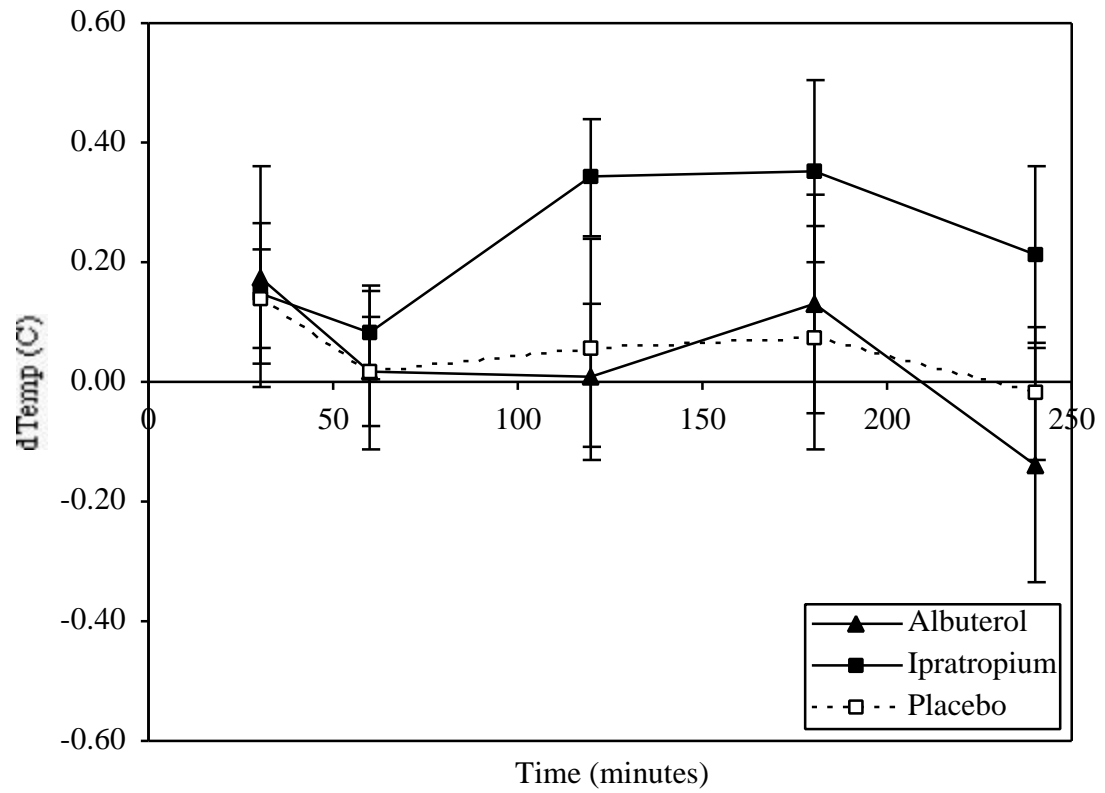


Figure 10. Mean difference in arterial oxygen tension from baseline of horses with COPD after treatment with aerosolized albuterol, ipratropium or placebo.

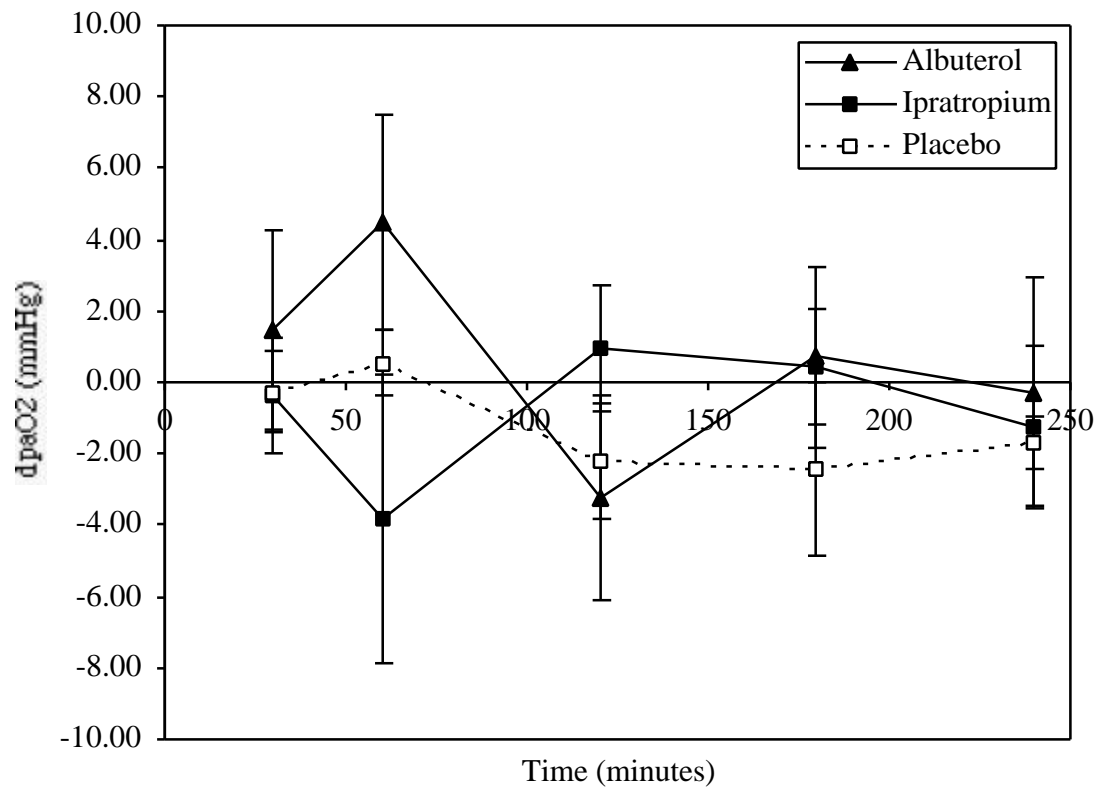


Figure 11. Mean arterial carbon dioxide tension of horses with COPD after treatment with albuterol, ipratropium and placebo.

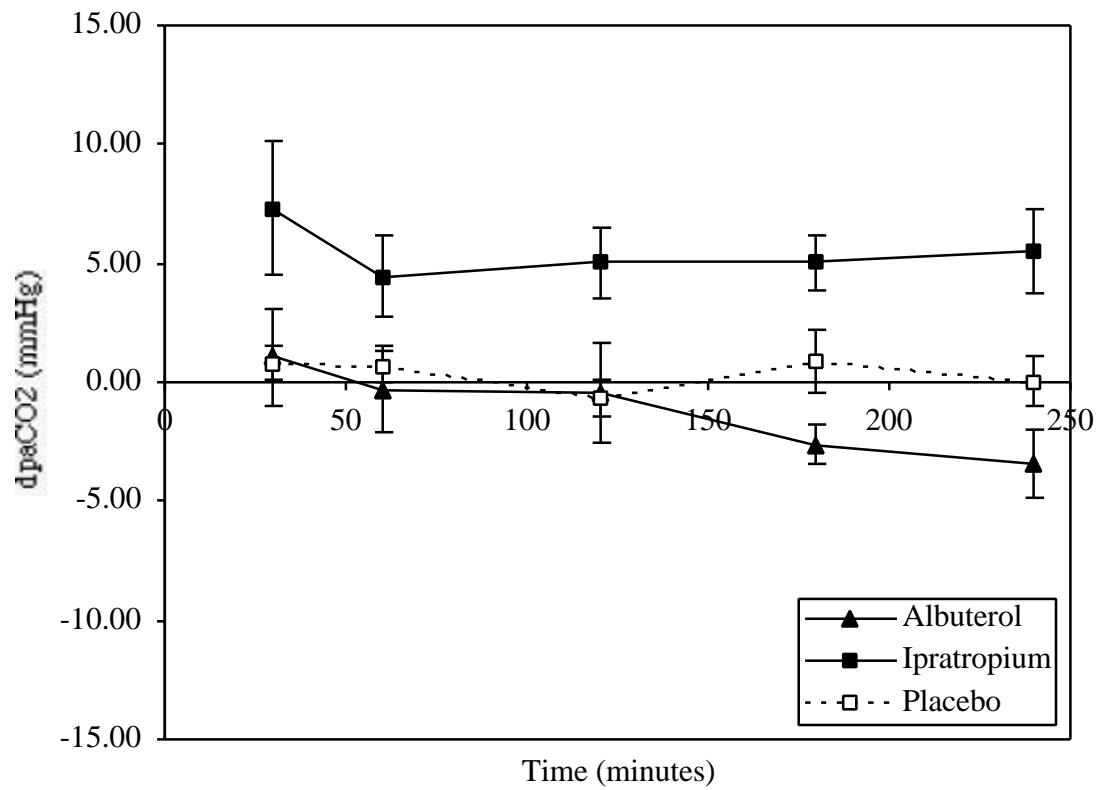


Figure 12. Mean difference in end-tidal carbon dioxide tension from baseline of horses with COPD after treatment with albuterol, ipratropium or placebo.

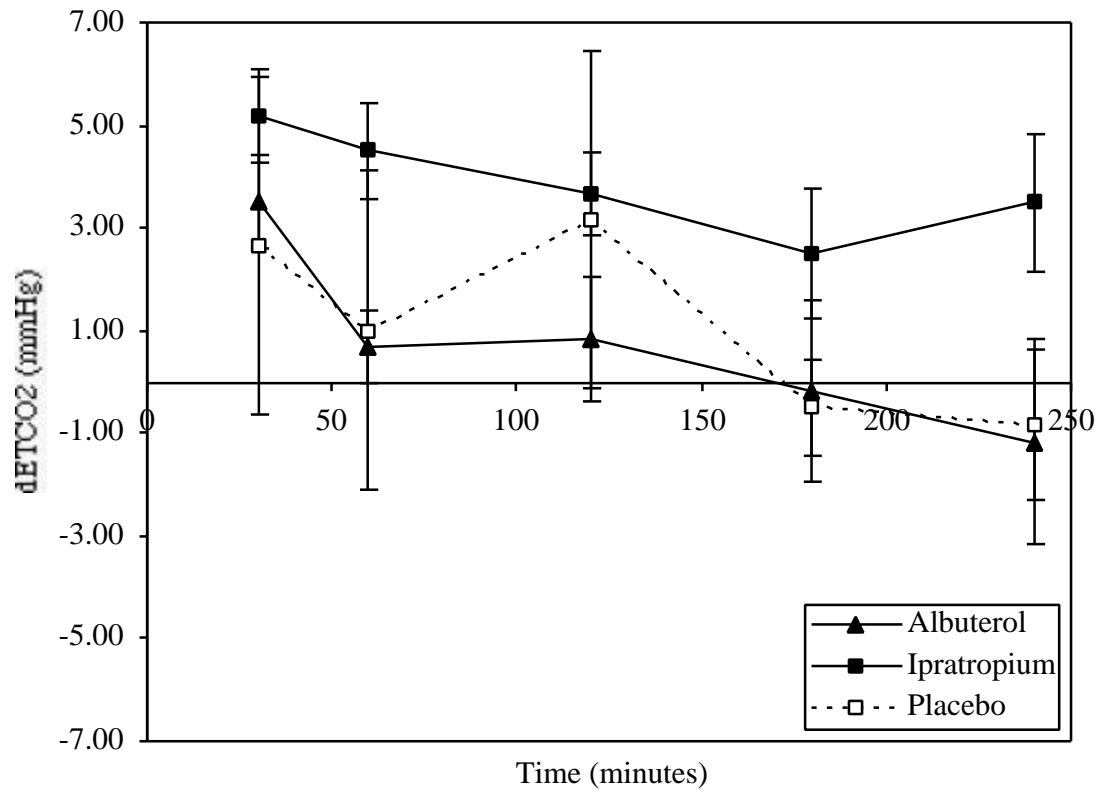


Figure 13. Mean difference in physiologic shunt fraction from baseline of horses with COPD after treatment with albuterol, ipratropium and placebo.

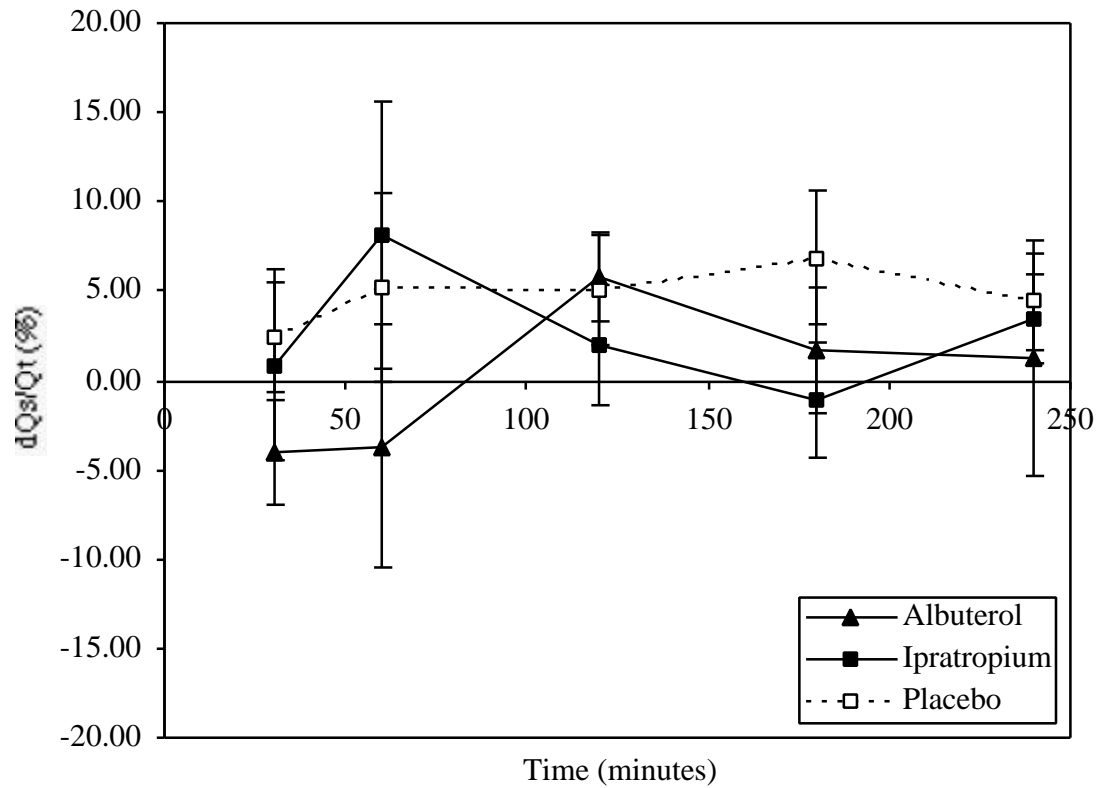


Figure 14. Mean difference in alveolar dead space fraction from baseline of horses with COPD after treatment with aerosolized albuterol, ipratropium and placebo.

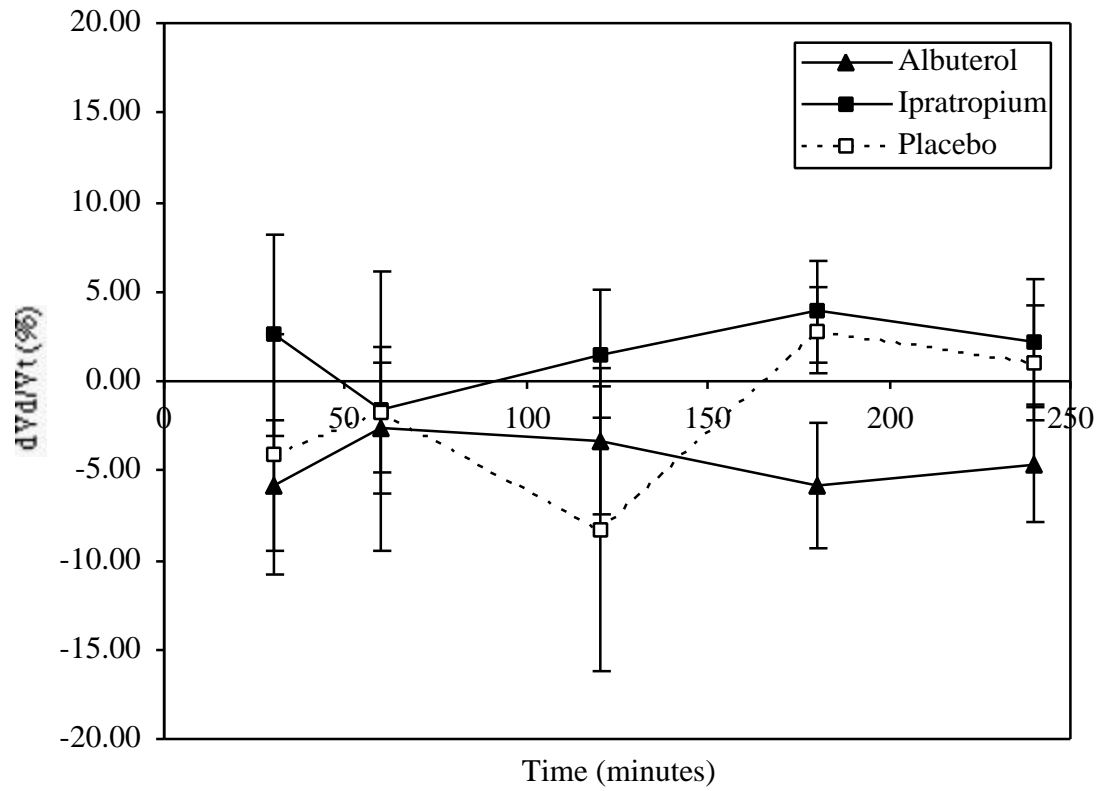
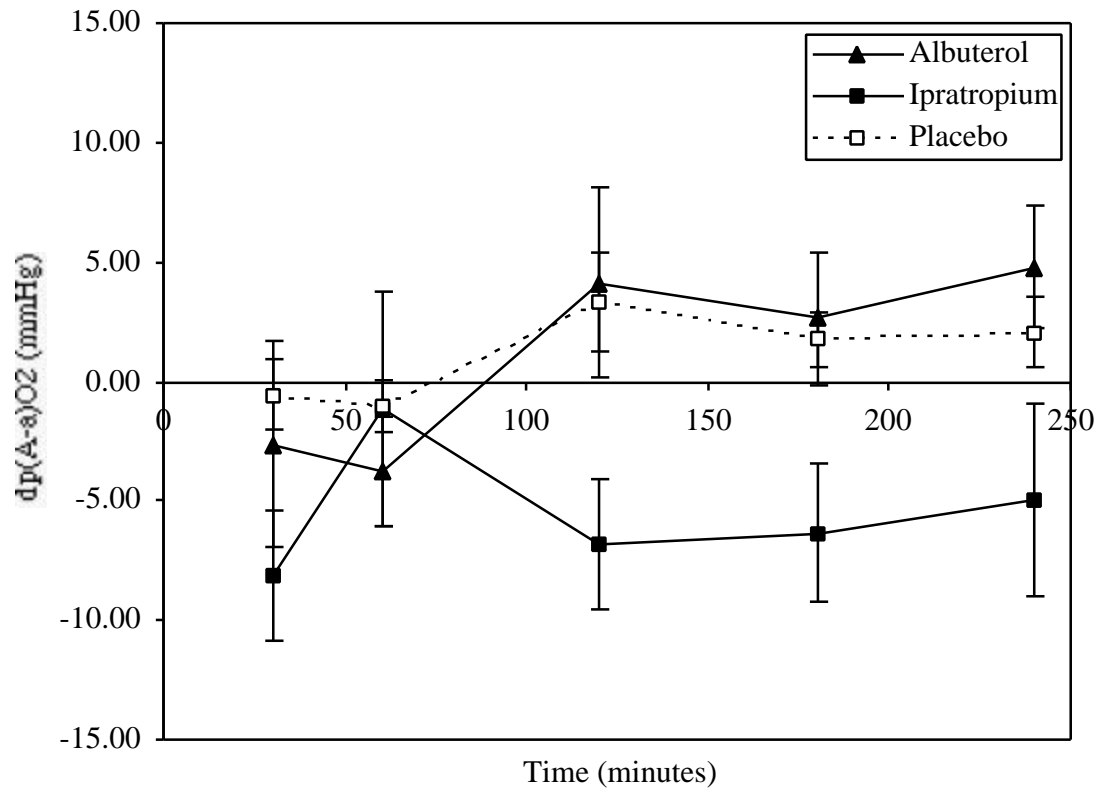


Figure 15. Mean difference in alveolar to arterial oxygen tension difference from baseline of horses with COPD after treatment with albuterol, ipratropium or placebo.



## CHAPTER 4: DISCUSSION

The horses used in the study exhibited typical signs of obstructive pulmonary disease after environmental exposure to moldy, dusty hay. Fungi and actinomycetes in hay reliably induce pulmonary inflammation and bronchoconstriction in COPD-susceptible horses after inhalation exposure.<sup>51, 52, 97</sup> Baseline assessment of response variables prior to treatment indicated severe impairment of pulmonary gas exchange. Tachypnea (RR 28 bpm +/- 2.43) was attributed to hypercapnia, hypoxemia and vagal stimulation.<sup>67, 70</sup> Tachycardia (HR 59 bpm +/- 3.65) is not always observed in horses with COPD,<sup>47, 125</sup> but was attributed to hypoxemia and elevated circulating catecholamines associated with physiological stress. The degree of hypoxemia observed in the study horses at baseline (paO<sub>2</sub>, 59.7 mmHg +/- 2.06) was severe compared to previous reports in COPD affected horses.<sup>47, 63, 70</sup> Mean arterial oxygen tension (paO<sub>2</sub>) of horses was below normal before induction of acute airway obstruction, and subsequently decreased by 17.3 mmHg during disease. This decrease in paO<sub>2</sub> after induction of COPD is slightly greater than previously reported.<sup>63, 70</sup> Hypoxemia in these horses could be caused by alveolar hypoventilation, right to left vascular shunts, V<sub>A</sub>/Q mismatching and diffusion impairment, associated with bronchospasm, inflammation, mucus plugging and edema of peripheral airways, and thickening and fibrosis of the alveolocapillary membrane.<sup>50, 126</sup>

Marked baseline elevation of alveolar to arterial oxygen tension difference (p<sub>(A-a)</sub>O<sub>2</sub>, 42.88 mmHg +/- 1.04) indicates severe impairment of gas exchange due to increased physiologic shunting of mixed venous blood, V<sub>A</sub>/Q alterations or diffusion impairment.<sup>71, 74</sup> The physiologic shunt fraction (Q<sub>S</sub>/Q<sub>T</sub>) is a measure of the amount of venous admixture required to produce the observed p<sub>(A-a)</sub>O<sub>2</sub> if all alveoli were functioning ideally.<sup>71</sup> Mean Q<sub>S</sub>/Q<sub>T</sub> at baseline (35.15% +/- 3.56) was markedly elevated from normal values and 1.3 times higher than previously reported means in horses with COPD.<sup>44, 74</sup> This indicates a large amount of venous admixture, from perfused but poorly ventilated alveoli, with end-pulmonary capillary blood from ideally functioning alveoli. Administration of 100% oxygen to affected horses has previously demonstrated decreased V<sub>A</sub>/Q ratio and increased physiologic rather than anatomic shunting of blood as the cause of increased Q<sub>S</sub>/Q<sub>T</sub> in horses with COPD.<sup>74</sup> Alveolar hypoxia minimizes

changes in  $V_A/Q$  to a limited extent via a local vasoconstrictor effect on pulmonary vessels, directing blood flow to normoxic regions of lung. This compensatory effect of decreasing right to left shunts and venous admixture, is maximal at an alveolar oxygen tension of 80mmHg and becomes less efficient as  $p_AO_2$  increases or decreases.<sup>2</sup>

Occlusion of small airways by constriction, mucus plugs and edema with subsequent uncompensated alveolar hypoxia will result in increased percentage venous admixture ( $Q_S/Q_T$ ) and hypoxemia in horses with severe COPD.

Hypercapnia ( $paCO_2$ , 51.38mmHg +/- 1.66) in study horses at baseline was more severe than previously reported in COPD-affected horses<sup>63, 70</sup> and may be due to abnormal  $V_A/Q$  ratio and hypoventilation. Tachypnea and increased respiratory effort observed in these horses indicate increased ventilatory drive in an attempt to maintain tidal volume and eliminate  $CO_2$ . Elevated end-tidal  $CO_2$  at baseline ( $ETCO_2$ , 48.06 mmHg +/- 1.17) could be explained by predominance of lung units with decreased  $V_A/Q$  ratios and relative alveolar hypoventilation,<sup>14</sup> and has not been previously reported in COPD-affected horses.<sup>69</sup> Airway obstruction causes inequality of time constants between different lung regions and  $ETCO_2$  tension represents the alveolar gas from the regions with the longest time constants.<sup>2, 71</sup> In horses, collaterally ventilating pathways are of limited value in maintaining ventilation to an obstructed airspace as collateral airway time constants are long and resistance is high.<sup>2</sup> As a result, alveolar gas of obstructed airways will equilibrate with mixed venous (pulmonary arterial) blood and elevated alveolar  $CO_2$  tension can subsequently cause arterial hypercapnia.<sup>14</sup>

Decreased  $ETCO_2$ , previously reported in horses with lower airway disease, is caused by increased alveolar dead space ventilation and dilution of alveolar carbon dioxide with air.<sup>44</sup> Alveolar dead space fraction at baseline ( $V_D/V_T$ , 5.65% +/- 2.16) was normal or elevated compared to previously reported normal values in horses (means -18.2 to 6.1%)<sup>44, 69</sup> and was 3.5 times lower than the mean previously reported in COPD-affected horses.<sup>69</sup> The mild elevation of  $V_D/V_T$  observed suggested increased physiologic dead space, but was associated with concurrent elevations in  $paCO_2$  and  $ETCO_2$ . It is probable that areas of increased  $V_A/Q$  ratio existed and that diffusion impairment contributed to

hypercapnia, however, decreased  $\text{ETCO}_2$  would be expected if these processes were predominant. Mismatching of ventilation and blood flow due to predominance of lung units with decreased  $V_A/Q$  ratio and relative alveolar hypoventilation is therefore the most likely cause of  $\text{CO}_2$  retention in these horses with severe COPD.

Aerosolized ipratropium (0.35 ug/kg) significantly decreased  $\text{paCO}_2$  and  $\text{ETCO}_2$  by 30 minutes following treatment. There were no significant treatment by time interactions and the effect lasted for the 4 hour experimental duration. Effects of ipratropium treatment on  $\text{paCO}_2$  and  $\text{ETCO}_2$  in COPD-affected horses have not been previously reported. In horses severely affected with COPD, decrease in  $\text{paCO}_2$  and  $\text{ETCO}_2$  toward normal values following administration of a muscarinic receptor antagonist could be explained by airway smooth muscle relaxation, bronchodilation and improved ventilation of perfused alveoli. Mean  $\text{ETCO}_2$  values did not decrease below normal, indicating that increased dead space ventilation of non-perfused alveoli was not predominant after ipratropium. Ipratropium treatment improved  $\text{paCO}_2$  from baseline significantly more than albuterol (1ug/kg) and vehicle aerosol administration, both of which did not detectably improve ventilation and  $\text{CO}_2$  elimination of obstructed airways.

Aerosolized ipratropium (0.35 ug/kg) significantly increased  $p_{(A-a)}\text{O}_2$  by 30 minutes following treatment, persisting for the 4 hour experimental duration. This effect could be caused by increased alveolar oxygen tension ( $p_A\text{O}_2$ ) or worsening of hypoxemia, however, there was no significant decrease in  $\text{paO}_2$  following ipratropium. Significantly increased  $p_A\text{O}_2$  has been observed in COPD-affected horses during recovery from exercise, 15 minutes after ipratropium (2400 ug DPI) pretreatment.<sup>113</sup> Treatment of COPD-affected horses with ipratropium could increase  $p_A\text{O}_2$  by improving alveolar ventilation via antagonism of muscarinic receptor-mediated bronchospasm in large and small airways. Improved  $p_A\text{O}_2$  should result in improved  $\text{paO}_2$ , but hypoxic vasoconstriction of vessels supplying previously poorly ventilated alveoli, diffusion impairment and increased ventilation of non-perfused alveoli (increased  $V_A/Q$  ratio) would contribute to hypoxemia and subsequently increased  $p_{(A-a)}\text{O}_2$ . Ipratropium treatment did not decrease physiologic shunt fraction ( $Q_S/Q_T$ ), indicating that significant

improvement in  $V_A/Q$  matching was minimal. There was, however, a non-significant trend for decreased  $Q_S/Q_T$  at 1 hour after ipratropium administration in this study. Severe elevation of baseline mean  $Q_S/Q_T$  and the small number of horses used may contribute to the statistical non-significance of this finding.

Improvements in pulmonary function have been detected 15 minutes after ipratropium administration and can last for 4 hours after treatment.<sup>15, 65, 123</sup> Decreased  $\Delta PpL_{max}$  and  $R_L$  in COPD-affected horses after nebulized ipratropium (500 ug) was indicative of bronchodilation in larger airways<sup>65</sup>, but significant responses have also been observed in smaller airways via improvement of  $C_{dyn}$ .<sup>123</sup> Decreased cholinergic innervation and a slighter decrease in contractile response to ACH occur in peripheral airways.<sup>16, 20, 21</sup> Responses to muscarinic receptor antagonist, ipratropium, observed in this study cannot differentiate between effects in large or small airways. Ipratropium dry powder inhalation produced optimal bronchodilation at a dose of 1200 ug in COPD-affected horses.<sup>123</sup> The dosage of aerosol ipratropium (0.35 ug/kg, range 144 to 216 ug) used in horses in this study was relatively low, but can produce maximal bronchodilation in horses with tracheobronchial disease.<sup>15</sup> Severe airway inflammation and bronchospasm may have prevented adequate peripheral deposition of aerosol ipratropium. It is possible that a higher dosage of aerosol ipratropium (> 0.35 ug/kg) may have produced more significant improvements in pulmonary gas exchange in horses severely affected with COPD in this study. However, despite evidence that ipratropium improved bronchodilation in these horses, evidence of small airway obstruction and impaired alveolar gas diffusion persisted, most likely due to severe inflammation.

There is no obvious explanation for the significant decrease in mean heart rate at 2 and 3 hours from baseline or decrease in mean rectal temperature from baseline across time. Decreased excitement and sympathetic stimulation after psychological adjustment to stall confinement and experimental procedures is a possible explanation.

A previously observed response to  $\beta_2$  agonist administration in horses is transient exacerbation of hypoxemia followed by overall improvement in arterial oxygen tension

from baseline.<sup>100, 117</sup> Although non-significant, there was a numerical trend in  $\text{paO}_2$  decreasing from baseline at 30 minutes and 1 hour after albuterol, then increasing above baseline at 2 hours after albuterol aerosol administration. This phenomenon is associated with suddenly increased ventilation of previously obstructed alveoli supplied by vessels with hypoxic compensatory vasoconstriction, with subsequent improvement of  $V_A/Q$  matching, gas exchange and arterial oxygen tension.<sup>114</sup> Bronchodilation using aerosolized albuterol in horses has been optimally achieved at a dosage of 360  $\mu\text{g}$ ,<sup>112</sup> which is less than the dose used in this study (1  $\mu\text{g}/\text{kg}$ , range 450 to 630  $\mu\text{g}$ ). It is therefore unlikely that a higher dose of albuterol ( $> 1 \mu\text{g}/\text{kg}$ ) would have produced significant beneficial responses in the horses in this study. Aerosolized albuterol (1  $\mu\text{g}/\text{kg}$ ) administered via Aeromask decreased  $Q_S/Q_T$  in horses with lower airway disease that had elevated baseline  $Q_S/Q_T$  ( $>1.5\%$ ).<sup>76</sup> Marked elevation in mean baseline  $Q_S/Q_T$  (35.15%) in COPD-affected horses in this study may have contributed to lack of detectable effects of albuterol treatment.

Lack of significant changes in response variables after albuterol aerosol (1  $\mu\text{g}/\text{kg}$ ) may be due to the severity of obstructive pulmonary disease that the affected horses demonstrated. Beta adrenergic receptors should be activated in COPD-affected airways,<sup>34, 35</sup> however, severe inflammation and epithelial damage will increase sensitivity of smooth muscle to spasmogens and may decrease  $\beta$  agonist induced relaxation of airway smooth muscle in COPD-affected horses.<sup>34</sup> In addition, bronchoconstriction, mucus plugging, mural edema and inflammatory cellular infiltration impair distribution of aerosolized drugs to small peripheral airways.<sup>119</sup> Excitatory  $\beta_2$  adrenoceptors augment ACH release independently of inhibitory nerve function and intact epithelium.<sup>29, 36</sup> Postjunctional spasmolytic effects of  $\beta_2$  agonist, albuterol, usually predominate over prejunctional excitatory effects.<sup>38</sup> Although  $\beta_2$  excitatory adrenoceptor function is not reported to be up-regulated in COPD-affected horses,<sup>94</sup> an imbalance in the dichotomous effects of albuterol could limit its efficacy as a bronchodilator in horses severely affected with COPD.

Results from this study indicate that one dose of an aerosolized bronchodilator will not resolve clinical signs and may not significantly improve pulmonary gas exchange in horses severely affected with COPD. Despite improved pulmonary function in horses with COPD after administration of bronchodilators, persistently significant differences from normal horses indicate residual airway obstruction and inflammation.<sup>65, 100, 112, 123</sup> This emphasizes the importance of concurrent antiinflammatory therapy in effective treatment of equine COPD. Corticosteroids can reduce pulmonary inflammation, may potentiate efficacy of  $\beta$  adrenergic agonists and can be delivered via aerosol to avoid systemic side effects.<sup>119</sup> Reduced airway inflammation should improve epithelial functions and decrease plugging of airways with mucus and cellular infiltrate, and enhance pulmonary gas exchange. Used in combination, bronchodilators and corticosteroids promote more uniform distribution of aerosolized drugs to peripheral airways and provide the most efficacious treatment for acute airway obstruction in COPD-susceptible horses.<sup>96, 99, 100</sup> However, complete resolution of clinical signs can only be obtained if exposure to etiological antigens is prevented.<sup>54, 55, 97</sup> Suitable environments include pasture or a low-dust barn with wood shavings bedding and a completely pelleted diet.<sup>54, 97</sup> Access to hay should be prevented, except for commercially processed and packaged hay that can be included in a controlled diet.

Arterial blood gas analysis and measurement of pulmonary indices of gas exchange are convenient tests that have been recommended to assess horses with COPD and mild lower respiratory disease.<sup>44, 63, 69, 70, 74</sup> Arterial blood gas analysis and measurement of physiologic shunt fraction and alveolar to arterial oxygen tension difference were useful at detecting marked alterations in pulmonary gas exchange in severely COPD-affected horses in this study. Alveolar dead space fraction was only mildly elevated in horses with severe obstructive pulmonary disease, but is more difficult to interpret due to wide variation in established normal values.<sup>44, 69</sup>

Response to administration of bronchodilators for treatment of COPD can be assessed by monitoring of clinical signs and pulmonary function testing.<sup>64, 65, 100, 112, 119, 123</sup> Physiologic shunt fraction was useful in monitoring response of horses with lower airway

disease to aerosol albuterol administration.<sup>76</sup> Arterial blood gas analysis has been used to monitor response of horse with COPD to bronchodilator therapy.<sup>100, 113, 117</sup> Arterial blood gas analysis was useful in monitoring response of horses with severe COPD to aerosol ipratropium in this study, detecting improved alveolar ventilation and CO<sub>2</sub> elimination. Calculation of alveolar to arterial oxygen tension difference indicated failure of ipratropium to improve oxygen exchange across the alveolocapillary membrane, despite improved alveolar ventilation and carbon dioxide exchange. Measurements of physiologic shunt fraction and alveolar dead space fraction were less sensitive than arterial blood gas analysis in monitoring response of horses with severe COPD to treatment with bronchodilators. However, lack of change in these calculated indices may also reflect inadequate smooth muscle relaxation and persistent airway obstruction after aerosolized albuterol (1 ug/kg) or ipratropium (0.35 ug/kg), or failure of these treatments to improve pulmonary gas exchange due to severe inflammation.

## **CHAPTER 5: CONCLUSIONS**

Arterial blood gas analysis, alveolar to arterial oxygen tension difference, physiologic shunt fraction and alveolar dead space fraction were used to assess response of horses with severe COPD to treatment with aerosolized ipratropium (0.35 ug/kg) and albuterol (1 ug/kg). Horses showed marked impairment of pulmonary function at baseline via severe derangements in arterial blood gas values and indices of pulmonary gas exchange. Alveolar dead space fraction was less useful in evaluating efficiency of gas exchange than arterial blood gas analysis, physiologic shunt fraction and alveolar to arterial oxygen tension difference.

Arterial blood gas analysis was the most sensitive test for monitoring response of horses with severe COPD to bronchodilator therapy, while physiologic shunt fraction and alveolar dead space fraction did not significantly change. Minimal or no detectable improvement in efficiency of gas exchange after bronchodilators in horses with severe COPD may represent lack of significant improvement in airway caliber, rather than lack of sensitivity of the testing methods. Possible reasons for this include insufficient dose, inability of bronchodilator agents to reach peripheral airways and overwhelming residual inflammation and small airway obstruction.

Comparison of aerosolized bronchodilators in the treatment of horses with severe chronic obstructive pulmonary disease indicated that ipratropium (0.35ug/kg) significantly improved CO<sub>2</sub> elimination and alveolar ventilation, while albuterol (1ug/kg) failed to produce detectable bronchodilation or improve pulmonary gas exchange. At these dosages, ipratropium is more efficacious than albuterol for bronchodilation in acute exacerbations of COPD in severely affected horses, but bronchodilator therapy alone is inadequate for relief of airway obstruction as detected by arterial blood gas analysis and indices of pulmonary gas exchange.

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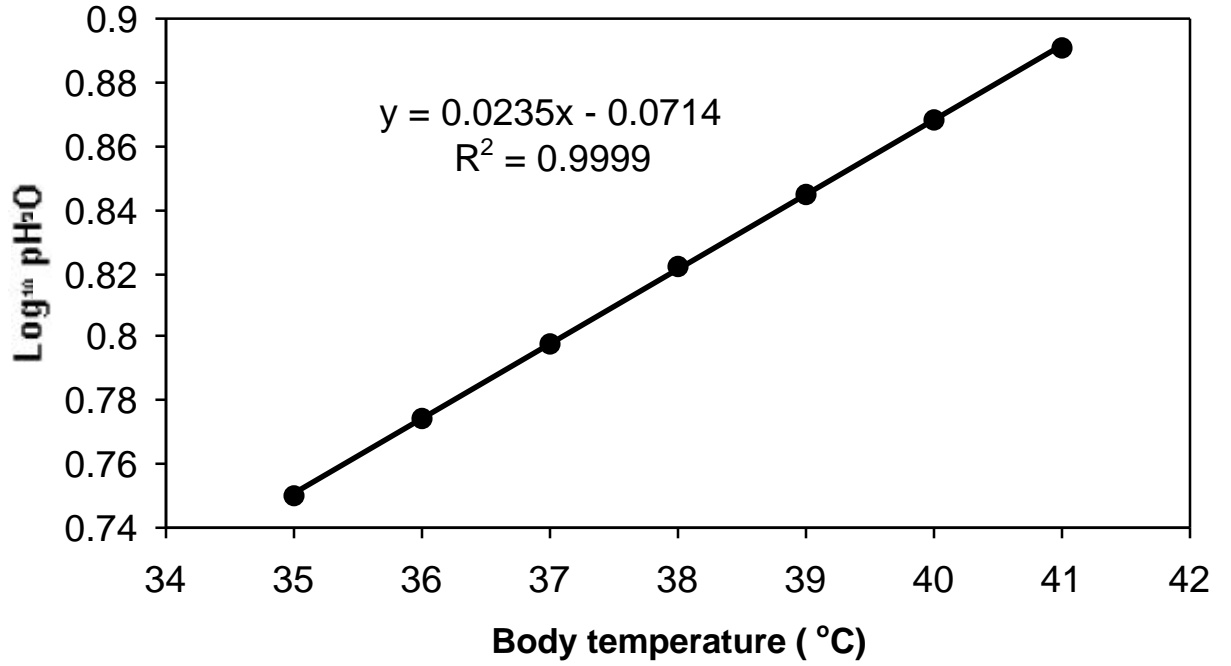
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## APPENDIX A

Partial pressure of water vapor corrected for temperature



## VITA

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#### Degrees:

Bachelor in Veterinary Science (BVSc) 1995  
University of Sydney, Australia

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Virginia Polytechnic Institute and State University

#### Professional Experience:

Residency in Large Animal Medicine 1997-2000  
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Private Practice Large Animal Internship 1996-1997  
Ourimbah Veterinary Hospital, NSW Australia

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#### Specialty Board Certification Status:

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#### Publications:

Friday PA, Scarratt WK, Elvinger F, Timoney P; Ataxia and Paresis with Equine Herpesvirus Type 1 infection in a Herd of Riding School Horses. *Journal of Veterinary Internal Medicine* :14(2), March 2000

Fox J, Friday PA, Duncan RB, Scarratt WK; Cerebello-olivary and lateral (accessory) cuneate degeneration in a juvenile American Miniature Horse. Accepted, *Veterinary Pathology* 1999

#### Research Activities:

Comparison of Two Aerosolized Bronchodilators in the Treatment of Equine Chronic Obstructive Pulmonary Disease. Veterinary Memorial Fund May 1998, \$7408; Friday PA, Broadstone RV, Buechner-Maxwell V and Crisman MV.

Pharmacokinetic Characterization of Nitroglycerin after Intravenous and Transdermal Administration to Healthy Adult Horses. AAEP 1998, \$4700; Animal Health and Disease VT 1998, \$5966; Virginia Veterinary Medical Association 1998, \$4776; Friday PA, Wilcke J, Crisman MV and Howard R.