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Lung Biopsy Pathology and Exercise Tolerance in Horses with Chronic Bronchiolitis

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ABSTRACT Treadmill exercise tolerance was studied in 34 Standardbred trotters and 20 saddle horses with mild or moderate signs of chronic obstructive pulmonary disease (COPD). Respiratory gas exchange during exercise was studied in 21 of the horses, 12 Standardbreds and 9 saddle horses. Relationships between exercise tolerance and pathology in lung biopsies were assessed. Exercise tolerance data differed between the breeds, the Standardbreds showing an increased red cell volume in relation to body weight and a restricted respiratory minute volume, while the saddle horses had an excessive heart rate response to exercise. In the Standardbreds but not in the saddle horses, oxygen uptake and pulmonary ventilation capacities correlated inversely with the morphological grade of small airway disease, and oxygen uptake capacity and tidal volume were inversely related to the height of the bronchiolar epithelium. Red cell hypervolaemia may be a form of compensation for impaired ventilation due to COPD in trained Standardbreds. Differences between the breeds in responses to exercise are probably associated with differences in physical activity.

Key words: Blood lactate; COPD; heart rate; horses; oxygen uptake

INTRODUCTION

In chronic obstructive pulmonary disease (COPD) in the horse, chronic inflammatory lesions of the bronchioles are generally the major abnormality causing obstruction of the conducting airways.[2,23,24] Advanced lesions may be present before clinical signs of airway obstruction become severe[15] and functional derangement may not be observable at rest in patients with only moderate signs of disease. Since reduced work performance and reluctance to exercise are common signs of COPD,[4,9–12,14] exercise testing is often used to evaluate the severity of illness. An excessive heart rate response to exercise has been claimed to be a consistent sign of COPD, although this was not apparent in some studies.[1,10,11,13,20] In most studies on the effects of COPD on exercise tolerance, morphological characterization of the disease was not carried out. Percutaneous lung biopsy has been found useful for this purpose.[8,21,24] According to Viel,[24] the pathology in lung biopsies from COPD subjects correlates well with necropsy findings. Biopsy pathology could therefore be of interest in clinical physiological studies of COPD.

The aims of this study were twofold: First, to assess exercise tolerance in horses with clinically mild or moderate signs of COPD and with chronic small airway disease found on biopsy. Secondly, to evaluate the relationship between the severity of the pathology on lung biopsy and the degree of impairment of exercise tolerance.

MATERIALS AND METHODS

Horses

Fifty-four adult horses, 34 Standardbred trotters in training and 20 saddle horses, were studied (Table 1). All had chronic small airway disease on lung biopsy, a history of

Table 1. *Data on horse material used, including breed, age and sex distribution*
N = Number of horses; SD = standard deviation

	Reference material					COPD material				
	Standardbreds			Saddle horses		Standardbreds			Saddle horses	
	♀	♂	(♂)	♀	(♂)	♀	♂	(♂)	♀	(♂)
N	55	55	42	14	26	11	12	11	6	14
Mean age (years)	4.3	4.7	7.5	9.2	9.3	6.4	5.3	7.6	10.2	9.8
SD	1.8	2.3	3.8	2.8	3.9	3.8	1.8	2.9	1.1	3.0

chronic cough and mild or moderate signs of COPD, including abnormal pulmonary auscultation sounds, cough after tracheal compression, and abnormal tracheobronchial secreta on endoscopy at rest and/or after exercise. Signs of pulmonary emphysema/hyperinflation (heaves) were not present. Standard exercise tests measuring a number of variables were performed (Table 3). Due to breed variations in exercise tolerance data,[16] two groups of reference horses were used (Table 1). The first included 152 Standardbred trotters in various states of training and without signs of disease likely to affect work capacity.[17,19] The other group comprised 40 saddle horses regularly used for pleasure or show riding. These were either sound or had only minor complaints not considered to affect exercise tolerance. Five of those and 10 other horses with no signs of respiratory disease were healthy controls in the evaluation of lung biopsy findings.

Biopsy procedure and morphological methods

Percutaneous cutting needle biopsy of the lung was performed as previously described[3] within 14 days of exercise tolerance testing. Paraffin sections were stained with haematoxylin and eosin, alcian blue-PAS and Masson's trichrome, and were examined without knowledge of the identity of the animals. At microscopic examination, a calibrated eyepiece micrometer was used to measure a range of morphological variables. All bronchioles were counted and examined. Their lumen and epithelium were included in diameter measurements. The mean (BEH_{mean}) and maximal (BEH_{max}) bronchiolar epithelial height (BEH, μm) were determined for each case. The diagnosis of bronchiolitis was based on the presence of inflammatory mucosal cellular infiltrates or luminal exudates. Alveolitis was defined as inflammatory involvement of the alveolar tissue beyond the vicinity of conducting airways. The bronchiolar epithelium was considered hyperplastic when showing stratification. Goblet cell metaplasia was diagnosed when there were goblet cells in the terminal bronchioles or bronchioles of lower order less than 500 μm in diameter.[24] The severity of some features was graded as minimal or above minimal.

Exercise tolerance test

Exercise tolerance testing was done with a high speed treadmill at a 6.25% slope (Sikob, Stockholm). Two standardized tests at a trot, one without ($n=54$) and one with ($n=21$) a respiratory mask, were performed on different days. The procedures were detailed previously.[5,17,22] The heart rate (HR) response to incremental exercise was expressed as the treadmill velocity (V, m s^{-1}) corresponding to a HR of 200 bpm (V_{200}), extrapolated

Table 2. *Morphological grouping of clinical cases from lung biopsy findings*
Mean numbers of available bronchioles in each category also shown. N = number of horses

Category	Criteria for grouping	N	Mean number bronchioles per case
I	Peribronchiolitis. No other bronchiolar lesions	5	5.4
II	Bronchiolitis. No bronchiolar epithelial hyperplasia, goblet cell metaplasia or luminal mucus	6	4.5
III	Bronchiolar luminal mucus and/or goblet cell metaplasia. No epithelial hyperplasia	7	7.1
IV	Bronchiolar epithelial hyperplasia. No luminal mucus or goblet cell metaplasia	2	4.0
V	Bronchiolar epithelial hyperplasia with luminal mucus and/or goblet cell metaplasia. No alveolitis	23	6.5
VI	Bronchiolar epithelial hyperplasia with luminal mucus and/or goblet cell metaplasia. Alveolitis	11	5.8
I–VI		54	6.1

from the linear pulse/velocity relationship in the test without the mask. The blood lactate (LA, mmol l^{-1}) response to exercise without a mask was expressed as V at LA=4 mmol l^{-1} (V_{LA4}), extrapolated from the exponential LA/V relationship.[17,18,22] Respiratory minute volume ($\dot{V}E$, l min^{-1}), measured with a flowmeter (GD-100, Fluid Inventor, Stockholm), and expired oxygen and carbon dioxide concentrations, determined by mass spectrometry (Centronic 200 MGA, Kjellbergs Successors, Stockholm), were recorded continuously. Oxygen uptake and VE at V_{200} ($\dot{V}O_2$-200, ml min^{-1} kg^{-1} and $\dot{V}E$-200, l min^{-1} kg^{-1}) were calculated and the respiratory rate (RR) was monitored. Tidal volume (TV) was calculated from $\dot{V}E$ and RR. As relationships between V and TV were not consistent,[17] we report the mean exercise value of TV. The total red cell volume (CV), calculated from the Evans blue dye space (PV = plasma volume), and the haematocrit were determined after mobilization of the splenic erythrocyte reservoir by the exercise test.[16]

Statistical analysis

Using a computer and the SAS program, tests of differences between groups were carried out with Student's t-test and functional/morphological relations were analysed using the ANOVA procedure. Mean values and standard deviations (SD) are presented unless otherwise stated.

RESULTS

Pathology

There were 3–12 bronchioles in the biopsies from each horse. The bronchioles were <300 µm in diameter in 95% of those measurable (89%). Peribronchiolitis and alveolitis of minimal degree were features of 80% and 27%, respectively, of the controls and were therefore disregarded in the COPD horses. After consideration of all other changes found, we designed a grading system dependent on certain obligatory morphologic features and including 6 categories of increasingly severe pathology (Cat. I–VI, Table 2). Grading was carried out independent-

Table 3. *Age (years), bodyweight (BW, kg), plasma volume/BW (PV/BW, ml kg^{-1}), red cell volume/BW (CV/BW, ml kg^{-1}), treadmill velocity at pulse 200 (V_{200}, m s^{-1}), treadmill velocity at blood lactate 4 mmol l^{-1} (V_{LA4}, m s^{-1}), oxygen uptake/BW at V_{200} ($\dot{V}O_2$-200/BW, ml min^{-1} kg^{-1}), respiratory minute volume/BW at V_{200} ($\dot{V}E$-200/BW, l min^{-1} kg^{-1}), exercise tidal volume/BW (TV/BW, ml kg^{-1}), and ventilatory equivalent at V_{200} ($\dot{V}E/\dot{V}O_2$-200) in COPD-afflicted and reference Standardbred trotters and saddle horses*

$\bar{X}$ = mean value; SD = standard deviation; N = number of horses; p = degree of significance

	Standardbred trotters								Saddle horses		
	Reference group				COPD group				COPD group		
	$\bar{X}$	SD	N	$p<$	$\bar{X}$	SD	N	$p<$	$\bar{X}$	SD	N
Age	5.3	3.0	152		6.4	3.0	34	0.001	9.9	2.6	19
BW	433	35	152		436	26	34	0.001	511	89	20
PV/BW	52.2	4.6	152		52.3	4.4	34	0.05	49.6	4.7	20
CV/BW	68.7	11.5	152	0.001	80.8	19.0	34	0.001	61.2	8.4	20
V_{200}	8.42	0.95	152		8.22	0.79	34	0.001	7.41	0.75	20
V_{LA4}	8.64	1.19	130		8.31	1.21	34	0.001	7.38	0.73	19
$\dot{V}O_2$-200/BW	109	16	91		104	13	12	0.05	92	12	9
$\dot{V}E$-200/BW	2.53	0.37	91	0.05	2.30	0.32	12		2.25	0.54	9
TV/BW	26.8	3.9	48	0.01	23.9	2.5	12		21.7	2.5	9
$\dot{V}E/\dot{V}O_2$-200	23.4	2.8	91		22.2	2.4	12		24.6	5.8	9

ly of BEH values. The mean numbers of bronchioles examined varied only somewhat betwen categories (Table 2). Besides features of each category inherent by definition, bronchiolitis was found in all cases in Cat. IV and VI, 86% of cases in Cat. III and 87% of cases in Cat. V; peribronchiolitis in all cases of Cat. II, IV, V, and VI, and 86% of cases in Cat. III; and alveolitis in 29% of cases in Cat. III. Mean values for BEH_{max} and BEH_{mean} were significantly higher in Cat. IV–VI than in Cat. I–III ($p<0.001$), and means for BEH_{max} increased significantly with category order (Fig. 1).

Exercise tolerance related variables

As seen in Table 3, both body weight (BW) and age were lower in the Standardbreds than in the saddle horses. The effects of COPD differed between the two types of horse. In the Standardbreds, CV/BW was 18% larger in COPD subjects than in the reference horses, and $\dot{V}E$-200/BW and TV/BW were less. In the saddle horses, means of PV/BW and V_{200} were lower in COPD subjects than in the controls. In both breeds, V_{LA4} and $\dot{V}O_2$-200/BW were normal in comparison with the respective reference group. In the COPD-afflicted saddle horses, PV/BW, CV/BW, V_{200}, V_{LA4} and $\dot{V}O_2$-200/BW were lower than in the Standardbreds with COPD, although PV/BW, V_{200}, and $\dot{V}O_2$-200/BW did not differ significantly between the breeds in the normal horses. The ventilatory equivalent at V_{200} ($\dot{V}E/\dot{V}O_2$-200) in COPD subjects was similar in both breeds and agreed with that of the Standardbred controls.

Relationships between histopathology and exercise tolerance related variables

As seen in Table 4, in the Standardbreds but not in the saddle horses, $\dot{V}O_2$-200/BW and $\dot{V}E$-200/BW were significantly inversely related to morphological grading, and $\dot{V}O_2$-200/BW and TV/BW were significantly in-

	Reference group		
$p<$	$\bar{X}$	SD	N
	9.3	3.5	39
	524	62	40
0.01	53.4	4.7	40
	61.6	8.3	40
0.01	8.17	0.86	18
	7.72	0.56	18
	102	–	3
	2.47		3

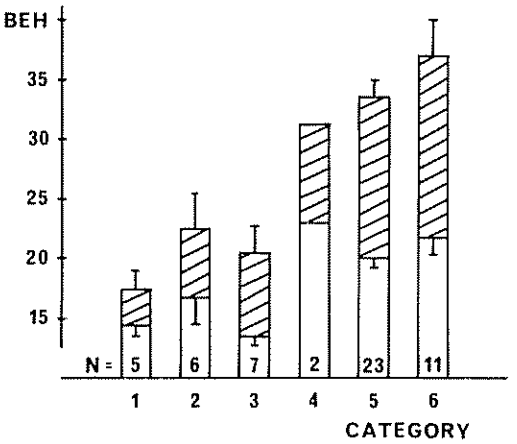

Fig 1 Relationship between bronchiolar epithelial height (BEH, μm) and morphological grading (categories 1–6) in lung biopsies. Means of values and standard deviations are shown for each category. Empty bars = mean height (BEH_{mean}); hatched bars = maximal height (BEH_{max}); N = number of horses

versely related to BEH_{max} and BEH_{mean} respectively. Significance was, however, also reached when both types of horses were studied together and in this case $\dot{V}O_2$-200/BW and TV/BW also correlated inversely with both BEH variables. Morphological grading, BEH_{max} and BEH_{mean} were independent of age and breed and did not correlate with $\dot{V}E/\dot{V}O_2$-200.

DISCUSSION

The pathology of the conducting small airways ranged from peribronchiolitis alone to marked chronic bronchiolitis with epithelial hyperplasia and metaplasia. The lesions are in accordance with those described repeatedly in COPD.[2,23,24] Though small airway disease involves the lungs diffusely in this syndrome,[24] the sampling error in biopsy examinations must be considered. Viel,[24] using a semi-quantitative grading system, considered biopsy reliable in reflecting the grade of disease in the lung as a whole. However, in a study of mild clinical disease only, biopsy diagnosis was less accurate[8] and it seems clear that biopsies with only minor lesions must be interpreted cautiously.

Since we were interested in the possible relevance of biopsy morphology in relation to exercise tolerance, we found it necessary to use a simple grading system with objective criteria for grouping. The different categories probably represent variations in progression and severity of the same principal morphological entity and not different subsets of airway disease. The height of the bronchiolar epithelium was used as an additional variable independent of grouping. Conducting airways of the orders found in the biopsies normally display a simple low columnar or cuboidal epithelium. The marked difference in BEH between categories with and without bronchiolar epithelial hyperplasia indicates that this variable essentially denoted hyperplastic change. The increase of BEH_{max} with category order suggests that the degree of hyperplasia increased along with the overall severity and complexity of the pathology, a

Table 4. *Exercise tolerance-related variables (abbreviations as in Table 3) with correlations to morphologic grading (Category, see Table 2) and to maximal and mean bronchiolar epithelial height (BEH_{max} and BEH_{mean}, μm) in lung biopsies in COPD-afflicted horses*

* $p<0.05$, ** $p<0.01$ N = number of horses

		$\dot{V}O_2$-200/BW	$\dot{V}E$-200/BW	TV/BW	$\dot{V}E/\dot{V}O_2$-200
All horses	Category	−0.56**	−0.45*	−0.22	−0.05
$N=21$	BEH_{max}	−0.61**	−0.41	−0.44*	−0.01
	BEH_{mean}	−0.48*	−0.30	−0.57**	−0.01
Standardbreds	Category	−0.61*	−0.64*	0.11	−0.14
$N=12$	BEH_{max}	−0.72**	−0.52	−0.31	0.15
	BEH_{mean}	−0.47	−0.39	−0.60*	0.02
Saddle horses	Category	−0.25	−0.34	−0.53	−0.23
$N=9$	BEH_{max}	−0.29	−0.39	−0.33	−0.32
	BEH_{mean}	−0.36	−0.23	−0.45	−0.14

finding corroborating previous results of studies on whole lungs.[25]

Our results indicate that clinically mild or moderate COPD can negatively affect performance potential. The markers used for respiratory and cardiocirculatory functions have previously been postulated to reflect aerobic power, pulmonary ventilation capacity and anaerobic threshold in the horse.[17,18] In addition we used $\dot{V}E/\dot{V}O_2$-200 as a marker for the gas exchange efficiency during work at the anaerobic threshold and CV/BW as an expression for both the cardiovascular dimension and the oxygen transport capacity. We expressed HR, blood lactate, and respiratory gas exchange responses to exercise as values at a work load corresponding to HR=200 bpm and LA=4 mol l^{-1}, respectively, since this represents a near maximal, mainly aerobic work level attainable for all horses.[17,18]

Although severity of disease as judged from lung biopsy morphology was similar in the Standardbred and saddle horse groups, exercise tolerance data differed. The most striking result was the red cell hypervolaemia found in the Standardbreds. This sequel, previously reported in association with overtraining, exercise-induced pulmonary haemorrhage and T-wave abnormalities in the ECG, has been considered to be compensatory for tissue hypoxia caused by cardiorespiratory dysfunction during heavy exercise.[16,17,19] In contrast, CV/BW was not changed in the saddle horses. Littlejohn et al.[11] reported normal haemoglobin values in horses with COPD and haematocrits have been reported to be slightly increased only in severe cases of COPD.[6,7] We interpret the difference between Standarbreds and saddle horses in the effect of COPD on CV/BW as probably being due to prolonged and intense training for racing in the former breed despite respiratory distress; conditions not pertaining to the diseased saddle horses. This could explain the breed differences regarding the other training dependent exercise tolerance related variables as well.

The normal HR response to submaximal exercise in COPD-afflicted Standardbreds in this study agrees with previous studies of mild small airway disease.[1,13,20] The excessive HR response in the diseased saddle horses may reflect diminished physical activity required of these horses on account of their illness, as compared with the control horses which were exercised regularly.

It is likely that epithelial hyperplasia of the small airways would restrict air flow. This assumption seems valid as $\dot{V}E$-200/BW was

diminished in the COPD horses, at least the Standardbreds, due to a reduced exercise TV, and respiratory gas exchange variables correlated inversely with pathology grading and BEH in the Standardbreds. Further, oxygen uptake capacity is probably reduced, as the ventilatory equivalent was normal in both breeds.

In conclusion, even mild or moderate COPD seems to limit pulmonary ventilation and oxygen uptake during exercise, leading to a compensatory increase in CV in regularly trained and raced Standardbreds but not in physically less active saddle horses. In the former, gas exchange variables appear to be dependent on the severity of lung biopsy pathology, including the degree of small airway epithelial thickening, whereas HR and blood lactate responses to submaximal exercise are unaffected. In saddle horses an imposed inactivity may lead to an excessive HR during exercise. Overall, exercise intolerance seems poorly predictable from the degree of pathology in lung biopsies in saddle horses.

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