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Physiologic changes during maximal treadmill exercise of poorly performing Standardbred horses with or without tracheal blood post–exercise

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Summary

From a group of 46 young racing Standardbreds presented for problems of decreased athletic performance, eight with complete physiologic data were found to have blood in the trachea (POS) within one hour post-maximal exercise test on a high speed treadmill. This group was compared to those horses from the larger group of 46 which had no tracheal blood post-treadmill exercise (NEG). Parameters measured during the treadmill test included maximal speed (V_{max}), heart rate (HR_{max}), respiratory and stride frequency (RF, SF respectively) at maximal speed, aerobic capacity ($VO_{2\ max/peak}$), and post-exercise plasma lactate concentration (Lac). Within one hour of the exercise, bronchoscopy and bronchoalveolar lavage were also performed. Of the physiologic parameters measured there were no differences between the groups in V_{max}, HR_{max}, SF, $VO_{2\ max/peak}$, or Lac, but there was a significant decrease in RF at end exercise (85±5.9 versus 96±10.9 breaths per minute, $p<0.05$) in the POS group of horses. The bronchoalveolar findings were suggestive of increased airway inflammation in more of the POS horses, whereas all horses had hemosiderophages present.

This study suggests that some horses with post-exercise blood in the trachea may have an associated decrease in respiratory rate at the point of fatigue during exercise. However, it also brings into question the validity of using tracheal blood as the sole criterion for sanctioned, yet empirical treatment of race horses for exercise-induced pulmonary haemorrhage.

Keywords: exercise test, horse, tracheal blood, EIPH

Physiologische Veränderungen während maximaler Belastung auf dem Laufband bei leistungsschwachen Trabern mit oder ohne Blutansammlung in der Trachea nach Belastung

Von 46 Trabern im Renntraining im Alter von 2 bis 4 Jahren, die wegen verminderter Leistungsfähigkeit auffielen, zeigten 8 Tiere innerhalb einer Stunde nach einer maximalen Belastung auf dem Laufband (Aufwärmphase, 3 min bei 4 m/s, dann 8 m/s und Steigerung um 1 m/s bis maximal 13 m/s) Blutablagerungen in der Trachea (POS). Von den übrigen wurden viele Tiere wegen unvollständiger Daten nicht in die Studie einbezogen, sodaß zum Vergleich ebenfalls 8 Tiere ohne tracheale Blutung nach maximaler Belastung auf dem Laufband (NEG) zur Verfügung standen. Während der Belastung wurden folgende Parameter gemessen: die maximale Laufgeschwindigkeit (V_{max}), die maximale Herzfrequenz (HR_{max}), Atem- und Schrittfrequenz (RF und SF) bei maximaler Laufgeschwindigkeit, aerobe Kapazität ($VO_{2\ max/peak}$) sowie die Laktatkonzentration im Plasma nach Belastung (Lac). Innerhalb von 30 bis 60 Minuten nach Belastungsende wurde eine endoskopische Untersuchung der Trachea mit anschließender bronchoalveolärer Lavage durchgeführt.

Es gab keine Unterschiede bezüglich der gemessenen Vmax, HR_{max}, SF, $VO_{2\ max/peak}$ oder Lac zwischen den Gruppen. Die POS-Gruppe wies jedoch eine signifikant geringere mittlere Atemfrequenz bei Belastungsende (85±5,9 versus 96±10,9 Atemzüge / min; $p<0,05$) auf. Die bronchoalveoläre Lavage zeigte bei mehr POS- als NEG-Tieren deutliche Anzeichen einer Entzündung der Luftwege, die Lavageflüssigkeit aller Pferde wies jedoch, außer in einem Fall, Hämosiderophagen und freie Erythrocyten auf.

Die Studie legt nahe, daß Pferde mit trachealer Blutung nach Belastung eine verminderte Atemfrequenz zum Zeitpunkt des Belastungsabbruches infolge von Ermüdung haben. Außerdem stellt sich die Frage, ob eine tracheale Blutablagerung eine ausreichende Indikation für eine Behandlung des belastungsinduzierten Lungenblutens darstellt.

Schlüsselwörter: Leistungstest, Pferd, tracheale Blutung, belastungsinduziertes Lungenbluten

Introduction

Despite increasingly intense investigation (*Erickson,* 1995), exercise induced pulmonary haemorrhage (EIPH) in horses has remained an enigma for veterinary scientists. Post-exercise endoscopic examination of the trachea for evidence of blood is the current accepted method to diagnose EIPH. Using this method, it has been shown that up to 80 percent of all race horses bleed into the airways during intense exercise (*Sweeney* and *Soma,* 1983). Hemorrhage associated with exercise appears to originate mainly from the caudal lung lobes (*O'Callaghan* et al., 1987), sites considerably distant from the tracheal view as examined by endoscopy.

The apparent severity of bleeding into the lower respiratory tract as judged by endoscopy can be highly variable and appears inconsistently ; horses previously shown to have post-exercise tracheal blood do not consistently show this sign after every race (*Collatos,* 1995). Further, while some horses may win races despite showing evidence of blood in the trachea, suggesting little relationship with athletic performance (*Collatos,* 1995), severe respiratory impairment, and even death (*Gelberg* et al., 1985) have also been associated with lung haemorrhage during intense exercise. Thus, there appears to be divergent thought on the relationship of finding post-exercise tracheal blood to athletic performance. It is indeed possible that sole use of post-exercise tracheal blood may be an imprecise marker of performance limiting events occurring in the lung parenchyma. The purpose of the study reported herein was to compare and contrast, based on standard physiologic parameters of athletic performance from a treadmill exercise test, horses with without tracheal blood post-exercise.

Materials and methods

Horses presented were from a group of horses presented to our facility between 09/94 and 06/95 for clinical evaluation of reduced athletic performance. All were Standardbreds, 2 to 4 years old, and racing or in training, with chief complaints of reduced athletic performance, either associated with recent respiratory viral infection, or suspected EIPH. Further, they were reported to be clinically normal and had no reported lameness problems. Horses were weighed, then had the safety harness, their racing hopples and heart rate electrodes fitted on them. After adjustment to the treadmill, each horse underwent an incremental maximal exercise test based on a previously published protocol (*Geor* et al., 1995). Briefly, after a warm up of 2 min at 1.8 ms^{-1}, followed by 3 min at 4 ms^{-1}, the horses were taken to 8 ms^{-1} and run for one minute, and had speed progressively increased by 1 ms^{-1} each minute through to 13 ms^{-1} or until the horse could no longer maintain its position on the treadmill despite humane encouragement. Treadmill angle was maintained at 3°C throughout the exercise. Measurements taken on each horse during treadmill exercise included; oxygen consumption (VO_2), maximal speed (V_{max}) (including the time maintained at the highest running speed), respiratory frequency at V_{max} (RF), stride frequency at V_{max} (SF), and post exercise plasma lactate (Lac). VO_2 was measured using an open flow through system (Oxymax, Columbus Instruments, Columbus Ohio) which was validated each day of use by the nitrogen dilution technique (*Fedak* et al., 1981). Oxygen and carbon dioxide sensors were calibrated daily with a certified gas mixture of 20.5% oxygen and 5% carbon dioxide, and the zero calibration performed with 100% nitrogen.

RF and SF were determined, respectively, by signals from a nasal thermistor and a highly damped accelerometer on the dorsal wall of the left front hoof (Scientific Solutions, Eden Mills, Ontario), fed into a multichannel physiograph (Gould) as previously described (*Young* et al., 1995). Heart rate was recorded with a computerized onboard heart rate monitor (Equine Performance Technology, Seven Valleys PA). Blood samples were drawn from the jugular vein into sodium fluoride/ potassium oxalate tubes immediately prior to exercise and within 2 minutes of the completion of the exercise test. These were held on ice until processing (within one hour) by centrifugation at 1500 g (Beckman TJ-6 Centrifuge) for 10 minutes, and the plasma analyzed for lactate concentration within 5 minutes by an autoanalyser (Model 705, Hitachi Canada Inc. Scarborough Ontario; lactate reagents, Boehringer Mannheim; Laval Quebec).

Endoscopy and bronchoalveolar lavage (BAL)

Within 30 to 60 minutes post-exercise, endoscopic examination of the trachea to the level of the carina was performed. Horses were recorded as tracheal blood positive (POS) if there was readily observed blood free on the mucosal surface at any site in the trachea, from immediately distal to the laryngeal structures to the carina. Lack of observed blood at this examination resulted in the horse being placed in the tracheal blood negative (NEG) group. This sole criterion was chosen to distinguish between the two groups of horses because it the currently accepted mode for determining EIPH in race horse for the purposes of sanctioned prerace treatment with furosemide.

Following endoscopy, a bronchoalveolar lavage was then performed, using a commercially available cuffed tube (Equine bronchoalveolar lavage catheter, Bivona Inc., Gary IN) with 5 aliquots of 60 ml 0.9% sterile saline at room temperature. Approximately 60 to 70% of the fluid was recovered using this method (data not shown), with any coloration to the recovered fluid recorded. Subsequent laboratory analysis was performed on a cytocentrifuged preparation (Shandon Cytospin 2, VWR Scientific, Montreal Quebec) of an aliquot of the recovered fluid, stained with Wright Giemsa (Miles Hema-Tek 1000 stainer) with a cell differential count obtained from counting 500 cells.

Data analysis

The physiologic parameters of the tracheal blood-positive group to the tracheal blood-negative groups were compared by t-test, using a statistical software package (Statmost, 1994 DataMost Corp.), with significance set at $p<0.05$. Broncholaveolar lavage results are reported on an observational basis only.

Results

Of 46 horses examined over this time period, 12 were found to have tracheal blood after a maximal treadmill exercise. Of these, only 8 had sufficiently complete physiologic data for comparative evaluations, as technical problems resulted in loss of oxygen consumption data on 12 of the 46 horses. Of the remaining horses in the NEG group, 8 horses had complete data available from all aspects of the examination to allow comparison to the same parameters from the 8 completed POS horses with fully complete data on their exercise tests. Of the 26 horses in the NEG group that were excluded from comparison, five were found during the course of the evaluation to have underlying disease; two were noted to be lame on treadmill work, one had an upper respiratory noise at work, one was recovering from pleuritis and one had a history of chronic obstructive airway disease. Three trotters in this group were excluded because it was considered inappropriate to compare them to pacing horses. Three other horses refused to work at high speed on the treadmill. The remaining 15 horses in the NEG category were excluded because of one or more technical problems that occurred during the testing procedure, resulting in missing physiologic data, yet unrelated to the animal.

Of the parameters measured, only the respiratory frequency at maximal effort (RF) was found to be significantly less in the POS group in comparison to the NEG group (Tab. 1). The remaining measurements had high individual variation and considerable overlap between the two groups. Based on previous work on this horse population (*Geor* et al., 1995), the aerobic capacity of both groups of our study horses was clearly below our established normals (Tab. 1). Further, while the running speed attained may appear higher in the NEG group, the difference was small and not significant when the number of seconds at the highest running speed was used in the calculations; the end of 11 ms^{-1} being only seconds away from moving into 12 ms^{-1}. The BAL results also show considerable variation between horses (Tab. 2). More of the POS horses had grossly discolored fluid from the lavage. Further, while all horses but one in the NEG group had hemosiderophages and free red cells in the lavage, there was a subjective impression of larger percentages of both cell types in the POS group. Unfortunately, quantitative assessment was not done on this aspect of the cytology. The cytologist, however, was unaware of the results of the exercise test or bronchoscopy .

Tab. 1: Physiologic parameters from a treadmill exercise test in horses with (POS) and without (NEG) tracheal blood visible on post-exercise endoscopy.

Tracheal blood (n)	$VO_{2\ max/peak}$ ml.kg.$^{-1}$min^{-1}	V_{max} ms^{-1}	HR_{max} beats.min^{-1}	RF resp.min^{-1}	SF min^{-1}	Lac mmol.L^{-1}
POS (8)	123.7±11.9	end 11±0.4	225±8.8	85±5.9[a]	130±5.5	10.7±5.0
NEG (8)	123.8±10.8	12±1.1	216±13.5	96±10.9[a]	128±6.7	10.1±4.7
Population normals[b](8)	144±3.4	end of 11 m.s^{-1}	215±8	101±3	127±4	9.4±0.8

$VO_{2\ max/peak}$ =maximal /peak oxygen consumption; V_{max} =maximal treadmill speed; HR_{max} = maximal heart rate; RF = respiratory frequency at V_{max}; SF = stride frequency at V_{max}; and Lac= plasma lactate concentration post-exercise. Data are mean ± SD; *Geor* et al. data are ± SE. The third group is included from a separate study from similarly aged horses for comparison to the background population for the region, but which were considered to have no performance problems. Unfortunately, endoscopy post exercise was not performed in this group.

[a] significantly different ($p \leq 0.05$)

[b] *Geor* et al., 1995

Tab. 2: Bronchoalveolar findings in horses with and without blood in the trachea after treadmill exercise

	Gross appearance	Cytology		
		Free red cells	Hemosiderophages	Cell differential
POS (n=8)	4 pink/tan, 4 cloudy, colourless	8 +, abundant	8+	2> 15% neut, 4> 40% lymph
NEG (n=8)	1 pink/tan, 7 cloudy, colourless	7+	7+	5> 40% lymph
Normal[4] (n=62)	cloudy, colourless	present[5]	present[5]	mac[1] 59%±10%, lymph[2] 31%±9%, neut[3] 9%±6%

[1] Macrophage ± 95 percentiles, [2] Lymphocyte ± 95 percentiles, [3] Neutrophil ± 95 percentiles

[4] *McKane* et al. 1993, [5] both increase with age

We observed higher percentages of lymphocytes in both groups than recently reported for horses in racing (*McKane* et al., 1993). Elevated neutrophils in the BAL were observed in two of the horses in the POS group, but in none of the horses in the NEG group.

Discussion

We were initially surprised to find that some of our study horses showing tracheal blood post exercise, as this is said to be diagnostic for EIPH (*Sweeney* and *Soma,* 1983). *Erickson* et al. (1990) also previously reported the presence of blood in the tracheal in 3 of 7 horses after undergoing high intensity exercise. They found no differences in intrathoracic or vascular pressures between the two groups, but in contrast to our work they had fewer animals to compare, and examined differing physiologic parameters. Therefore, post-treadmill exercise endoscopy warrants consideration for inclusion in future studies, as there is currently no repeatable model of EIPH with which to study this syndrome, and its use may assist in more accurate categorization of horses under study.

Our finding of a significant reduction of end exercise respiratory rate in the POS group may point to a significant physiologic change in these horses if they are experiencing EIPH. In a previous study of horses with no history of performance problems, we observed greatly decreased respiratory rates in the same horse at exercise when faced with increased respiratory effort with a closed face mask and valves, when compared to an open system that has minimal interference to breathing. (*Geor* et al., 1995). In a similar manner, problems within the lung, such as an acute bleeding episode may also manifest in such a reduction of respiratory rate. Additional information regarding mechanics of breathing during this change in respiratory frequency would be of great interest, but were not possible with our current system of measuring oxygen consumption with the open flow through system.

While it may appear that horses in the POS group had a lower running speed (Tab. 1) this was an insignificant difference and unlikely to be a factor in the difference in respiratory frequency observed in the POS versus the NEG groups of horses. Further, in our exercise protocol, the respiratory frequency is stable beyond 10 ms^{-1} for an individual horse (*Geor* et al. 1995, *MacMillan,* unpublished data).

As in most studies in equine exercise physiology, this study was plagued by high individual variation in physiologic readings obtained from each group. This included the range in respiratory rates at maximal exercise, with considerable overlap of values in the POS and NEG horses. Within the POS group, it was also clear that some horses had an aerobic capacity in excess of those in the NEG group; horses with poor performance of no detectable cause, yet did not show endoscopically visible blood . This is likely a reflection of the large overlap in many of the currently measured indices used to evaluate athletic potential. Despite this high variability it appeared that there was a far lower end exercise respiratory rate in some of the horses in the POS group that contributed to the statistical difference between the two groups. Therefore, it is possible that only a portion of the POS horses; those with markedly lowered end exercise respiratory rates has clinically significant EIPH causing this change. However, we currently have no readily available method to determine the severity of EIPH in a clinical setting.

To avoid this problem of individual horse variation that plagues many such studies, it would be preferable to have baseline information on horses when they are performing optimally, with which to compare when they suffer reduced performance. Serendipitously, one of the horses in the POS group was previously examined on the treadmill for a separate study (*MacMillan* et al. 1996). In the intervening time this horse had suffered from a respiratory viral infection and was reported to have performance problems persisting after the resolution of clinical and into resumption of race training. The changes observed in this horse in the exercise test after which blood in the trachea were observed were dramatic, with marked reduction in aerobic capacity from a previous high of 156 $ml.kg^{-1}$ min^{-1} VO_2 peak to a peak of 129 $ml.kg.^{-1}$ min^{-1}. Further, while her respiratory frequency at high speed was the same at 92 breaths per minute for both tests, during the exercise test after which tracheal blood was observed , there was a sudden and dramatic decrease in the respiratory frequency to 78 per minute at the highest speed attained. At this point the horse also appeared very distressed. It is tempting to speculate that the blood in the trachea noted on the second exercise period was associated with both reduced aerobic capacity and end exercise respiratory frequency. Unfortunately, neither post-exercise endoscopy, nor BAL were performed when this horse was in the first study (*MacMillan* et al., 1996).

As tracheal blood is only a marker of possible events deeper in the lung, not all horses in the POS group may have had exercise limiting EIPH. It seems therefore, a more specific mode of diagnosis would help refine testing such as this. Our addition of BAL to the evaluation of these horses was intended to assist in providing more such information of these more distal lung events (*Viel* 1983). However, frank blood and cytological evidence of bleeding were found in both groups of horses. Subjectively, there was more evidence of both bleeding and inflammation in the POS group.

The results of this study suggest there may be a change in end-exercise respiratory rate in horses that suffer EIPH, as diagnosed by post-exercise endoscopic examination of the trachea. However, it also suggests the need to uncover more definitive diagnostic criteria for EIPH, rather than solely basing its diagnosis on post-exercise endoscopy.

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