Effects of pre-exercise intrapulmonary blood inoculation on equine pulmonary function during supramaximal exercise

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Abstract
This study was designed to determine the effect of 200 ml of autologous blood instilled in the lungs of healthy horses had on gas exchange, maximal oxygen consumption ($\text{VO}_{2\text{max}}$) and breathing mechanics during supramaximal exercise, as a model of exercise-induced pulmonary haemorrhage (EIPH). The subjects were six healthy well-conditioned Thoroughbred horses. On four occasions over a 6-week period, six horses were subjected to two bouts of exercise to fatigue per day, each at speeds equated with an oxygen requirement that was 110% of $\text{VO}_{2\text{max}}$. Prior to the second bout of exercise on each day, the horses underwent bilateral bronchoscopy during which either nothing (control), or 200 ml of saline, plasma or blood was inoculated into the distal airways, divided equally between each lung. Run time to fatigue, $\text{VO}_{2\text{max}}$ and measurements of pulmonary gas exchange and breathing mechanics were made and analysed for the effects of the four treatments (control, saline, plasma and blood). Inoculation of blood significantly reduced pulmonary oxygen exchange, $\text{VO}_{2\text{max}}$ and run time to fatigue. Plasma inoculation caused intermediate effects, adversely affecting arterial oxygen tension but without significantly reducing $\text{VO}_{2\text{max}}$ or run time to fatigue. Saline treatment had no effect. None of the treatments had an effect on ventilatory mechanics. The results of this study suggest that volumes of blood in the order of 200 ml can impair gas exchange and interfere with the ability of horses to exercise at supramaximal intensities. Blood volumes of this magnitude are readily evident endoscopically, and probably reflect a moderate-to-severe level of EIPH, in contrast to previously published studies where the unilateral instillation of 100 ml of blood or less did not affect performance.

Keywords: equine; EIPH; supramaximal exercise; breathing mechanics; gas exchange; blood inoculation

Introduction
Most research on exercise-induced pulmonary haemorrhage (EIPH) has centred around the aetiology and possible prevention of the condition, rather than its effects on lung function and exercise tolerance. It has been presumed that EIPH interferes with the ability of racehorses to perform, and a recent study supports this contention, with higher grades of EIPH associated with reduced competitiveness8. Although it has been demonstrated that the prevalence of pulmonary haemorrhage among Thoroughbred racehorses is more than 90% when based on a single post-race examination17, and virtually all racehorses are expected to have at least one episode of EIPH during a racing career of 3 years duration4,5,17, there is a debate about the volume of pulmonary haemorrhage required to impair athletic performance13,14,16,19. The volume of haemorrhage associated with grade III or grade IV EIPH (on a scale of 0–IV) remains unknown. Not all horses with higher grade EIPH (based on endoscopy) suffer from poor performance. One reason for this may be that the volume of haemorrhage...
vares between horses, and this may not be exactly reflected in the amount of blood seen with endoscopy of the large airways. Also, low volumes of blood may not have a significant impact on racing performance\textsuperscript{12}. The loss of racing performance in horses displaying episstaxis is usually dramatic, but the exact relationship between the volume of EIPH and capacity to compete remains undetermined. A previous study indicated that volumes of less than 100 ml did not affect supramaximal treadmill performance\textsuperscript{12}. Unfortunately, there is currently no accepted method of accurately determining the volume of blood associated with an episode of EIPH and this has made it difficult to assess the relationship between the volume of EIPH and performance. Therefore, the present study was designed to further evaluate the effect of a larger (greater than 100 ml) known volume of blood in the airways on pulmonary function and exercise performance when horses galloped on a treadmill at intensities on a par with those associated with hard training and racing.

\textbf{Materials and methods}

\textbf{Horses}

Six Thoroughbred horses with a mean age of 7 years (range 4–9) and mean weight of 480 ± 12.1 (SEM) kg (range 423–572 kg) with several years experience running on a high-speed equine treadmill were used. The complete history of the horses with respect to EIPH was not known, although occasional evaluation had failed to discern any endoscopic evidence of this condition. Two weeks prior to commencement of the study, each horse underwent an incremental exercise test on a 10% incline to determine its maximal oxygen consumption (\(\text{V}\text{O}_{2}\text{max}\)). The test consisted of a 3-min warm-up at 4 m s\(^{-1}\), followed by 1 min at each of 6, 8, 9, 10, 11 and 12 m s\(^{-1}\). Expired air samples were collected in the last 10 s of exercise at each speed, and analysed to determine the oxygen consumption (\(\text{V}\text{O}_2\)) and the carbon dioxide production (\(\text{V}\text{C}\text{O}_2\)) at each speed. Regression analysis of the linear portion of the \(\text{V}\text{O}_2\)-speed curve was used to calculate the speed equated with an oxygen requirement that was 110% of the \(\text{V}\text{O}_2\text{max}\) for each horse. The calculated speeds ranged from 10.8 to 12.2 m s\(^{-1}\).

\textbf{Experimental protocol}

The horses were each assigned a number corresponding to a particular pattern of procedures distributed in a Latin square arrangement. The procedures consisted of two exercise bouts on each of 4 testing days. Each exercise bout involved a 2-min warm-up at 4 m s\(^{-1}\) followed by running at their calculated 110\% \(\text{V}\text{O}_2\text{max}\) speed until fatigued. During each test, horses had arterial blood sampled and \(\text{V}\text{O}_2\), \(\text{V}\text{C}\text{O}_2\) and breathing mechanics measured in the last 15 s of the warm-up and each subsequent minute of high-intensity exercise. Prior to each exercise, bout bronchoscopy was conducted. On the first run of each day, nothing was instilled into the lung during bronchoscopy (baseline runs); however, prior to the second run (treatment run) either nothing (control), 100 ml of saline, 100 ml of autologous plasma or 100 ml of autologous blood was instilled into the most caudodorsal region of the caudal lobe of each lung. There was a rest period of 1.5 h for each horse between the baseline and treatment runs on each testing day. Testing days for each horse were spaced so that there was 1 week of rest following the control or saline inoculation tests and 2 weeks of rest following inoculation of either plasma or blood. Each horse received either a control or saline inoculation on the testing day 2 weeks after either blood or plasma inoculation. This pattern of performing the baseline and treatment runs on each testing day was conducted to provide assurance that baseline data for each testing day had not been affected by prior treatments. Plasma for inoculation was obtained from 500 ml of autologous whole blood that was collected into a bag containing sodium citrate and dextrose, 3 h prior to the first run of that testing day. Whole blood was collected from the carotid artery catheter at the time of inoculation and instilled into the airways immediately after its collection, without the addition of an anticoagulant.

\textbf{Instrumentation}

At the beginning of each testing day, horses were instrumented with an 18 g catheter placed in a left carotid arterial loop that had previously been translocated subcutaneously. A no. 7F Swan–Ganz catheter was introduced into the pulmonary artery \textit{via} the right jugular vein to provide measurement of mixed venous blood temperature. Heart rate measurements were obtained \textit{via} a bipolar telemetry system (Polar Electronics, Woodbury, NY) that was fitted to the horse using an elastic girth strap. The same bronchoscopic procedure was used with both the control and treatment protocols, with the bronchoscope being passed to the same position in the respiratory tree for each horse. This was ensured by video recording the passage of the endoscope on every occasion. Each horse was sedated with 0.6 mg kg\(^{-1}\) xylazine intravenously and then underwent bronchoscopy during which seven 2 ml volumes of 2% lidocaine were inoculated \textit{via} a 2.7 m long, 1.9 mm outer diameter polyethylene tube (Critchley Electrical Products, Silverwater, NSW, Australia) passed through the
biopsy channel of a 2.5 m long video endoscope (Olympus America, Melville, NY). The lidocaine was used to suppress coughing and was infused at the bifurcation of the trachea and at the first three branches of the lateral segmental bronchi of each caudal lobe. When indicated by the protocol schedule, saline, plasma or blood was infused via the polyethylene catheter prior to the second exercise bout of each testing day. Immediately after bronchoscopy, an oesophageal balloon catheter was placed via the right ventral nasal meatus so the balloon tip rested in the thoracic oesophagus caudal to the heart, enabling the measurement of transpulmonary pressure during exercise. The horse was then walked onto the treadmill and fitted with an open-flow mask system for collection of expired air samples and measurement of ventilatory airflow\(^2\).

**Measurements**

Aterial blood samples were drawn into heparinized syringes, capped and stored on ice to minimize alterations in gas partial pressures following sampling. Arterial blood gas tension (P\(_{\text{O}_2}\)) was determined using a self-calibrating portable blood gas analyser (Radiometer America, Westlake, OH) immediately after each run. Blood gas measurements were temperature corrected. The analyser automatically calculated the values for the percentage saturation of haemoglobin with oxygen (%O\(_{2}\)sat) and the oxygen content of the respective blood samples (C\(_{\text{O}_2}\)).

A venous blood sample was taken 5 min after exercise. These samples were transferred immediately to tubes containing lithium fluoro-oxalate to prevent coagulation and suspend the glycolytic activity of the erythrocytes. The tubes were stored on ice and centrifuged at the end of each day, and the plasma supernatant analysed for lactate concentration using an automated lactate analyser (Yellow Springs Instruments, Yellow Springs, OH).

An open circuit flow-through expired gas collection system with flow rates of 6000–7000 l min\(^{-1}\) was used to collect portions of the expired air into a large (1500 l) Tissot spirometer. Measurement of the O\(_2\) and CO\(_2\) concentrations of the mixed air within the spirometer was used to determine both VO\(_2\) and VCO\(_2\). This face mask design and use have previously been described in detail\(^5\,\,10\). Briefly, the animal was fitted with a bias flow face mask, and room air was drawn through side ports at a rate of at least 6000 l min\(^{-1}\) during exercise. At the end of each minute of exercise, a shutter-type-biased flow entry port on either side of the mask was briefly shut (5 s maximum) for measurement of ventilatory mechanics. With the closure of these ports, airflow was drawn through two identical 160 mm diameter pneumotachographs (Mercury Electronics, Glasgow, UK) and the pressure drop across one pneumotachograph was measured using a differential pressure transducer (Validyne Engineering, Northridge, CA) at a time when the biased flow had ceased. Transpulmonary pressures were measured with a differential pressure transducer (Validyne Engineering, Northridge, CA) by comparing the pressures in the mask cranial to the nares with pressures recorded from the oesophageal balloon catheter. The pressure and flow signals were phase matched to 10 Hz and recorded digitally. Breathing frequency, tidal volume, minute ventilation, peak inspiratory and expiratory flow rates, maximum change in transpulmonary pressure, total pulmonary resistance, dynamic compliance and work of breathing were all determined using a physiological data analysis system (Laboratory Software Associates, Heidelberg, Victoria, Australia). Alveolar ventilation and alveolar oxygen tension (P\(_{\text{A}_2}\)) were calculated from the respective standard equations. The alveolar–arterial oxygen tension difference (AaDO\(_2\)) was taken to be the difference between P\(_{\text{A}_2}\) and P\(_{\text{A}_2}\).

**Analysis of data**

All data were expressed as mean ± SEM. First, the data from all the treatment runs for each horse were analysed using a two-way repeated measures ANOVA and the Student–Newman–Keuls *post hoc* pairwise comparison was used to identify significantly different means. Secondly, the data collected at the end of the first minute of exercise at 110% VO\(_{2\max}\) for each treatment run were compared using a one-way ANOVA and Student–Newman–Keuls *post hoc* analytical test.

In an additional analysis, the data collected at the end of 60 s of exercise at 110% VO\(_{2\max}\) for the four baseline runs were compared using a one-way ANOVA to assess whether any differences existed, which could have been attributable to residual effects of prior treatments. Finally, data gathered at the end of 60 s of exercise at 110% VO\(_{2\max}\) for each of the four treatment runs were compared with those from the corresponding baseline run using paired *t*-tests, to check further for changes associated with the treatments. All statistical analyses used a commercial analysis package (SigmaStat 2.05, SPSS Science, Chicago, IL), with significance for all tests set at *P* < 0.05.

**Results**

There were no significant differences between the means of the four baseline recordings for any of the measurements taken during the course of the study. Blood inoculation resulted in decreases in P\(_{\text{A}_2}\) and %O\(_{2}\)sat (Table 1). VO\(_{2\max}\) and run time to fatigue both decreased significantly from the pre-blood inoculation baseline values of 155.4 ± 5.7 ml min\(^{-1}\) kg\(^{-1}\) and 131.5 ± 18.9 s, to the post-blood inoculation values of
148.7 ± 5.0 ml min⁻¹ kg⁻¹ and 116.5 ± 18.1 s, respectively. However, neither alveolar ventilation nor \( P_AO_2 \) was altered by this treatment although \( AaDO_2 \) was wider (Table 1). Plasma inoculation was associated with a lower \( P_AO_2 \) and a greater \( AaDO_2 \), but had no other significant effects. The magnitude of these changes was less than those measured following instillation of blood (Table 1). Saline treatment had no effect.

Maximal heart rates and post-exercise plasma lactate concentrations did not differ over any of the eight exercise runs.

The fluid inoculations into the lung had no detectable effects on the ventilatory mechanics of the horses at rest or during intense exercise (Tables 1 and 2). Minute ventilation and heart rate values returned to pre-exercise levels before the second run.
commenced for all horses on each testing day. Run time to fatigue was not affected by exercising the horse twice in 1 day when the control, saline and plasma treatments were administered. No adverse clinical signs were seen in any of the six horses in the weeks following the inoculations.

**Discussion**

Infusion of 25, 50 and 100 ml of blood into the right lung immediately prior to exercise has been reported to have no impact on ventilation, gas exchange or supramaximal performance on a treadmill. A study examining the effects of intrapulmonary inoculation of autologous blood in resting horses found that repeated instillation of 400 ml of blood over a period of time resulted in decreased dynamic compliance and increased resistance. However, this difference appeared to be due to unusually high pre-infusion measurements of dynamic compliance in the group of horses treated with blood, as post-treatment compliance was not different from those in normal controls, and was similar to that recorded before and after the infusion of blood in our study. To further clarify these issues and to bring the relevance of previous work into clearer focus for the horse racing industry, the present study investigated the effects on pulmonary function during supramaximal exercise of intrapulmonary inoculation of 100 ml of blood into each lung.

Horses performing intense exercise develop arterial hypoxaemia. When \( P_{\text{O}_2} \) drops below c. 75 mmHg, this is usually associated with a significant reduction in %O\(_2\)sat. As this variable is a critical determinant of C\(_\text{O}_2\) and therefore, the volume of oxygen available for aerobic metabolism in the working muscles, any added further reduction to oxygen uptake at the alveolar–pulmonary capillary interface would be physiologically significant to the supramaximally exercising horse because it would reduce the amount of energy the horse could derive from aerobic metabolism. In other words, VO\(_{2\text{max}}\) would be lowered. In this study, infusion of 100 ml of blood into the dorsocaudal region of each lung was associated with an increase in the severity of exercise-induced arterial hypoxaemia and oxyhaemoglobin desaturation, and a lower C\(_\text{O}_2\).

At submaximal exercise intensities, increases in cardiac output may compensate somewhat for lowered C\(_\text{O}_2\). However, by definition, during maximal and supramaximal exercises there is no ability to further increase heart rate, stroke volume or the extraction of oxygen from the blood by working muscles. Hence, it logically follows that a reduction in C\(_\text{O}_2\) results in a reduced VO\(_{2\text{max}}\) as was demonstrated in this study.

Although the speed at which the horses ran was calculated to have an oxygen requirement that was 110% of the VO\(_{2\text{max}}\) under baseline, control and saline treatment conditions, the reduction in VO\(_{2\text{max}}\) following inoculation of blood into the airways had the effect of increasing the relative oxygen requirement of the exercise test to about 117% of the VO\(_{2\text{max}}\). Not surprisingly, this increase in exercise intensity was associated with a shorter run time to fatigue. This may account for the loss of speed in the latter parts of races that is commonly associated with episodes of EIPH.

Treatment had no effect on the partial pressure of carbon dioxide, minute ventilation, alveolar ventilation or \( P_{\text{O}_2} \), indicating that ventilation was not affected by any treatment. Also, there was no change in pulmonary mechanics due to any treatment although this was not unexpected, given that the tests used to assess the entire respiratory tract, whereas any mechanical changes associated with instillation of fluid would be relatively local. Inoculation of isolated regions deep within the lung is unlikely to significantly alter the total resistance of the lung, since it is the larger airways that provide the greatest resistance to airflow in the lung. Dynamic compliance was similarly unlikely to be greatly changed since it is a measurement of the entire lungs’ distensibility and reflects the sum of the compliance of many segments that have a much greater volume than the small regions inoculated with blood.

Although there was no indication that ventilation was compromised by any treatment, AaDO\(_2\) was significantly wider following the inoculation of blood and plasma, suggesting that while their presence in

<table>
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<th>Post-inoculation</th>
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the distal airways had no effect on ventilation, plasma and blood did interfere with the movement of oxygen from alveoli to erythrocytes in the pulmonary capillaries. Instillation of plasma had effects that appeared to be intermediate between those of saline and autologous blood. Following plasma infusion, VO_{2max} was decreased by 2.3% ($P = 0.08$) and run time to fatigue was c. 11% less ($P = 0.18$) as compared with reductions of 4.3 and 11.4%, respectively, after blood inoculation. The AaDO$_2$ was greater following inoculation with blood and this might at least partially explain the greater degree of hypoxaemia and haemoglobin desaturation observed with this treatment, and thus the more marked decrease in VO$_{2max}$. The effects of plasma infusion should not be discounted in view of the recently reported finding of evidence of pulmonary oedema in conjunction with episodes of EIPH\textsuperscript{18}.

The wider AaDO$_2$ was presumably a result of a greater diffusion limitation and/or greater mismatching of perfusion and ventilation secondary to blood in the alveoli and small airways. It was not possible to differentiate between the two as causes of the hypoxaemia observed in this study, although alveolar ventilation and $P_aO_2$ were not affected by treatment and it can be assumed that the distance over which oxygen had to diffuse was increased in the airways infused with blood and plasma.

The results of this study indicate that the presence of 100 ml of blood in each lung of strenuously exercising horses interfered with their ability to perform on a high-speed treadmill. How this finding relates to the impact of EIPH on performance of horses at the racetrack is not completely clear. Currently, there is no accurate way of determining the exact volume of haemorrhage present in the air spaces of the lung following a bout of EIPH. There was no worsening of the degree of exercise-induced arterial hypoxaemia when seven horses were exercised supramaximally on a treadmill 6 min after completing a bout of exercise known to induce EIPH\textsuperscript{15}. No estimate of the volume of EIPH was given in this report. However, extrapolation from data associated with the counting of red blood cells in bronchoalveolar lavage fluid collected soon after supramaximal treadmill exercise suggests that the volume of EIPH is probably considerably less than 200 ml in most cases\textsuperscript{6,11}. It has also been presumed that the volume of EIPH is linked to the grade of EIPH, and the results of a recent study demonstrate that there is probably a definite link between performance and EIPH. In that investigation of a large number of Thoroughbred racehorses, there was good evidence that horses with EIPH ≤ grade I were likely to perform significantly better than those with EIPH ≥ grade II\textsuperscript{9}. It has also been shown that infusions of 25, 50 and 100 ml of autologous blood into the right lung of six Thoroughbreds under conditions similar to those existing in this study were not associated with any significant reductions in oxygen exchange, VO$_{2max}$ or treadmill run time to fatigue\textsuperscript{12}. Therefore, while 100 ml of blood in each lung of horses impairs their ability to perform, the results of this study and those cited above suggest that the volume of EIPH may be critical in determining its effect on performance. It is widely accepted that epistaxis following strenuous exercise is an indication that EIPH of a relatively large volume has occurred, and it has been demonstrated that it has a negative effect on performance\textsuperscript{18,20}. It is expected that the time of onset of EIPH in relation to exercise is also a relevant factor. In the current study, 200 ml of blood was present in the lungs at the time when exercise began, and this may not be the situation in many, if any, naturally occurring cases of EIPH, although EIPH is quite common in racing Quarter Horses, which usually complete their event in less than 20 s\textsuperscript{7}. However, EIPH is definitely known to develop during exercise and, based on the results of this study, its impairment of the diffusion of oxygen across the alveolar–pulmonary capillary interface is likely to be significant if it is voluminous enough. Under these conditions, EIPH will interfere with the performance of the exercising horse.

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