Ventilatory responses of ponies and horses to exercise

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Abstract
Because athletic horses become hypoxaemic and hypercapnoeic during high-intensity exercise but ponies do not, six Thoroughbred horses and five ponies performed an incremental exercise test at speeds with calculated energy requirements that were 40, 60, 80 and 115% of VO$_{2\text{max}}$ with the objective of comparing their blood gas and ventilatory responses to exercise. Expired gas and blood samples were taken and breathing mechanics were assessed before exercise and during the last 15 s at each intensity. Maximal VO$_2$ and VCO$_2$ in horses were 153 ± 5 (SEM) and 187 ± 4 ml kg$^{-1}$ min$^{-1}$, respectively, while corresponding values in ponies were 92 ± 4 and 112 ± 7 ml kg$^{-1}$ min$^{-1}$ . During heavy and supramaximal exercise, horses, but not ponies, became hypoxaemic and hypercapnic. There was no significant difference for VE kg$^{-1}$ between groups during maximal exercise, but PAO$_2$, P aO$_2$ and PvO$_2$ were lower and P aCO$_2$ and [(A - aO$_2$)D] were greater in horses than in ponies. Additionally, the horses' maximal transpulmonary pressure difference was higher and their total pulmonary resistance and ventilatory equivalent lower than in ponies. Flow-volume loops suggested that horses experienced expiratory flow limitation but that ponies did not. These results indicated that horses like Thoroughbreds appear to be expiratory flow-limited and become hypoxaemic and hypercapnic when the demand for gas exchange associated with their high VO$_{2\text{max}}$ and VCO$_{2\text{max}}$ is greater than can be met by their ventilatory system. Ponies, which are less capable athletes, could better match their ventilatory response with their metabolic capabilities and so were able to maintain P O$_2$ in the pre-exercise range and decrease P aCO$_2$ to a tension that was more compatible with acid–base homeostasis.

Keywords: ventilation; gas exchange; exercise-induced hypoxemia and hypercapnia; expiratory flow limitation

Introduction
At work intensities ≥ ~ 65% of the maximum oxygen consumption (VO$_{2\text{max}}$), athletic horses like Thoroughbreds and Standardbreds exhibit arterial hypoxaemia and oxyhaemoglobin desaturation, while hypercapnia becomes evident at exercise intensities ≥ ~ 90% VO$_{2\text{max}}$ . In contrast, during exercise intensities of the same relative magnitude, ponies maintain arterial oxygen tensions close to those at rest and become hypocapnic . Reasons for these different responses have been postulated, but not definitively explained . The most widely accepted explanations are differences at the alveolar–pulmonary capillary interface (i.e. diffusion limitations) and differences in ventilation . In an effort to clarify why the pony and the horse have different blood gas responses to heavy and supramaximal exercise, we compared the blood gas and ventilatory responses of ponies and horses to exercise of varying intensities. We hypothesized that ponies and horses would exhibit different ventilatory responses to heavy and supramaximal exercise, thus contributing to the dissimilar blood gas responses observed between the two groups exercising at similar intensities.

Materials and methods

Experimental animals
Six healthy adult Thoroughbred horses (mean body weight 501 ± 27 kg) and five healthy adult ponies

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(mean body weight 164 ± 18 kg), ranging in age from 3 to 14 years, were studied. All animals were judged to be healthy by haemogram and physical examination, which included upper airway endoscopy. All subjects were acclimatized to standing and running on a high-speed equine treadmill (Sato, Equispeed Technologies, Kansas City, MO) and had gone through an intensive, controlled training programme for 1 month prior to the experiment. The treadmill was housed in a temperature- and humidity-controlled room.

Protocols/exercise tests
All exercise was conducted with the treadmill inclined at 10%. One week before the study, VO$_{2\text{max}}$ of each animal was determined using an open-circuit flow-through gas collection system and a previously described incremental step test$^{15}$. A regression equation was determined for the linear portion of the VO$_{2\text{max}}$-speed curve of each subject and used to estimate speeds yielding oxygen requirements that were 40, 60, 80 and 115% of VO$_{2\text{max}}$. These intensities were classified as mild, moderate, heavy and supramaximal, respectively. For the actual study, all animals completed consecutive 2 min steps at each of these pre-determined speeds, except at the supramaximal workload where they ran for at least 1 min. The treadmill was stopped when the subject could no longer maintain its speed. Arterial and mixed venous samples were taken immediately before exercise and during the last 15 s of exercise at each intensity. Expired gas was collected at these same times for the measurement of oxygen consumption (VO$_2$) and carbon dioxide production (VCO$_2$). After collecting these samples and before increasing the speed of the treadmill (or stopping it), signals were collected for assessment of breathing mechanics$^{5,14}$.

Instrumentation
Catheterizations of the pulmonary and left carotid arteries were performed in order to obtain blood samples during exercise for the measurement of blood gas tensions, and haemoglobin and lactate concentrations. All animals had had the left carotid artery translocated to a subcutaneous position in the neck at least 2 months prior to the experiment. Arterial samples were collected anaerobically from an 18 g catheter (Novalon, Becton–Dickinson, Sandy, UT) placed aseptically in the subcutaneous carotid loop. A 110 cm Swan–Ganz catheter (Model 93A1317E American Edwards Laboratories, Santa Ana, CA) was introduced via the right jugular vein and used to collect mixed venous samples from the pulmonary artery. The position of the Swan–Ganz catheter tip was verified from pressure waves displayed on an oscilloscope. Catheterizations were performed 1–2 h prior to the exercise test.

Collection and analysis of expired gas
Each horse wore a snugly fitting face mask that allowed measurement of airflow rates without compromising gas exchange$^{6,14}$. A similar mask was designed for the ponies. The masks incorporated features that allowed air to be drawn through them at high-biased flow rates for measurement of VO$_{2\text{max}}$, while also allowing the flow entry and exit ports on the masks to be briefly closed for the measurement of airflow generated by the subject. For both horses and ponies, air was drawn through the mask and around the muzzle into a 20 m long, 15 cm inner diameter flexible hose that led to a motor-driven blower. For horses, open-circuit flows were ~3000 l min$^{-1}$ for pre-exercise measurements and ~6000 l min$^{-1}$ during exercise. For the ponies, measurements were taken with open-circuit flows ~1500 l min$^{-1}$ before exercise and ~3000 l min$^{-1}$ during exercise. The flows for the ponies had been determined previously by comparing arterial blood gas tensions during rest and exercise when the ponies wore no mask with those measured at a variety of open-circuit flows while the ponies wore the face mask. Biased flow was determined with an anemometer (TSI, Inc., Series 2210, St Paul, MN) that was calibrated yearly in a wind tunnel (traceable to the National Institute for Science and Technology) and weekly using the nitrogen dilution technique$^{15}$. The O$_2$ (Ametek SSA Oxygen Analyzer, Paoli, PA) and CO$_2$ (Beckman LB-2, Beckman, Irvine, CA) analysers were calibrated daily with room air and certified gas mixtures of 18.5% O$_2$ and 0.1 and 3.0% CO$_2$. Expired gas samples (F$_{\text{E}}$O$_2$ and F$_{\text{E}}$CO$_2$) were collected at the same time that blood was sampled, then used to calculate VO$_2$ and VCO$_2$, which were converted to STPD units. The respiratory exchange ratio ($R$) was then determined.

Airway mechanics/measurements
The face mask allowed the flow-through entry and exit ports to be briefly closed (5 s maximum) for the collection of airflow data from 6 to 12 consecutive breaths. As a result of the closure of these ports, airflow generated by each horse moved back and forth across two custom-made 160 mm diameter pneumotachographs that were positioned dorsolateral to the external nares$^{6,14}$. For the ponies, one pneumotachograph was used rather than two and was positioned dorsal to the nares (Fig. 1). Each pneumotachograph had linear pressure-flow characteristics for flows up to 80 l s$^{-1}$. The linearity of the differential pressures measured was checked in 5 l s$^{-1}$ increments. Flow was measured by recording changes in pressure across one pneumotachograph with a differential pressure transducer (Validyne, Northbridge, CA). The pneumotachographs were calibrated daily using the anemometer. Accuracy of airflow measurements was verified by electronic integration of flows generated
using 3.8 l (SRL Medical, Inc., Dayton, OH) and 12.8 l (custom-made, Washington State University, WA) precision syringes. The same test was used to calibrate volume signals.

Transpulmonary pressure ($P_{tp}$) was recorded as described previously\(^{16,17}\); after placement of an oesophageal balloon-tipped catheter in the ponies, the position was checked radiographically to ensure that the balloon portion of the catheter resided within the thoracic cavity, between the heart and the cardia of the stomach. Mask pressure was measured just cranial to the nares. Transpulmonary pressure was defined as the difference between oesophageal and mask pressures. Pressure transducers were calibrated with a water manometer and the mask pressure and oesophageal catheter signals and pneumotachograph–transducer system were phase-matched to a frequency of 10 Hz.

All of the pressure and airflow signals were streamed through an A-D board to a 160 MB computer hard disk. A customized software program (MS-DOS version 3.01 driven rtdLinX Driver, model #i430FX; software written in support of the A-D driver by Robert Kirkpatrick, Biomedical Engineer, Washington State University, WA) was used to calculate tidal volume ($V_T$), minute ventilation ($V_E$), breathing frequency ($f_b$), maximal changes in $P_{tp}$ ($\Delta P_{tp_{max}}$), total pulmonary resistance ($R_L$), dynamic compliance ($C_{dyn}$), mechanical work of breathing per breath cycle ($W_{rm}$, J per breath) and flow-volume loops. Dynamic compliance was determined by relating the change in elastic pressure to the change in volume between consecutive points of zero flow\(^{18}\), using an inertial pressure value that was calculated from inertance values of 0.020 cmH\(_2\)O l\(^{-1}\)s\(^{-2}\) for the ponies\(^{19}\) and 0.026 cmH\(_2\)O l\(^{-1}\)s\(^{-2}\) for the horses\(^{20}\). Elastic pressure was taken to be the difference between inertial pressure and transpulmonary pressure. The absolute work of breathing/min ($W_{rm}$/min), work per litre of ventilation ($W_{rm}$/l, J l\(^{-1}\)) and work per litre of O\(_2\) consumed ($W_{rm}$/VO\(_2\), J l\(^{-1}\)) were also calculated.

**Blood gas analysis**

Arterial and mixed venous blood samples were collected anaerobically into 3 ml heparinized syringes immediately before exercise and during the last 15 s at each exercise intensity. Syringes were capped, ensuring that there were no air bubbles and then stored in an ice bath. Samples were analysed within 1 h of collection. Arterial blood samples for determination of plasma lactate concentrations were collected into tubes containing sodium fluoride. These samples were taken at rest, during the last 15 s of exercise at each work intensity and 5 min after stopping exercise. All samples were stored in an ice bath and analysed within 2 h of collection. Arterial and mixed-venous oxygen ($P_{aO_2}$ and $P_{vO_2}$) and carbon dioxide ($P_{aCO_2}$ and $P_{vCO_2}$) tensions, pH and arterial and mixed venous haemoglobin saturations ($S_{aO_2}$ and $S_{vO_2}$) were determined using a portable blood gas analyser (IRMA, Diameetrics Medical, Inc., St Paul, MN) and were temperature corrected. Temperature in pulmonary arterial blood was recorded with the Swan–Ganz catheter at the same time that blood was collected. Haemoglobin concentrations were recorded with an IL 282 (Instrumentation Laboratory, Lexington, MA) and plasma lactate concentrations were measured in duplicate using an autoanalyser (YSI 2300, Yellow Springs Instruments, Yellow Springs, OH).

**Determination of other variables**

Arterial and mixed venous O\(_2\) content ($C_{aO_2}$ and $C_{vO_2}$) and the respiratory exchange ratio ($R$) were calculated using standard formulae. Alveolar oxygen tension ($P_{aO_2}$) was calculated using the ‘ideal’ gas equation and the alveolar–arterial oxygen tension difference [(A–a)O\(_2\)D] was subsequently determined. Heart rate (HR) was recorded using a digital-display cardiotachometer (Hippocard PEH200, Versailles,
Statistical analysis
All the results were expressed as means ± SE and were analysed using a two-way analysis of variance to test for differences due to species and exercise intensity (with exercise intensity being a repeated measures factor), followed by the Bonferroni’s t-test. All differences were considered to be statistically significant when P ≤ 0.05.

Results
Metabolic and cardiovascular responses to the four exercise intensities are summarized in Table 1. VO2max for the horses was 152.6 ± 4.6 ml kg⁻¹ min⁻¹ and for the ponies was 92.0 ± 3.8 ml kg⁻¹ min⁻¹ (Table 1). The actual work intensities at which the horses completed each bout of exercise were 40.9 ± 0.02, 64.9 ± 0.02, 91.3 ± 0.04 and 111.5 ± 2.6% of VO2max, respectively; the average treadmill speed at each of these intensities was 3.0 ± 0.25, 5.1 ± 0.27, 7.2 ± 0.25 and 10.9 ± 0.28 m s⁻¹, respectively. The actual workloads at which the ponies exercised were 49.5 ± 0.05, 66.6 ± 0.04, 91.8 ± 0.03 and 109.6 ± 1.7% of VO2max, respectively, with the average treadmill speeds being 1.8 ± 0.12, 2.9 ± 0.27, 5.0 ± 0.22 and 8.2 ± 0.41 m s⁻¹, respectively. Minimum speed at which the treadmill would operate was 1.7 m s⁻¹.

At the heavy and supramaximal exercise intensities, horses developed an arterial hypoxaemia (70.5 ± 3.3 and 67.8 ± 2.7 mmHg, respectively), whereas the arterial O₂ tensions for the ponies remained near resting values (93.8 ± 5.9 and 95.4 ± 5.1 mmHg; respectively) (Fig. 2). At supramaximal exercise intensities, horses developed a concurrent arterial hypercapnia (49.8 ± 1.3 mmHg). In contrast, the ponies became hypocapnic (35.0 ± 2.1 mmHg) (Fig. 3). The corresponding pH was higher in ponies (7.32 ± 0.04 vs. 7.23 ± 0.03). For both horses and ponies, PₐO₂ was lower than before exercise at the mild to moderate exercise intensities. For the horses, PₐO₂ continued to decrease as workload increased (12.0 ± 3.3 mmHg at ~112% VO2max), whereas there was no further change in the pony group (19.4 ± 1.3 at ~67% VO2max vs 19.6 ± 1.5 at ~90% VO2max and 21.4 ± 2.6 mmHg at ~110% VO2max). Although PₐCO₂ increased in both horses and ponies as workload increased, there was a significant difference in peak values between the two groups at supramaximal exercise intensities, with the horses reaching an average of 127.5 ± 6.9 mmHg and the ponies 81.8 ± 4.3 mmHg (Fig. 3).

HR increased in a similar fashion in both horses and ponies as work intensity increased (Table 1), reaching a maximal HR of 213.8 ± 7.3 beats per minute (bpm) in ponies and 208.3 ± 4.3 bpm in horses at supramaximal exercise intensities. When comparing absolute Q and SV, there were significant differences between the ponies and horses at all exercise intensities. In contrast, there were no significant differences between mass-specific Q and SV in the two groups of animals, despite the fact that both mass-specific Q and SV appeared to be substantially higher in the horses at supramaximal exercise (Table 1).

In conjunction with each increase in exercise intensity, fₑ increased in both groups, with the ponies averaging 150.9 ± 3.2 breaths/min and the horses averaging 120.7 ± 2.1 breaths/min at the highest work intensity. The fₑ in horses did not increase significantly with exercise at intensities above ~91% VO2max, whereas the incremental change in fₑ was significant at each intensity for the ponies. There was a significant species-exercise interaction for Vₑ, with the horses averaging 16.5 ± 0.61 and the ponies 4.3 ± 0.21 at the supramaximal workload. When comparing Vₑ on a mass-specific basis (i.e. Vₑ kg⁻¹), a difference between the two groups was observed at all exercise intensities (Table 2). There was a significant difference between

### Table 1: Cardiovascular responses before exercise and after exercising for 2 min at mild, moderate and heavy work intensities and after exercising for 1 min at supramaximal work intensity

<table>
<thead>
<tr>
<th>Intensity (%VO2max)</th>
<th>Group*</th>
<th>VO₂ (ml kg⁻¹ min⁻¹)</th>
<th>VCO₂ (ml kg⁻¹ min⁻¹)</th>
<th>RER</th>
<th>Q (ml kg⁻¹ min⁻¹)</th>
<th>HR (beats min⁻¹)</th>
<th>SV (ml kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-H</td>
<td>H</td>
<td>6.20 ± 0.4</td>
<td>4.84 ± 0.4</td>
<td>0.81 ± 0.01</td>
<td>108.6 ± 6.5</td>
<td>46.7 ± 5.2</td>
<td>2.44 ± 0.24</td>
</tr>
<tr>
<td>Pre-P</td>
<td>P</td>
<td>6.27 ± 0.4</td>
<td>4.77 ± 0.33</td>
<td>0.84 ± 0.01</td>
<td>105.8 ± 9.8</td>
<td>37.2 ± 6.2</td>
<td>3.19 ± 0.67</td>
</tr>
<tr>
<td>~40</td>
<td>H</td>
<td>62.6 ± 4.2</td>
<td>57.0 ± 6.8</td>
<td>0.95 ± 0.05</td>
<td>402.7 ± 50.7</td>
<td>141.8 ± 10.7</td>
<td>2.81 ± 0.16</td>
</tr>
<tr>
<td>~50</td>
<td>P</td>
<td>44.9 ± 2.6</td>
<td>35.7 ± 3.8</td>
<td>0.92 ± 0.01</td>
<td>318.5 ± 18.4</td>
<td>152.0 ± 5.9</td>
<td>2.04 ± 0.13</td>
</tr>
<tr>
<td>~65</td>
<td>H</td>
<td>99.3 ± 5.5</td>
<td>96.1 ± 6.2</td>
<td>0.98 ± 0.03</td>
<td>492.7 ± 33.4</td>
<td>183.1 ± 7.3</td>
<td>2.89 ± 0.12</td>
</tr>
<tr>
<td>~67</td>
<td>P</td>
<td>61.0 ± 3.4*</td>
<td>59.4 ± 7.7</td>
<td>1.02 ± 0.06</td>
<td>362.2 ± 6.0</td>
<td>173.3 ± 12.7</td>
<td>2.05 ± 0.13</td>
</tr>
<tr>
<td>~91</td>
<td>H</td>
<td>139.5 ± 8.2</td>
<td>146.4 ± 11.9</td>
<td>1.05 ± 0.04</td>
<td>586.8 ± 53.7</td>
<td>195.7 ± 6.7</td>
<td>2.98 ± 0.18</td>
</tr>
<tr>
<td>~92</td>
<td>P</td>
<td>84.1 ± 2.1*</td>
<td>98.4 ± 7.7*</td>
<td>1.17 ± 0.08</td>
<td>448.9 ± 10.7</td>
<td>201.5 ± 11.5</td>
<td>2.13 ± 0.07</td>
</tr>
<tr>
<td>~112</td>
<td>H</td>
<td>152.6 ± 4.6</td>
<td>187.0 ± 4.3</td>
<td>1.23 ± 0.02</td>
<td>670.0 ± 42.2</td>
<td>208.3 ± 4.3</td>
<td>3.20 ± 0.14</td>
</tr>
<tr>
<td>~110</td>
<td>P</td>
<td>92.0 ± 3.8*</td>
<td>112.7 ± 7.3*</td>
<td>1.22 ± 0.06</td>
<td>490.7 ± 8.8</td>
<td>218.6 ± 7.3</td>
<td>2.24 ± 0.04</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significantly different from horses (P < 0.01). **Group H = six Thoroughbred horses, Group P = five ponies. VO₂, oxygen uptake; VCO₂, carbon dioxide production; RER, respiratory exchange ratio; Q, cardiac output; HR, heart rate; SV, stroke volume.
figure 2: Alveolar, arterial and mixed venous oxygen tensions before exercise and after exercising for 2 min at mild, moderate and heavy work intensities and after exercising for 1 min at supramaximal work intensity. H = horses, P = ponies. Values are means ± SEM. *Significantly different from horses, P ≤ 0.01.

figure 3: Arterial and mixed venous carbon dioxide tensions before exercise and after exercising for 2 min at mild, moderate and heavy work intensities and after exercising for 1 min at supramaximal work intensity. H = horses, P = ponies. Values are means ± SEM. *Significantly different from horses, P ≤ 0.01.

The two groups with respect to VE. At the highest work intensity, the horses averaged 1991.5 ± 56.11 mm Hg and the ponies 648.4 ± 26.11 mm Hg. However, these values were not significantly different between the two groups at any of the exercise intensities when expressed on a mass-specific basis. The ventilatory equivalent for carbon dioxide (VE/VCO2max) was significantly different between ponies and horses at all exercise intensities, with VE/VCO2 higher in ponies at all exercise intensities (Table 2).

Despite the increased VE, PpO2 increased only slightly in the horses, levelling off after they reached ~40% VO2max despite further increases in work intensity (103.4 ± 1.11 mm Hg at ~112% VO2max) (Fig. 2). In contrast, the PpO2 increased progressively with rising workload in ponies to 114.9 ± 2.22 mm Hg at ~110% VO2max (Fig. 2). The [(A–a)O2] widened progressively in conjunction with each increase in exercise intensity in horses, reaching 35.6 ± 2.4 mm Hg during supramaximal exercise, while the [(A–a)O2] widened from pre-exercise up to ~65% VO2max in the ponies (22.5 ± 1.4 mm Hg), but subsequently did not change further (19.5 ± 3.4 mm Hg at ~110% VO2max). Overall, the relationship between [(A–a)O2] and both PpO2 and PaO2 was common to both groups of animals, with [(A–a)O2] widening as PpO2 decreased and PaO2 decreasing as [(A–a)O2] widened (Fig. 4).

There was a significant species–exercise interaction with respect to Wrm, with the horses reaching a mean of 94.0 ± 5.7 J per breath at ~112% VO2max and the ponies a mean of 11.9 ± 1.2 J per breath at the same work intensity (Fig. 5). When expressed as Wrm l−1 and Wrm min−1, there were again statistically significant species–exercise interactions apparent between the two groups, but when expressed as Wrm/VO2 no statistical differences were found between the two groups at any of the work intensities (Fig. 5).

All exercise intensities were associated with a significant difference between groups with respect to

Table 2: Breathing mechanics and ventilatory responses before exercise and after exercising for 2 min at mild, moderate and heavy work intensities and after exercising for 1 min at supramaximal work intensity

<table>
<thead>
<tr>
<th>Intensity</th>
<th>Group**</th>
<th>fE min−1</th>
<th>VE kg−1 ml kg−1</th>
<th>VE kg−1 ml kg−1</th>
<th>VE/VCO2max</th>
<th>PL (cmH2O l−1 s−1)</th>
<th>Cdyn (cm H2O l−1)</th>
<th>Ptpmax (cm H2O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-H</td>
<td>15.7 ± 1.9</td>
<td>11.1 ± 1.5</td>
<td>171.6 ± 26.0</td>
<td>36.6 ± 6.2</td>
<td>0.26 ± 0.02</td>
<td>1.7 ± 0.17</td>
<td>4.9 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>Pre-P</td>
<td>34.0 ± 3.0*</td>
<td>5.9 ± 0.5</td>
<td>200.5 ± 23.5</td>
<td>43.4 ± 6.8*</td>
<td>0.43 ± 0.05</td>
<td>0.61 ± 0.06*</td>
<td>2.9 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>~41</td>
<td>76.1 ± 7.0</td>
<td>25.8 ± 2.0</td>
<td>1921.2 ± 162.0</td>
<td>34.7 ± 2.3</td>
<td>0.25 ± 0.01</td>
<td>0.29 ± 0.02</td>
<td>34.1 ± 3.0</td>
<td></td>
</tr>
<tr>
<td>~50</td>
<td>94.3 ± 3.6*</td>
<td>18.7 ± 0.5</td>
<td>1734.3 ± 99.1</td>
<td>50.9 ± 6.3*</td>
<td>0.34 ± 0.04</td>
<td>0.29 ± 0.05</td>
<td>13.5 ± 1.3*</td>
<td></td>
</tr>
<tr>
<td>~65</td>
<td>95.4 ± 8.3</td>
<td>29.5 ± 2.7</td>
<td>2735.2 ± 177.4</td>
<td>28.5 ± 0.8</td>
<td>0.29 ± 0.02</td>
<td>0.21 ± 0.03</td>
<td>50.9 ± 2.0</td>
<td></td>
</tr>
<tr>
<td>~87</td>
<td>100.9 ± 5.6*</td>
<td>26.2 ± 0.3</td>
<td>2863.2 ± 143.4</td>
<td>47.3 ± 6.3*</td>
<td>0.39 ± 0.03</td>
<td>0.28 ± 0.04</td>
<td>21.5 ± 2.1*</td>
<td></td>
</tr>
<tr>
<td>~91</td>
<td>113.2 ± 2.2</td>
<td>31.1 ± 1.3</td>
<td>3520.4 ± 152.1</td>
<td>24.5 ± 1.3</td>
<td>0.35 ± 0.01</td>
<td>0.16 ± 0.02</td>
<td>68.9 ± 3.1</td>
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</tr>
<tr>
<td>~92</td>
<td>130.0 ± 2.7*</td>
<td>25.8 ± 0.6</td>
<td>3553.3 ± 64.0</td>
<td>37.0 ± 2.9*</td>
<td>0.55 ± 0.04*</td>
<td>0.19 ± 0.01</td>
<td>35.2 ± 2.3*</td>
<td></td>
</tr>
<tr>
<td>~112</td>
<td>120.7 ± 2.1</td>
<td>33.3 ± 1.5</td>
<td>4014.2 ± 191.0</td>
<td>21.5 ± 0.8</td>
<td>0.39 ± 0.01</td>
<td>0.14 ± 0.01</td>
<td>82.9 ± 4.3</td>
<td></td>
</tr>
<tr>
<td>~110</td>
<td>150.9 ± 3.2*</td>
<td>25.4 ± 0.5</td>
<td>3834.9 ± 106.5</td>
<td>34.7 ± 2.8*</td>
<td>0.61 ± 0.06*</td>
<td>0.17 ± 0.03</td>
<td>42.2 ± 4.3*</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significantly different from horse group (P ≤ 0.01). **Group H = six Thoroughbred horses, Group P = five ponies. fE, breath frequency; VE kg−1 ml kg−1, tidal volume; VE kg−1 ml kg−1, minute ventilation; VE/VCO2max, ventilatory equivalent for oxygen; PL, total pulmonary resistance; Cdyn, dynamic compliance; Ptpmax, maximal changes in transpulmonary pressure.
to $\Delta P_{\text{p Tmax}}$ with peak values reaching 82.9 $\pm$ 4.3 cmH$_2$O in horses and 42.2 $\pm$ 4.3 cmH$_2$O in ponies (Table 2). There was a significant species–exercise interaction for $R_l$ for both horses and ponies at heavy and supramaximal exercise intensities (Table 2). $R_l$ was higher in ponies at each measurement time. The horses had a significantly higher $C_{\text{dyn}}$ at rest (1.68 $\pm$ 0.17 cm$^{-1}$H$_2$O) than the ponies (0.61 $\pm$ 0.06 cm$^{-1}$H$_2$O). With the onset of exercise, $C_{\text{dyn}}$ for both groups decreased quickly, reaching 0.14 $\pm$ 0.01 cm$^{-1}$H$_2$O for the horses and 0.17 $\pm$ 0.03 cm$^{-1}$H$_2$O for the ponies at supramaximal exercise intensities.

Flow-volume loops generated before and during all exercise intensities suggested that at exercise intensities of $\sim$ 90 and $\sim$ 112% VO$_{2\text{max}}$, horses experienced expiratory flow limitation (Fig. 6). Flow-volume loops generated for the ponies at the same exercise intensities showed no flow limitation (Fig. 6).

**Discussion**

**Gas exchange**

All horses developed an arterial hypoxaemia with a concurrent hypercapnia during heavy and supramaximal exercise, whereas the ponies remained normoxaemic and developed an associated hypocapnia. The arterial hypoxaemia in the horses was most likely due to a combination of diffusion limitation and an inadequate ventilatory response to exercise$^1$–$^11$.

It has been suggested that diffusion limitation is responsible for at least 50% of the wide [(A–$\alpha$)O$_2$D] in horses exercising at heavy and supramaximal work intensities and, therefore, is the primary cause of exercise-induced arterial hypoxaemia$^1$. Alveolar hypventilation resulting in a lowered P$_{\text{aO2}}$ (driving pressure), a reduction in red blood cell capillary transit time and an accumulation of alveolar interstitial fluid all have been implicated as causes of diffusion limitation$^{11,21-25}$. Although ponies do not become hypoxaemic or experience any drop in the saturation of haemoglobin with O$_2$, the widening of their [(A–$\alpha$)O$_2$D] indicates that some diffusion limitation may exist in ponies exercising at moderate, heavy and supramaximal work intensities. That the ponies did not become hypoxygenated was due to a significant increase in P$_{\text{aO2}}$, which allowed them to maintain P$_{\text{aO2}}$ in the face of the increasing [(A–$\alpha$)O$_2$D]. In contrast, the horses’ P$_{\text{aO2}}$ did not change at workloads $\cong 65\%$ VO$_{2\text{max}}$ and as a result, P$_{\text{aO2}}$ continued to decrease as the [(A–$\alpha$)O$_2$D] widened. The alveolar–pulmonary capillary O$_2$ difference, and more specifically the P$_{\text{vO2}}$, is an important factor in determining the rate of diffusion of O$_2$ from alveoli to the pulmonary capillary blood$^{23}$. At supramaximal exercise intensities, the horses in our study had a mean P$_{\text{vO2}}$ that was $\sim$ 12 mmHg lower than the mean P$_{\text{vO2}}$ in the ponies (Fig. 2), theoretically resulting in a smaller driving force for diffusion of O$_2$. This is of significance because it has been postulated that larger animals have a lower driving force for O$_2$ diffusion$^{25,26}$. Additionally, because of their larger lungs, larger animals also have longer diffusion path lengths, which lead to greater stratified inhomogeneity and larger differences in PO$_2$ between proximal and terminal
alveoli in an acinus\textsuperscript{25,26}. Because the calculated \(P_{A}O_2\) reflects the average of the local \(PO_2\) values at the surface of the perfusion units and the comparatively greater distances for diffusion of \(O_2\) results in a lower average \(P_{A}O_2\), a lower driving force for diffusion is created\textsuperscript{25,26}.

Physiologically, horses are able to increase their oxygen-carrying capacity by almost doubling their packed cell volume (PCV) due to splenic contraction\textsuperscript{27,28}. Consequently, \(C_aO_2\) increases with exercise despite the desaturation of oxyhaemoglobin that accompanies the hypoxaemia. However, this haemoglobin concentration, along with an increased \(Q\), may also contribute to the development of diffusion limitation and arterial hypoxaemia by contributing to pulmonary and systemic hypertension, leading to a reduction in mean capillary red blood cell transit time and equilibration of \(O_2\) across the capillary–alveolar interface\textsuperscript{29–30}. It is possible that the development of arterial hypoxaemia by the horses and not the ponies in this study could be partially due to the horse’s ability to increase their PCV to a greater degree than the ponies, resulting in a more significant diffusion limitation. Unfortunately, PCVs were not measured in this study, so direct comparison between ponies and horses could not be made. However, although not significantly different, the horses in this study did have a higher \(C_aO_2\) (26.5 ± 1.4 ml100 ml\(^{-1}\)) than the ponies (22.8 ± 0.84 ml100 ml\(^{-1}\)) at the highest work intensity, which may reflect a greater oxygen-carrying capacity due to higher haemoglobin concentrations, which could then contribute to greater diffusion limitations. In addition, although not statistically different, the horses did have a higher mass-specific \(\dot{Q}\) (670.0 ± 42.2 vs 490.7 ± 8.8 kg\(^{-1}\) min\(^{-1}\)) and \(SV\) (3.20 ± 0.14 vs 2.24 ± 0.04 ml kg\(^{-1}\)) than the ponies at the highest work intensities. Although pulmonary capillary blood volumes have not been measured in ponies or horses during exercise, given the greater \(\dot{Q}\) that we observed in the horses it seems likely that transit times in these animals would have been considerably less than in the ponies. Therefore, while the mean pulmonary capillary transit time in horses exercising at high intensities has not been measured\textsuperscript{7}, it is possible that the arterial hypoxaemia observed in horses, but not in ponies, could be partially explained by a shorter pulmonary capillary erythrocyte transit time.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{flow-volume_loops.png}
\caption{Representative individual flow-volume loops from a pony (a) and a Thoroughbred horse (b) before exercise and after exercising for 2 min at mild, moderate and heavy work intensities and after exercising for 1 min at supramaximal work intensity. As end-expiratory lung volume could not be measured, loops are centred around a volume that is \(-50\%\) of total lung capacity.}
\end{figure}
Beyond ~65% \( \dot{V}O_2 \text{max} \), the \([A-a]O_2D\) did not change in the ponies, whereas it continued to widen as exercise intensity increased in the horses. Although there are a number of factors which limit diffusion, our data suggest that the difference in the \([A-a]O_2D\) responses of the two groups during heavy and supramaximal exercise may be partly related to differences in \(P_{\text{a}O_2}\). \(P_{\text{a}O_2}\) also plays an important role in determining the rate of diffusion of O\(_2\) across the alveolar–pulmonary capillary interface via its influence on the effective slope of the oxyhaemoglobin dissociation curve\(^{51-54}\). The \(P_{\text{a}O_2}\) in horses fell progressively as workload increased, whereas in ponies there was no significant difference in \(P_{\text{a}O_2}\) at any exercise intensity (Fig. 2). Because of the differences in \(P_{\text{a}O_2}\), the alveolar–pulmonary capillary \(O_2\) gradient appeared to be similar in both groups during exercise (~91.4 mmHg for the horse group and ~93.5 mmHg for the pony group at supramaximal work intensities). Although it could be assumed that with similar gradients the rate of diffusion of \(O_2\) would be comparable in the two groups, this is not the case because of the differences in the effective slopes of their oxyhaemoglobin curves. The lower the \(P_{\text{a}O_2}\) and the closer the \(P_{\text{a}O_2}\) is to the steep part of the oxyhaemoglobin curve, the slower the rate of diffusion equilibration\(^{51,53}\). Therefore, a longer capillary transit time would be required to achieve equilibrium in \(P_{\text{a}O_2}\) between erythrocytes and alveolar air\(^{24,51,53}\). The minimum transit time that is sufficient for adequate diffusion to occur is not fixed. Rather, it is dependent upon a number of factors including the above-mentioned effective slope of the oxyhaemoglobin curve\(^{27,51-53,56}\). As stated earlier, given the substantially greater \(Q\) that we observed in the horses in this study, it seems likely that transit times in these animals would have been considerably less than in the ponies. Therefore, it is possible that the arterial hypoxaemia observed in horses, but not in ponies, could be partially explained by a shorter pulmonary capillary erythrocyte transit time coupled with a slower rate of oxygen diffusion.

Ventilation

Diffusion limitation, however, cannot account completely for exercise-induced hypoxaemia in horses. Inadequate alveolar ventilation has been implicated as another important factor in the development of hypoxaemia and the concurrent hypercapnia\(^{4,11}\). Studies of gas exchange in horses exercising under hyperoxic conditions have indicated that if \(P_{\text{a}O_2}\) could be increased to ~115-120 mmHg then the oxyhaemoglobin desaturation would be eliminated, but the hypercapnia would persist\(^{6,37}\). Our horses were only able to ventilate enough to maintain \(P_{\text{a}O_2}\) at slightly more than 100 mmHg, whereas ponies were able to increase their \(P_{\text{a}O_2}\) to a greater degree (~115 mmHg), maintaining \(P_{\text{a}O_2}\) near pre-exercise values and becoming mildly hypocapnic.

It is unclear as to why horses cannot mount a more appropriate ventilatory response to high-intensity exercise, whereas ponies can. Both groups of animals were able to increase ventilation considerably in response to exercise. All animals increased mass-specific \(V_{\text{E}}\) at least 20 times over pre-exercise \(V_{\text{E}}\), with no significant differences occurring between the two groups. At the lower exercise intensities (≤ ~65% \( \dot{V}O_2 \text{max} \)), both groups increased \(V_{\text{E}}\) by increasing both \(V_{\text{T}}\) and \(f_{\text{E}}\). However, after reaching ~65% \( \dot{V}O_2 \text{max} \), ponies did not significantly change their \(V_{\text{T}}\) in response to increases in workload, whereas the horses continued to increase their \(V_{\text{T}}\) at each increase in exercise intensity. Comparatively, after reaching ~90% \( \dot{V}O_2 \text{max} \) the horses did not significantly change their \(f_{\text{E}}\) in response to increases in exercise intensity. In contrast, \(f_{\text{E}}\) continued to increase in ponies as workload increased, with the ponies achieving a \(f_{\text{E}}\) almost 25% greater than the horses at heavy and supramaximal exercise intensities (Table 2). It therefore appeared that the two groups employed different breathing strategies to increase \(V_{\text{E}}\) during heavy and supramaximal workloads: horses did so principally by increasing \(V_{\text{T}}\), whereas ponies increased \(V_{\text{E}}\) by increasing \(f_{\text{E}}\) with relatively little change in \(V_{\text{T}}\).

Although \(V_{\text{E}}\) increased substantially as work intensity increased, the hypercapnia suggests that horses were unable to mount a ventilatory response that was appropriate for the metabolic load associated with exercise. A decrease in ventilatory equivalents in horses in response to increasing exercise intensity may reflect an inability to hyperventilate adequately during maximal workloads\(^{58}\). It has been reported that at comparable work intensities, horses mounted an inadequate ventilatory response in comparison to ponies as evidenced by their higher \(P_{\text{a}CO_2}\) and \(HCO_3^-\) and lower \(V_{\text{E}}/\dot{V}CO_2\) as well as having a more acidic arterial pH than the ponies, probably due to the hypercapnia\(^9\). In the current study, there was a significant difference between the two groups with respect to \(V_{\text{E}}/\dot{V}O_2\). \(V_{\text{E}}/\dot{V}CO_2\) was higher in ponies at each intensity and was sufficient to maintain normal gas exchange. In contrast, the horses' \(V_{\text{E}}/\dot{V}CO_2\) recorded at the highest exercise intensities were much lower than at rest (Table 2). This low value further indicated that the ventilatory response of the horses to high exercise intensities was worse than the ponies' and may not have been appropriate for the metabolic demands associated with exercise. Hence, horses did not ventilate as well as the ponies. Others have suggested that the decrease in \(V_{\text{E}}/\dot{V}CO_2\) in horses may result in a reduced cost of ventilation, delay in respiratory muscle fatigue and reduced \(O_2\) uptake by the respiratory muscles\(^{27,39}\). It has also
been suggested that rather than increase their $V_{E}/V_{CO2}$, Thoroughbreds have developed a powerful non-bicarbonate buffering system in the form of their extremely high Hb concentration, which helps to minimize the acid–base disturbances without having to increase their ventilation so as to minimize respiratory muscle work. However, in view of the hypoxaemia and reduced $S_aO_2$ that develops, it seems unlikely that reduced $V_{E}/V_{CO2}$ is truly beneficial.

**Mechanics of breathing**

There have been a number of theories as to why there is a lack of a compensatory hyperventilation in the horse during high-intensity exercise. Flow limitation, increased resistive forces and the high mechanical work of breathing may be prominent factors in determining horses' ventilatory capabilities while exercising at maximal and supramaximal exercise intensities. In the current study, even though all expressions of $W_{rm}$ increased substantially in both groups during exercise, horses exercising at heavy and supramaximal intensities had a significantly higher $W_{rm}$ than ponies. Additionally, $W_{rm} l^{-1}$ and $W_{rm}$ were significantly higher in the horses than in the ponies at all exercise intensities. In both horses and ponies, the relationship between $W_{rm} l^{-1}$ and $V_{E}$ was approximated by a straight line, while the relationship between $W_{rm}$ and $V_{E}$ was curvilinear (Fig. 7). Similar relationships exist in exercising people in whom it has been demonstrated that increases in the work of breathing during heavy exercise curtail performance. This occurs at least partly because a greater percentage of the cardiac output must be directed to the respiratory muscles to support this increased work. As a result, blood flow to the skeletal muscles is reduced and exercise performance is impaired. Thus, it is likely that the additional respiratory muscle work required to enable horses to increase their ventilation to the point that best matched their metabolic rate would be extremely large and detrimental to their speed. Estimates using the alveolar gas equation suggest that it may not be physically possible for horses to increase alveolar ventilation to significantly lower the $[\text{A}_{\infty-a}O_2D]$ or the $P_{aCO2}$.

$W_{rm}/VO_2$ was the only expression of $W_{rm}$ in which there were no differences between ponies and horses.

![Graph](attachment://graph.png)  
**Fig. 7** Relationship between work of breathing ($W_{rm}$) and minute volume per kg ($V_{E} \text{ kg}^{-1}$) (a) and work per litre ventilation ($W_{rm} l^{-1}$) and minute volume per kg ($V_{E} \text{ kg}^{-1}$) (b) before and during exercise in a group of six Thoroughbred horses and a group of five ponies.
$W_{rm}/\dot{V}O_2$ has been used to evaluate respiratory muscle $O_2$ uptake in comparison to total $O_2$ uptake, and it may be that because $W_{rm}/\dot{V}O_2$ continued to increase in the Thoroughbreds as exercise intensity increased, a 'critical level of ventilation' 1,27 may have been reached. It has been hypothesized that beyond this point any additional oxygen made available by an increase in ventilation would be solely used for ventilation rather than for locomotion or another form of work 1. If this was the case, then it may be another reason why the horses did not increase their ventilation further in conjunction with increases in work intensity.

$W_{rm}$ has been reported to increase during exercise for a variety of reasons, including increased elastic, flow-resistive and/or inertial work of breathing and displacement of abdominal contents 1,5,27,44. High elastic resistance to work may occur in horses during heavy exercise due to their stiff chest wall and high $f_{0e}$, resulting in the inability of the inspiratory muscles to develop the pressures required to overcome this resistance, especially in the brief time available for inspiration (~250 m s) and if end-expiratory lung volume is increased 1,27. $C_{dyn}$ provides an estimate of the elastic properties of the lung and thus elastic resistance and typically increases with increased lung inflation and decreases with increased $f_{0e}$ 27. In both groups, $C_{dyn}$ decreased to very low levels with increasing exercise intensity, presumably contributing noticeably to the increase in $W_{rm}$ observed in both horses and ponies. Even though $C_{dyn}$ was equally low in both groups and most likely contributed to the increase in their $W_{rm}$, $W_{rm}$ was significantly higher in the horses, possibly reflecting the generation of expiratory pressures in excess of those needed to produce maximal expiratory airflow.

Flow-resistive forces contribute to increased $W_{rm}$ and most likely limit ventilatory changes that horses can make during high-intensity exercise. Traditionally, it has been felt that physiological adjustments, such as bronchodilation and enlargement of laryngeal cross-sectional area decrease $R_L$ and thus facilitate ventilation during exercise 1,5,44,45. However, $R_L$ increases above pre-exercise levels during heavy and supramaximal exercise and is believed to be important in limiting any further increase in $V_{E}$ in horses exercising at high intensities, due mainly to frictional forces and turbulence of airflow 1,5,27,45. Although there was a significant exercise–species interaction with respect to $R_L$ (higher in the ponies) at all exercise intensities, $R_L$ does not relate well to flow limitation or ventilatory capacity during exercise because it is related primarily to large, cartilaginous airways. In contrast, it has been shown in elite human athletes, exercising at high workloads, that flow limitation is due to small airway compression during expiration and that this has little effect on $R_L$ 46,47.

Consistent with small airway compression, flow-volume loops made for the horses and ponies in the current study strongly suggested that horses developed expiratory flow-limitation at heavy and supramaximal exercise intensities, but that the ponies did not. This was indicated by the observation that the expiratory portions of the tidal flow-volume loops of the horses overlapped each other whereas those generated by the ponies did not. This mechanical constraint is a major reason some people cannot ventilate more when exercising 47,48 and it may be the primary reason that the horses were not able to ventilate more. Theoretically, horses may be able to increase their ventilation further if they could decrease their end-expiratory lung volume (EELV) while further increasing $V_{E}$. Again, however, the increased $W_{rm}$ associated with increasing $V_{E}$ may result in oxygen consumption of the respiratory muscles increasing at the expense of locomotion, with the resulting decrease in speed not being beneficial to the animal. Further investigation of this aspect of equine ventilation requires the development of methods for measuring EELV during strenuous exercise.

In conclusion, it appears that horses become hypoxaemic and hypercapnic because of diffusion limitation and because, relatively speaking, their metabolic capacity far surpasses their ventilatory capacity during heavy and supramaximal exercise. Ventilation is probably restricted by the high mechanical cost of breathing and expiratory flow limitation. Ponies are less capable athletes, utilizing less $O_2$ and producing less $CO_2$ and, therefore, can better match their ventilatory response to the metabolic demand of maximal exercise. Because of their superior ability to ventilate, any diffusion limitation that ponies may incur during exercise is offset by an increase in $P_{aO_2}$. The pony is a sedentary equid, whereas the Thoroughbred horse has been selectively bred to be an elite athlete. It may be that as a result of these breeding practices, the respiratory system has become a limiting factor in determining the equine athlete’s ability to perform, whereas ponies are still limited by cardiovascular or peripheral factors.

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Ventilatory responses of ponies and horses

References


