Reliability of cardiorespiratory measurements with a new ergospirometer during intense treadmill exercise in Thoroughbred horses

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Abstract

This study investigated the reliability of measurements with a new equine ergospirometer (Quadflow). Heart rate and blood lactate responses during exercise in horses wearing the Quadflow and an open flow mask were also compared. The mean percentage error of the oxygen uptake measurements was 8.2% (range 2.1–12.5%). Percent error for peak expiratory flow rates ranged from 6.1% to 9.4%, and for minute ventilation from 2.5% to 7.4%. The coefficients of variation of the means of four measurements in two horses exercising continuously at 9.0 m/s were <5% for variables related to pulmonary ventilation, and was 7.7% for oxygen uptake. The Quadflow mask resulted in small increases in blood lactate concentration and relative heart rate during submaximal exercise. It was concluded that between- and within-test reliability statistics for important measurements in equine clinical exercise testing were acceptable for routine use in a veterinary practice or research laboratory.

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1. Introduction

Maximal oxygen uptake (VO_2max) is an important measurement during clinical exercise testing in horses (Rose et al., 1990; Morris and Seeherman, 1991). It expresses the aerobic capacity of the horse. Poor exercise performance might be associated with low maximal oxygen uptake, but further measurements are needed to determine whether the limit to oxygen transport has a cardiovascular or respiratory origin. Oxygen uptake measurements in combination with measurements of heart rate (oxygen pulse) and minute ventilation (ventilatory equivalent for oxygen) assist with evaluation of cardiovascular and respiratory limits to exercise performance (Weisman and Zeballos, 2001). Oxygen pulse is a valuable measurement in clinical exercise testing because it correlates with stroke volume, and the ratio of minute ventilation to oxygen uptake has also been recommended for clinical evaluation of respiratory function in horses (Hörnicke et al., 1983).

There has been limited use of the measurements of VO_2max with heart rate and minute ventilation during clinical exercise testing in horses because of limited availability of the technology to measure simultaneously minute ventilation and oxygen uptake during intense exercise. Ultrasonic flow meters have been used in combination with mass spectrometry to enable simultaneous measurement of oxygen uptake and respiratory gas flow rates during exercise in horses. Butler et al. (1993) described pulmonary ventilation, heart rate and oxygen uptake in seven Thoroughbred horses using an ultrasonic flow sensor placed in a mask sited on the nose. However, Marlin and Roberts (1988) noted that...
ultrasonic flow sensors can be subject to rapid drift in zero and gain. We also found that an ultrasonic flow sensor for equine spirometry described by Beadle et al. (1995) was subject to rapid drift of zero and gain. The performance of these sensors can also be influenced by water vapour and small changes in the placement and orientation of the transducers can affect accuracy of the measurements. Another limitation is that design constraints mean that the ultrasonic path only covers a fraction of the expired and inspired gas flow.

We therefore decided to construct and evaluate a new system for simultaneous measurement of respiratory airflow rates and oxygen uptake in exercising Thoroughbreds. The aims of the study were to investigate the between- and within-test reliability of measurements of ventilation and oxygen uptake during treadmill exercise with the new system. A comparison was also made between the heart rate and blood lactate concentrations during exercise in horses wearing either the new Quadflow mask or an open flow mask.

2. Materials and methods

2.1. Horses

Six Thoroughbred horses were selected for use on the basis of their tractability and absence of cardiac murmurs. The horses had no evidence of upper airway disease during endoscopy performed during treadmill exercise at 12 m/s. Horses were acclimatised to treadmill exercise while wearing a face mask and had been in regular treadmill training for eight weeks, at speeds of 7–12 m/s, for five days per week. Characteristics of the horses and results of tracheal washes performed two weeks before exercise tests commenced are presented in Table 1.

The Animal Use Committee of the Japan Racing Association approved all experiments.

2.2. Quadflow pneumotachometer

A flow sensor was constructed from four symmetrically arranged, bi-directional pitot tube flow meters housed in a plastic tube with internal diameter 9.1 cm. The characteristics and performance of the pitot tube sensors for measuring gas flow during exercise have been described (Porszasz et al., 1994). The flow sensors were placed in a plastic tube attached to a facemask in a position directly rostral to the nostrils. Low and high range pressure sensors were used for the pitot tubes (Honeywell). The angle between the two plastic tubes holding the left and right sensors was 53°, chosen to replicate the angle between right- and left-sided expiratory flows at rest in horses using photographs of expired airflow on a cold morning. The dead space in the two plastic tubes holding the pitot tube sensors and the one-way valves was 1.1 L. Fig. 1 shows the major components of the system.

The weight of the mask with two flow sensors attached was 2.3 kg. The resistance to air flow through one flow sensor was measured by pressure differences across an orifice plate. The resistance was 0.0424 cm H₂O/L/s at a flow rate of 25 L/s. During all experiments expired gas was extracted from the mask at approximately 100 mL/min for a separate study.

Rubber sheet of 0.5 mm thickness (Waki) was used to create a seal between the mask and the horse’s face. The slightly stretched rubber sheet was secured tightly to the mask with Velcro strips. The oval hole cut in the rubber sheet measured 12 cm vertically and 6 cm left to right. The horse’s nose and mouth were passed through this hole in the rubber as the mask was placed on the horse. The presence of a tight seal between the rubber sheet and the horse’s face was confirmed by visual inspection.

2.3. Experiment 1. Between-test reliability study

Two Thoroughbred horses (A, B) were used to investigate the between-test reliability of measurements with the Quadflow mask, using an incremental speed exercise test. Both horses were exercise tested twice in a four-day period.

2.4. Treadmill exercise test and measurements

The treadmill (Mustang) was set at a 10% slope, and velocity and slope were confirmed by independent

<table>
<thead>
<tr>
<th>Horse</th>
<th>Age (years)</th>
<th>Body weight (kg)</th>
<th>% Neutrophils</th>
<th>% Monocytes</th>
<th>% Lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>M</td>
<td>7</td>
<td>499</td>
<td>13</td>
<td>65</td>
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<tr>
<td>B</td>
<td>G</td>
<td>7</td>
<td>462</td>
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<tr>
<td>C</td>
<td>G</td>
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<td>G</td>
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<td>F</td>
<td>M</td>
<td>8</td>
<td>469</td>
<td>6</td>
<td>73</td>
</tr>
</tbody>
</table>

M, male and G, gelding.
measurements. Before all tests, the rectal temperature of the horse under test was recorded, and it was confirmed that the horse had a normal appetite and was not lame during trotting. Horses were fed normally at 06:00 h, and no treadmill tests were conducted within 2 h of a large meal. All exercise tests were conducted between 13:30 and 15:30 h. Before each exercise test room temperature and relative humidity were noted using a wet and dry bulb thermometer (Livingstone International). The oxygen and carbon dioxide analyzers were calibrated with air collected outside the laboratory (0.04% CO₂, 20.95% O₂). A precision gas with 16.00% O₂ and 2.00% CO₂ (Sumitomo Seika Chemicals) was used to calibrate the gas sensors.

The exercise test consisted of 3.5 min at 3.5 m/s, then a 30 s period of acceleration to 9 m/s. Horses then completed 75 s exercise at 9 and 10 m/s and variable periods of exercise at 11 m/s. The exercise test was stopped when the horse could not maintain the same speed as the treadmill. Treadmill velocity was then immediately decreased to 1.5 m/s and the horse walked for 2 min, and the Quadflow mask was then removed. Horses then walked on the treadmill for a further 3 min. The Quadflow recorded airflow rates (L/s) continuously during exercise. Heart rates were logged continuously during exercise by Polar HR meters (Polar, S610). Heart rates were recorded during the last 10 s of each step of the exercise test, and the highest heart rate measured over any 10 s period during the exercise test was recorded as maximal heart rate.

Flow calibrations were conducted with reference to ISO 7066 and AS 2360.7 standards. Initially, a custom-built orifice plate was calibrated in a wind tunnel to flows of 35 L/s (Department of Aeronautical Engineering, University of Sydney). A pitot tube was placed in a flow straightener tube, upstream of the orifice plate. It was adjusted to each of nine points in a target pattern across the cross section of the tube and a flow velocity measurement was made at each point. The flow profile across the tube was then plotted, and flow rate was measured using integration techniques detailed in the ISO standard. This was repeated at a range of flow rates of 0–34.1 L/s to produce a calibration curve for the orifice plate. The orifice plate is known to engineers as a stable and repeatable flowmeter design. When the calibration coefficients are known, a formula linearizes the flowmeter output, as described in ISO 7066. This allows the orifice plate to act as a flowmeter standard in extrapolated, sub-sonic, steady state turbulent flows. In all flow calibrations with the orifice plate, all tube internal diameters were 76.5 mm, and a pipe of 2 m length was used to ensure a steady state flow.

The Quadflow device was further calibrated from the orifice plate using four vacuum pumps capable of a total flow of 81 L/s. The voltage across the pumps was adjusted by a variable mains auto-transformer (Variac). A table of orifice plate measured flows against Quadflow output voltage was constructed over a range of flows up to the voltage saturation point of the Quadflow sensor. This table was used to create a lookup table, so that Quadflow voltages could be automatically converted into flows by the recording software. It should be noted that it was the electronics that saturate; the flow head was capable of measuring flows at greater than 81 L/s. The electronics were chosen to give the best possible linearity over the required flow range. Further, the calibration conditions of barometric pressure, ambient temperature, and relative humidity were recorded with the lookup table so that the appropriate correction can be made on respiratory flows during a test.

A three point “calibration check” was used before each exercise test to ensure that the Quadflow was still tracking the calibration curve. The calibration check was performed at zero flow, and at a value between 50
and 55 L/s in both the inspiratory and expiratory directions. The calibration was regarded as acceptable if the total flow in the sensors differed from the reference by less than 3%. These flow calibrations were measured with a Fleisch screen and VISE Medical TF-5 digital manometer with a Validyne transducer as the calibration standard. For completeness, the orifice plate output was compared to the Fleisch screen over a range of flows (18–135 L/s). The maximum error between these systems was 0.48%.

Mixed expired gas was collected over a 30 s period using passive collection through two one-way human respiratory function one-way valves with 18 mm diameter (S and W) placed 1-2 cm downstream of pitot flow tubes in the left and right Quadflow sensors. These two one-way valves were connected to plastic tubing (internal diameter 6 mm, length 85 cm) to an airtight, evacuated plastic bag (Simpla S3 CE urine drainage bag, Coloplast) with internal diameter of 3 mm was used to connect the outputs from the two one way valves. At least 60 mL of gas was collected during the 30 s collection periods. The mean difference in flow rates in the flow tube after introducing the gas sampling tube was 0.8%.

During exercise tests with horses wearing a Quadflow mask, attempts were made to maintain inspired gas compositions similar to normal atmospheric conditions, and to limit the increase in laboratory air temperature due to accumulation of expired gas. A vacuum pump (VISE Medical) was used to draw air from the laboratory at greater than 180 L/s. The hose inlet, with 30 cm diameter, was positioned at approximately 50 cm from and at 90° to the side of the horse’s head. The gas was vented outside the treadmill laboratory via tubing of 20 cm diameter that narrowed to 15 cm at the outlet. Room gas was collected into a 100 mL syringe from the laboratory air, just upstream from the horse’s head during periods of collection of expired gas in order to measure inspired oxygen and carbon dioxide percentages.

After each Quadflow exercise test the flow sensors calibrations were checked, and the one-way valves were rinsed with water and checked visually for normal function. Gases collected during the exercise test were analyzed within 30–40 min of completion of the exercise test.

Tidal volume was calculated by integrating the instantaneous flows, and was expressed at body temperature and pressure, saturated conditions (BTPS). Previous unpublished data had demonstrated that mixed expired gas during exercise was less than 37 °C, and in this study it was assumed that the temperature of the expired gas at the flow sensors was 34 °C. Minute ventilation was expressed at BTPS, using averages of tidal volumes and respiratory rates in five consecutive breaths. All variables measured during an exercise test are based on data collected during the 30 s period when expired gas was collected. Artefacts due to swallowing were avoided when selecting breaths to analyze. The first 15 breaths after a disturbance to gas flow due to swallowing were not used for analysis because the peak expiratory flows during that period were lower than before the swallow. Moreover, breaths immediately before fatigue were not selected for analysis. Peak inspiratory and expiratory flow rates (L/s) and times for inspiration and expiration were measured.

Gas analysis in the Quadflow system used a paramagnetic oxygen analyzer (PM 1111E, Servomex) and an infra-red carbon dioxide analyzer (IR1507, Servomex). The oxygen sensor was configured in “standard” mode, rather than “rapid” mode. A micro-diaphragm gas sampling pump (NMP 02 L/V, KNF Neuberger) was used to draw gas through these analyzers, at flow rates of 150–200 mL/min. Flow rate was monitored with a suitable flow meter with range 0–500 mL/min (Dwyer).

Expired gas was dried before analysis by passing it through a Nafion drying tube (Permapure) placed in air that was dehumidified by drawing the air through a desiccant. Expired O2 and CO2 concentrations were measured from a plateau of 5 s of continuous recording.

Oxygen uptake and carbon dioxide output were calculated for each completed step of the exercise test, and expressed at standard temperature and pressure, dry conditions (STPD). The measured expired oxygen and carbon dioxide concentrations were corrected for the known dead space of the tubes that housed the sensors (1.1 L). The formula used to correct the measured expired carbon dioxide (Meas. CO2%) for the mask dead space (VDmask) was:

Corrected expired CO2% = (Meas. CO2% * VT) - ((1.1 * Meas. FICO2) / (VT − VDmask)),

where VT is the tidal volume and Meas. FICO2 is the measured fractional concentration of carbon dioxide in the inspired gas during the exercise test.

The formula used to correct the measured expired oxygen (Meas. O2%) for the mask dead space (VDmask) was

Corrected expired O2% = (Meas. O2% * VT) - ((Meas. FIO2 * VDmask) / (VT − VDmask)),

where Meas. FIO2 is the measured fractional concentration of oxygen in the inspired gas during the exercise test.

Maximal oxygen uptake was recorded as the highest value recorded during a test. Respiratory exchange ratio (VCO2/VO2), oxygen pulse (VO2/HR, mL/kg/beat) and ventilatory equivalent for oxygen (VE BTPS/VO2 STPD) were calculated with standard formulae (Ruppell, 1986).
2.5. Within-test reliability study

Two Thoroughbred horses (D, E) were used to investigate the reliability of measurements during a constant speed test exercise test on the same day between 09:00 and 11:30. The exercise test consisted of 3.5 min at 3.5 m/s, then a 30 s period of acceleration to 9 m/s. Horses then completed 195 s of exercise at 9 m/s. Treadmill velocity was then decreased to 1.5 m/s, and the horse walked for 2 min, after which the Quadflow mask was removed. Horses were then walked on the treadmill for a further 3 min, and then returned to stables. Expired gas was collected for four 30 s periods commencing at 45, 85, 125 and 165 s of exercise at 9 m/s.

2.6. Effect of mask study

Four Thoroughbred horses (C, D, E and F), were used to compare heart rate and blood lactates while wearing open flow and Quadflow masks. On day 1, two randomly selected horses undertook an exercise test with open flow mask, and the other two horses were exercise tested with the Quadflow mask. Four days later treatments were crossed over. All exercise tests were conducted between 09:00 and 11:30 h. The exercise test was the same as used for the between test reliability study. The weight of the open flow mask was 1.7 kg.

Prior to the exercise test, a catheter (Angiocath, 12GA 3IN, Becton–Dickinson) was placed in the jugular vein to enable blood collection during and after exercise. Blood (5 mL) was collected during the last 10 s of each completed step and 5 min after the exercise test. Blood was immediately placed into 5 mL Vacutainer tubes containing sodium fluoride and EDTA (Beckton and Dickinson). Tubes were stored on ice and assays for whole blood lactate were performed within one hour (YSI 1500 Sport, Nikkaki Bios). Heart rates were recorded continuously during exercise.

The following measurements were made to compare the cardiorespiratory responses in the two mask conditions: maximal heart rate (HRmax), HR-9 (the HR at 9 m/s); blood lactate concentration at 10 m/s (La-10) and 5 min after exercise (La-5°). The run time to fatigue (s) was recorded by the same person in each test.

2.7. Statistics

Results are presented as means and standard errors of the means (SEM). The differences in results with Quadflow and open flow masks were tested for significance with a two tailed paired t test, or a Mann–Whitney Rank Sum test if the data were not normally distributed (Sigmastat, Jandel Scientific). P Values <0.05 were considered statistically significant. The results in the between-test reliability study were described with means of the absolute differences divided by the pooled mean of the measurements, expressed as a percentage error of the measurement (Covey et al., 1999). The values obtained from the velocity that resulted in the highest maximal oxygen uptake (mL/min/kg) were used to evaluate test–retest differences. The reliability of four measurements during 195 s of exercise at 9 m/s in two horses was described by coefficients of variation. Results are presented as median and 25%, 75% quartiles for HR9/HRmax% because the data were not normally distributed.

3. Results

The five consecutive breaths selected for analysis in the study of between-test reliability occurred at 44–65 s after the commencement of each step (mean 56 s). Fig. 2

Fig. 2. Typical Quadflow record of respiratory gas flow rates and tidal volumes in a horse exercising on a treadmill at 10 m/s. Expiratory values are positive.
shows typical total flow rates and derived tidal volumes during exercise at 10 m/s. Mixed expired gas was not collected correctly in horse E during the constant speed test, and oxygen uptake and carbon dioxide output was not calculated for that horse.

The inspired oxygen and carbon dioxide concentrations near the horse’s nose during exercise were not constant. In some cases, the inspired oxygen percentage decreased to 20.7%, and the carbon dioxide concentrations increased to 0.06%.

Results for the paired measurements at 9 and 10 m/s in horses A and B are presented in Table 2. The mean percentage error of the oxygen uptake measurements was 8.2% (range 2.1–12.5%). Error rates were low in time-based measurements of pulmonary ventilation such as times for inspiration and expiration, and for the ratios of time based indices of lung function. Percent error for peak expiratory flow rates ranged from 6.1% to 9.4%, and for minute ventilation from 2.5% to 7.4%. Error rates were highest for respiratory frequency and tidal volume.

Table 3 shows the means, SEMs and coefficients of variation (CV %)) of four repeated measurements in two horses (D and E) during 195 s of exercise at 9.0 m/s. The coefficients of variation were less than 5% for variables related to pulmonary ventilation, and was 7.7% for oxygen uptake.

Table 4 shows the heart rates, blood lactate concentrations and run times to fatigue in horses wearing the open flow mask and the Quadflow mask. Horses wearing the Quadflow mask exercised at a 3% higher percentage of maximal heart rate, and had higher blood lactate concentrations during submaximal exercise than horses wearing the open flow mask. Run time was significantly decreased when wearing Quadflow.

### Table 2
Results for the paired measurements at 9 and 10 m/s in horses A and B

<table>
<thead>
<tr>
<th>Horse A at 9 m/s</th>
<th>Horse B at 9 m/s</th>
<th>Horse A at 10 m/s</th>
<th>Horse B at 10 m/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>AD</td>
<td>% Error</td>
<td>Mean</td>
</tr>
<tr>
<td><strong>TI</strong></td>
<td>0.247</td>
<td>0.00</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>TE</strong></td>
<td>0.259</td>
<td>0.00</td>
<td>0.8</td>
</tr>
<tr>
<td><strong>TI/TT</strong></td>
<td>0.488</td>
<td>0.00</td>
<td>0.4</td>
</tr>
<tr>
<td><strong>TE/TT</strong></td>
<td>0.51</td>
<td>0.00</td>
<td>0.3</td>
</tr>
<tr>
<td><strong>TE/TI</strong></td>
<td>1.050</td>
<td>0.01</td>
<td>0.7</td>
</tr>
<tr>
<td><strong>Rf</strong></td>
<td>118.7</td>
<td>0.5</td>
<td>0.4</td>
</tr>
<tr>
<td><strong>Rf/(TE/TI)</strong></td>
<td>113.0</td>
<td>1.2</td>
<td>1.1</td>
</tr>
<tr>
<td><strong>VT</strong></td>
<td>14.2</td>
<td>0.4</td>
<td>2.9</td>
</tr>
<tr>
<td><strong>VE</strong></td>
<td>1688</td>
<td>41</td>
<td>2.5</td>
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<tr>
<td><strong>PEF</strong></td>
<td>81.4</td>
<td>5.3</td>
<td>6.5</td>
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<td><strong>PIF</strong></td>
<td>69.9</td>
<td>3.1</td>
<td>4.5</td>
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<td><strong>ME CO2</strong></td>
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<td>0.008</td>
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<tr>
<td><strong>ME O2</strong></td>
<td>0.169</td>
<td>0.003</td>
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<tr>
<td><strong>FIO2</strong></td>
<td>0.208</td>
<td>0.001</td>
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<td><strong>FE O2</strong></td>
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<td>23.5</td>
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<td><strong>Vd</strong></td>
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<td><strong>VO2/kg</strong></td>
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<tr>
<td><strong>OP</strong></td>
<td>0.61</td>
<td>0.07</td>
<td>11.0</td>
</tr>
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</table>

AD, absolute difference; % Error, percentage error of the measurement; TI, inspiratory time (s); TE, expiratory time (s); TI/TT, ratio of inspiratory time to breath duration; TE/TT, ratio of expiratory time to breath duration; TE/TI, ratio of time for expiration to time for inspiration; Rf, respiratory frequency (breaths/min); Rf/(TE/TI), ratio of respiratory frequency to the ratio of TE/TI; VT, tidal volume (L, BTPS); VE, minute ventilation (L/min, BTPS); PEF, peak expiratory flow (L/s, BTPS); PIF, peak inspiratory flow (L/s, BTPS); FEO2, fraction of oxygen in mixed expired gas; FCO2, fraction of carbon dioxide in mixed expired gas; FIO2, fraction of oxygen in mixed inspired gas; FICO2, fraction of carbon dioxide in mixed inspired gas; FEO2 Vd, corrected fraction of oxygen in mixed expired gas; FCO2 Vd, corrected fraction of carbon dioxide in mixed expired gas; VO2 carbon dioxide output (L/min, STPD); VO2, oxygen uptake (L/min, STPD); VO2/kg oxygen uptake (mL/min/kg, STPD); VE/VO2 ventilatory equivalent for oxygen (BTPS/STPD); RER respiratory exchange ratio; HR Heart rate b/min; OP oxygen pulse (L); VO2 (L/min, STPD)/HR.
4. Discussion

The Quadflow mask had a significant effect on blood lactate concentration during submaximal exercise, and this was associated with exercise at significantly higher relative heart rates. This response was associated with a significant reduction in run time to fatigue. The increase in relative heart rate and blood lactate concentration could be due to the extra 600 g carried on the head. The Quadflow mask may have also adversely affected gait and economy of locomotion. Ideally the control condition should have been an open flow mask of the same weight. The effects of the Quadflow mask were small, and they do not detract from use of the mask for clinical exercise testing.

Variability in measurements of oxygen uptake could be related to variable function of the one-way valves used to collect the gas. However, the high reliability of measurements of mixed expired oxygen and carbon dioxide concentrations during the repeated measures within-test trial suggest that the valve function was not a major factor in the variability of values for oxygen uptake. It is also unlikely that the valves would have slow response times or leak during inspiration, given the high expiratory flow rates during exercise, and the rapid acceleration of flow rates at the commencement of expiration. However, small variations in valve performance at different air flow rates and in the positions of the valves could have contributed to the between-test variability. The samples of expired gas may not have always been a true representation of mixed expired gas.

In this experiment, inspired gas concentrations were measured and expired gas concentrations were corrected for legend to symbols see Table 2.

Table 3
Means, SD and coefficients of variation (CV %)) of four repeated measurements in two horses (horse D and E) during 195 s of exercise at 9.0 m/s

<table>
<thead>
<tr>
<th></th>
<th>Horse D</th>
<th>Horse E</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
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<td>0.002</td>
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<td>VE</td>
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<td>0.60</td>
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<td>PEF</td>
<td>83.4</td>
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<td>PIF</td>
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<td>ME CO₂</td>
<td>0.0300</td>
<td>0.0008</td>
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<td>ME O₂</td>
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<td>FIO₂</td>
<td>0.2084</td>
<td>0.0012</td>
</tr>
<tr>
<td>FICO₂</td>
<td>0.0019</td>
<td>0.0013</td>
</tr>
<tr>
<td>FE O₂</td>
<td>0.1732</td>
<td>0.0022</td>
</tr>
<tr>
<td>FE CO₂</td>
<td>0.0323</td>
<td>0.0008</td>
</tr>
<tr>
<td>VCO₂</td>
<td>42.2</td>
<td>2.56</td>
</tr>
<tr>
<td>V₀₂</td>
<td>50.7</td>
<td>3.89</td>
</tr>
<tr>
<td>VO₂/kg</td>
<td>107</td>
<td>8.2</td>
</tr>
<tr>
<td>VE/VO₂</td>
<td>34.0</td>
<td>3.5</td>
</tr>
<tr>
<td>RQ</td>
<td>0.84</td>
<td>0.09</td>
</tr>
<tr>
<td>HR</td>
<td>200</td>
<td>4</td>
</tr>
<tr>
<td>OP</td>
<td>0.54</td>
<td>0.05</td>
</tr>
</tbody>
</table>

For legend to symbols see Table 2.

Table 4
Heart rate, blood lactate concentrations and run times to fatigue in four horses wearing the open flow mask and the Quadflow mask (means ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>Open flow</th>
<th>Quadflow</th>
<th>p</th>
<th>P (%)</th>
</tr>
</thead>
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<tr>
<td>HRmax</td>
<td>217 ± 4.4</td>
<td>214 ± 3.6</td>
<td>0.6</td>
<td>5</td>
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<tr>
<td>HR-9</td>
<td>196, 194–202</td>
<td>199, 179–207</td>
<td>0.3</td>
<td>70</td>
</tr>
<tr>
<td>HR-9/HRmax (%)</td>
<td>91 ± 0.9</td>
<td>94 ± 0.4</td>
<td>0.02</td>
<td>51</td>
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<tr>
<td>La-9</td>
<td>4.7 ± 0.4</td>
<td>5.7 ± 0.2</td>
<td>0.04</td>
<td>51</td>
</tr>
<tr>
<td>La-5'</td>
<td>11.3 ± 1.5</td>
<td>11.8 ± 1.6</td>
<td>0.82</td>
<td>5</td>
</tr>
<tr>
<td>RTF</td>
<td>484, 479–494</td>
<td>434, 408–458</td>
<td>0.03</td>
<td>5</td>
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</tbody>
</table>

HRmax, maximal heart rate; HR-9, heart rate during exercise at 9.0 m/s; HR9/HRmax%, percentage of maximal heart rate; La-9, blood lactate during exercise at 10.0 m/s; La-5', blood lactate concentration 5 min after exercise; p, level of significance; P, power of the test (%).
for known mask dead space. When these corrections were not included in the calculations, the oxygen uptake rates were be 9.4–15.0% (5.3–8.7 L/min) lower. These corrections are therefore very important when masks with flow tubes are used. The true functional dead space in the mask is unknown, and would obviously be greater than 1.1 L dead space measured in the two sensor tubes. Thirty seconds of collection of expired gas at a speed of 4 m/s was not sufficient to provide sufficient volume of gas for analysis. It was therefore not possible to calculate oxygen uptake for exercise at that speed.

There was considerable error in the repeated measurement of mixed expired carbon dioxide percentage in horse A. The source of this error was not identified, but the magnitude of the difference and the reliability of the mixed expired oxygen concentrations suggest that there may have been a technical error, for example, in calibration or gas collection. Mixed expired carbon dioxide and other variables that use this measurement were highly repeatable in the continuous exercise test. High variability in the values for inspired carbon dioxide concentrations was also a problem. This variability would contribute to high variability in values for carbon dioxide output and respiratory exchange ratio. Future design of the mask should include a refined method of measuring gas concentrations in the inspired gas. A vacuum pump was used to remove room gas and to assist with temperature control in the laboratory during exercise. Changes in inspired gas oxygen and carbon dioxide concentrations during tests would contribute to lack of steady state conditions, and would probably result in exacerbated hypoxaemia during exercise. Corrected mixed expired oxygen concentrations in this study during maximal exercise were in the range 16.1–17.2%. These values are higher than the mixed expired oxygen percentages reported in a laboratory that uses an ultrasonic flow meter during an exercise test (Lekeux and Art, 1994). The difference could be due to absence of an exhaust fan to remove hypoxic expired gas from the laboratory. Pneumotachometers should be used in conjunction with a vacuum pump to exhaust gas from the laboratory as much as possible during exercise tests.

The exercise test focused on collection of data at velocities that were at or near those that result in maximal oxygen uptake, rather than on collection of 3–4 sets of data at submaximal velocities. The design maximised the opportunity for collection of important data during intense exercise. The distance run by a horse that completed 75 s exercise at 11 m/s in the exercise test in this study would be approximately 3100 m, 14% less than in the treadmill test used by Rose et al. (1990, 1995). Removal of hot and hypoxic expired gas from the laboratory during the test should promote welfare of horses during treadmill exercise tests.

A potential disadvantage of the test protocol was the variability in rates of acceleration in the 30 s ramp period when the horse accelerated from 3.5 to 9 m/s. In this period, the treadmill velocity was gradually increased, taking into account the horse’s individual response during acceleration. We believe that this approach was in the interests of the welfare of the horses during the exercise tests. This method should not affect the measurements of ventilation, oxygen uptake or heart rate at velocities of 9 m/s and greater. The approach could also be an advantage because there may be less likelihood of anxiety with a smooth transition from trotting to high-speed gallop exercise.

Reliability measurements are an important component of the performance of any measuring device. Ideally, accuracy of measurements should also be assessed. The gold standard technique for assessment of the accuracy in measurements of oxygen uptake is the Douglas bag technique. This study was not able to assess accuracy because a “gold standard” technique was not available. However, the mean rate of oxygen uptake during exercise at 9 m/s on the treadmill inclined at 10% was similar to the values measured in other laboratories (Eaton et al., 1995; Rose et al., 1990; Erickson et al., 1994).

It was concluded that the Quadflow mask causes small increases in blood lactate concentration and relative heart rate during submaximal exercise. Between and within test reliability statistics for important measurements in equine clinical exercise testing were acceptable for routine use in a veterinary practice for clinical exercise testing, or in a research laboratory. Quadflow breath-by-breath spirometry coupled with collection of expired gas with one-way valves provides measurements of pulmonary ventilation and oxygen uptake that are reliable. The technique is suitable for use in future studies of pulmonary ventilation during treadmill exercise in horses.

Acknowledgements

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References


