Evaluation of oxygen consumption during field exercise tests in Standardbred trotters

Emmanuelle van Erck¹*, Dominique-Marie Votion¹,², Didier Serteyn¹ and Tatiana Art¹
¹Equine Sports Medicine Centre, Equine Pole, Bat. B41, Faculty of Veterinary Medicine, University of Liège, Sart Tilman, B-4000, Liège, Belgium
²Equine European Centre of Mont-le-Soie, Mont-le-Soie, Vielsalm, Belgium
* Corresponding author: emmanuelle.vanerck@skynet.be

Submitted 14 February 2007: Accepted 9 May 2007

Abstract

Reasons for performing the study: In human exercise physiology, the current gold standard for evaluating aerobic capacity is the measurement of oxygen consumption (VO₂) and maximal oxygen uptake (VO₂max). The evaluation of VO₂ in horses is performed in some laboratories equipped with a treadmill but has only been exceptionally reported in field conditions because of the lack of adapted equipment.

Objectives: The aim of this study was (1) to assess the feasibility of VO₂ measurement on the track using a recently validated portable breath-by-breath gas analyser system adapted to horses (Cosmed K4b² and Equimask), (2) to compare these results with those obtained during a treadmill exercise test and (3) to study correlations between VO₂ and physiological parameters usually measured in field condition such as heart rate (HR), lactataemia (LA) and the speed at which HR equals 200 beats per minute (bpm) (V₂₀₀) or LA 4 mmol l⁻¹ (VLA₄).

Methods: Five healthy Standardbred trotters in training were submitted to two stepwise incremental exercise tests, one driven on the racetrack and the other on a high-speed treadmill with a 4% incline. Speed (v), HR, ventilatory parameters and VO₂ were continuously recorded throughout the duration of the tests and LA was evaluated after each step.

Results: All horses completed the test satisfactorily after an initial acclimatization to the mask. There were marked individual differences in ventilatory strategy, and breathing frequency (Rf) at the higher levels of exercise was noticeably low. The VCO₂ measurements were incoherent. There were no significant differences between track and treadmill maximal data obtained during the last step [VO₂peak (track: 139.9 ± 8.9 ml kg⁻¹ min⁻¹; treadmill: 139.9 ± 13.4 ml kg⁻¹ min⁻¹), LAmax (track: 6.5 ± 1.6 mmol l⁻¹⁻¹; treadmill: 7.3 ± 3.0 mmol l⁻¹⁻¹), HRmax (track: 229 ± 6.2 bpm; treadmill: 222 ± 13 bpm)], although the maximal speed required to reach similar workloads was significantly higher on the track (11.9 ± 0.6 m s⁻¹ vs. 9.7 ± 0.4 m s⁻¹). The correlation between VO₂ and HR (r = 0.87; P < 0.001) and VO₂ and LA (r = 0.75; P < 0.0001) during both tests was good but no correlation was found between VO₂peak and HRmax, LAmax, V₂₀₀ or VLA₄.

Conclusions: This is the first report of a practical portable system to measure VO₂ and ventilation continuously during high-speed field exercise tests. However, current mask design markedly influences ventilation and could have prohibited the attainment of VO₂max. Furthermore, consistent VCO₂ measurements should be implemented by the manufacturers. Potential relevance: Continuous breath-by-breath ventilation and VO₂ measurements can be recorded in horses in the field at submaximal levels. With necessary adaptations to the system entailed, this study opens new perspectives in the analysis of physiological and metabolic mechanisms of exercise in the equine species in genuine track conditions.

Keywords: horse; exercise; oxygen uptake; field; treadmill

Horses are outstanding elite athletes in comparison with all other species. They owe their remarkable athletic potential to an incomparable aerobic capacity¹,². Clinical exercise testing of equine athletes allows investigation of some of their physiological specificities, evaluating their level of fitness, following their adaptation to training and is a valuable tool to assess causes of poor performance³–⁵. Common variables readily measured during exercise are heart rate (HR), and blood or plasma lactate concentration (LA) according to speed (v). Some
In human exercise physiology, the current gold standard for evaluating aerobic capacity is the measurement of oxygen consumption (VO$_2$) and more especially maximal oxygen uptake (VO$_{2\text{max}}$). The VO$_{2\text{max}}$ parameter integrates the efficiency and coordination between respiratory, cardiovascular and neuromuscular systems and thus yields both physiological and metabolic information.$^{11}$ Measurements of VO$_{2\text{max}}$ can be used to predict aerobic power and endurance performance in human subjects. Contrary to human athletes, the measurement of oxygen uptake and ventilation in horses represent a true technical challenge and is thus less commonly performed.

Up until now, the measurement of VO$_2$ in that species was achieved in some laboratories equipped with a high-speed treadmill. A milestone was crossed in 1964 when a study of ventilation and VO$_2$ during a high-speed treadmill was performed.$^{12}$ The conditions in which this study was accomplished were epic. However, since then, the evaluation of VO$_2$ has only been exceptionally reported in field conditions because of the lack of adapted gear.$^{13-15}$ Recently, an adapted portable breath-to-breath gas analyser system and mask (Cosmed K4b$^2$ and Equimask$^R$) have been validated for the measurement of VO$_2$ in horses.$^{16,17}$ This technology opens a new perspective for field evaluation of exercising horses.

The advantage of evaluating horses on a track is to recreate familiar exercise conditions, similar to those encountered during training sessions or competition while on a treadmill; several factors such as stress, locomotion, harnessing and thermoregulation can influence the outcome of the exercise tests. These factors can create significant bias in HR, LA and stride length.$^{10,18,19}$ Because of this, treadmill exercise testing may not constitute an accurate reflection of the horse’s athletic ability on the track.

The aim of this study was (1) to assess the feasibility of VO$_2$ measurement on the track using the K4b$^2$ portable system, (2) to compare these results with those obtained during a treadmill exercise test and (3) to study correlations between VO$_2$ and physiological parameters usually measured in field condition, such as HR, V200, LA and VLA4.

**Material and methods**

**Horses**

Five driven Standardbred trotters in training were included in this study. The group contained three mares and two geldings, whose age ranged from 3 to 6 years and weighing 495 ± 31 kg. The horses were clinically healthy and sound. They were kept at the same stable but their level of training and racing differed.

**Exercise tests**

The horses were submitted to two stepwise incremental exercise tests, one on the racetrack and the other on a high-speed treadmill (Mustang, Kagra, Switzerland), in an unbalanced crossover study with two horses performing the track then the treadmill test and three horses performing the treadmill test first, all at a week’s interval.

The track test was performed on a 1000 m racetrack with the horse harnessed to a sulky. It consisted of an initial 5 min walk and 5 min trot warm-up phase. The horses were then exercised over 1000 m at incremental speeds of approximately 7.6, 8.5 and 10 m s$^{-1}$. Between steps, the horses were allowed to recuperate at a slow trot for 2 min. A last speed bout was performed over 500 m at maximal speed.

The treadmill test also consisted of a similar 5 min walk and 5 min trot warm-up phase. The exercise test consisted of three successive incremental phases at speeds of 7.4, 8.3 and 9.7 m s$^{-1}$ at a 4% incline. Between each phase, horses were trotted 2 min at 5 m s$^{-1}$ on a 0% incline. Since the horses were at fatigue, the last bout at maximal speed was not performed on the treadmill.

**Measurements**

The horses were weighed on each test day. For each test, v, HR, ventilatory parameters (BTPS) and VO$_2$ (STPD) were recorded. The K4b$^2$ system was equipped with a GPS and a heart rate Polar$^R$ system which recorded continuously speed and HR during the track exercise tests. The driver was able to control speed with the help of a tachymeter linked to the sulky wheels. An ECG telemetry unit (Lifescopes 8, Nihon Kohden, USA) was also used to control HR during the last 30 s of each exercise step.

Blood lactate concentration was evaluated 1 min after the end of each exercise step by sampling blood in the jugular vein and by using a portable analyser (Accutrend$^R$ Lactate, Roche, USA). Blood samples were collected 1 min after the end of each step.

A portable K4b$^2$ open gas analysis system (Cosmed, Rome, Italy) was used to measure breath-by-breath ventilatory variables. This system has been described and evaluated for its use in horses.$^{16,17}$ The K4b$^2$ system consists of a portable unit that comprises an O$_2$ and a CO$_2$ gas analyser. Respiratory gases are expired through two turbines fitted on an adapted airtight facemask. The sampling line is a semi-permeable Permapure Naflon$^R$ catheter designed to avoid condensation. Gas and delay calibrations were performed prior to each test.
The facemask (Equimask®) was made of a moulded plastic piece that covered completely the tip of the head and was prolonged over the forehead to increase stability. A hackamore bridle was adapted to the mask to allow control of the horse while ensuring airtightness. A silicone gasket was snugly fitted around the lower part of the mask. The mask was maintained over the horse’s head by the hackamore bridle and a headcheck (Fig. 1). A bidirectional turbine was inserted in each of both openings facing the nostrils. One of the turbines was fitted with a ventilation opto-electronic reader and the gas sampling line. The K4b² analyser and recording unit were fixed to a strap fastened around the horse’s thorax. Prior to the study, the horses were acclimatized to working with the facemask and hackamore bridle. All horses were driven by the same driver during the acclimatization sessions and the exercise tests on the track.

**Data analysis**

The data collected with the K4b² system were analysed with provided specific software. Data were averaged over a minimum of 20 s during a steady-state period at the end of each step. The VLA4 and V200 measurements were calculated by linear interpolation. Peak oxygen consumption (VO₂peak), maximal blood lactate concentration (LAmax), maximal heart rate (HRmax) and Vmax correspond to the average maximal values reached at the last step of each incremental test.

Statistical comparison of data recorded on the track and treadmill was performed by ANOVA and significance was considered for P < 0.05. For comparison of VO₂ measurements during the test, speed and individuals were included as covariates. Correlations between exercise indices were made using a Spearman rank correlation test on independently derived variables.

**Results**

Prior acclimatization to the mask and hackamore bridle was a necessary step to ensure safety and comfort of both the horse and driver. All horses completed the test satisfactorily and maintained a relatively constant speed during each step of the exercise test on the racetrack. (Fig. 2) is a typical example of the VO₂ and speed data recorded by the K4b² system during a field exercise test (Horse 2). This time tracing shows that the duration of the last step was insufficient to elicit a plateau in VO₂. Apart from variations in the scales, the recorded VO₂ tracings were similar in the other horses.

The parameters measured in each individual during the exercise tests both on the treadmill and track are summarized in Table 1. Maximal data were obtained during the last step of each test. The highest VO₂ reached varied considerably between individuals, suggesting that VO₂max had not been reached in most horses. The VO₂ data recorded at the last step were therefore designated as VO₂peak. Although the track test included an extra step at maximal speed, the LAmax, HRmax and VO₂peak achieved on the track and on the treadmill were not significantly different. However, the Vmax reached on the track was significantly higher in comparison with the treadmill. Minute ventilation was also comparable between track and treadmill tests, although the respiratory frequency (RF) was significantly higher during the last step of the treadmill test. Marked differences in ventilatory strategy were observed between individuals.

Although VCO₂ measurement was implemented in the K4b² system, the results obtained in our horses were markedly underestimated and incompatible with physiological reference data. Consequently, the calculated RER was inferior to 1 at all levels of exercise.

The derived exercise indices V200 and VLA4 determined from the treadmill test were significantly lower in comparison with those obtained from track data (Fig. 3). The inter-individual variability of V200 calculated from the treadmill data was greater than that from the track.

There was a good correlation between VO₂ and speed in both treadmill (r = 0.67; P < 0.01) and track tests (r = 0.88; P < 0.0001), but VO₂ at a given speed was significantly higher on the treadmill than on the
track (Fig. 4). The fact that both graphs demonstrate a parallel linear relationship shows that the increase in VO\textsubscript{2} with speed was proportional whatever the testing condition.

The correlation between VO\textsubscript{2} and HR (\(r = 0.87; P < 0.001\)) and VO\textsubscript{2} and LA (\(r = 0.75; P < 0.0001\)) was good during both tests, while no correlation was found between VO\textsubscript{2peak} and the indices measured at the highest level of exercise (i.e. HR\textsubscript{max} and LA\textsubscript{max}), nor with V200 or VLA\textsubscript{4} (Table 2).

**Discussion**

This is the first report of continuous breath-by-breath VO\textsubscript{2} and VO\textsubscript{2peak} measurements in trotters under genuine track conditions. The measurement of oxygen consumption and ventilation parameters with the K4b\textsuperscript{2} system and the Equimask\textsuperscript{w} can be performed during submaximal field exercise tests as well as treadmill tests.

The small portable equipment is easily fixed on the harness and can also be carried by a rider if the measurements are to be performed in a ridden horse\textsuperscript{17}. Two to three sessions were necessary for the horses to acclimate to exercising on the track with the mask and to respond satisfactorily to the hackamore bridle. The use of a headcheck proved to be particularly useful to limit excessive head movements of the horse and to maintain the mask in an adequate position throughout the test.

The Rf values measured both on track and treadmill were lower than the available physiological values for Standardbreds performing similar levels of exercise\textsuperscript{20}. These low Rf values, associated with increased tidal volumes, suggest an excessive resistance of the turbines and possibly deadspace, impeding ventilation as the flow rates increase. Similar results were found in the validation study performed by Art \textit{et al.}\textsuperscript{16}. Bayly \textit{et al.}\textsuperscript{21} have shown in a previous study that mask design of gas analysis systems without bias flow could significantly impede gas exchange and ventilation during exercise. Although mask design differed, theirs, fitted with two 100 mm openings, produced high resistance. The K4b\textsuperscript{2} turbines have an internal diameter equal to 60 mm and are each covered by a cap to limit flow damping. An estimation of the resistance of the system at varying flow rates up to those encountered during strenuous exercise should be performed. Further adaptation of the mask would be required to limit its interference with normal breathing at high flow rates and possible consequences on the measured VO\textsubscript{2}.

The several athletic indices measured in this study were comparable to previously published data in Standardbreds, even though exercise protocols varied\textsuperscript{10,22}. Although the track test included an extra step, the LA\textsubscript{max}, HR\textsubscript{max}, VE\textsubscript{max} and VO\textsubscript{2peak} reached on the track and treadmill were not significantly different, indicating that the horses were submitted to a comparable workload in both conditions. This required that the horses ran faster and for a longer period on the track. The discrepancy in vmax between the indoor and outdoor tests can be explained by factors inherent to differences in testing conditions. On the track, the horses were exercised in more familiar conditions and acclimatization to the mask and bridle had been initially performed by their trainer in similar conditions. For the treadmill tests, the horses were acclimated for a shorter period before a proper test was carried out. The greater variability in HR data measured on the treadmill could have been induced by the fact that some horses were more stressed when exercised indoors, but also to differences in muscle recruitment and cardiac output due to the treadmill incline\textsuperscript{23}. Although it is admitted that exercising on a treadmill is more stressful for horses, comparative studies of track and treadmill tests have shown that for identical speeds, HR and LA were lower on the treadmill, with an incline kept at 0%\textsuperscript{10,18}. The comparison of the VO\textsubscript{2} values measured for a given HR (VO\textsubscript{2}-200) or speed confirmed that, with the exception
of the youngest horse, the horses consumed more oxygen on the treadmill than on the track. This also influenced the outcome of the V200 and VLA4 measurements, which were lower on the treadmill in comparison with the track. In our study, a 4% treadmill slope may have imposed a greater workload than the one experienced by the horse pulling the sulky and driver on the track. Differences in oxygen uptake between incline and flat surfaces can be attributed to significant differences in muscle recruitment, locomotion pattern and cardiac output.

The inter-individual variations observed in the parameters measured during this study were mainly due to differences in age and in training status. The youngest and least experienced horse (Horse 2) reached higher VO2 values in the last step and was probably closer to VO2max than the older and more highly trained horses. On the other hand Horse 4, which had the highest level of training, displayed the lowest values in VO2peak, suggesting that further increase in workload would have been necessary to educe VO2max. The trainers’ classification of the horses according to age, level of training and subjective criteria was identical to the one brought forth by VO2peak on the track. The VLA4 and V200 parameters were less useful in establishing athletic discrimination between horses. The poor agreement between the V200 and VLA4 parameters and VO2peak or VO2-200 agrees with previous publications.

This study was designed to propose a practical field test including VO2 evaluation in working equine athletes rather than in research horses. Compliance of the horse, driver and trainer with the material and test were critical elements which had to be taken into account. Most horses were unable to reach higher speeds and performed the exercise tests at a submaximal level. Reaching higher levels of exercise could have been impeded by the high resistance of the mask. The criteria to establish VO2max were not achieved in most horses, i.e. a plateau in VO2 at the last steps of the test and rapidly accumulating blood lactates (>8 mmol l\(^{-1}\)) with increasing speed. The type of incremental exercise test applied in this study might also have been inappropriate for reaching VO2max in all horses, as the speed and lactate concentrations reached in the final step stayed relatively low in comparison with what could be expected in a maximal exercise. The determination of VO2max would require submitting the horses to a single, continuous maximal exercise or to longer bouts of exercise at higher speeds. For the horses used in this study, maximal exercise testing would have certainly required further acclimatization and would have been difficult to achieve in the younger
and less trained individuals. In humans, various standardized submaximal tests have been developed to predict VO₂max in order to bypass problems due to a lack of training or motivation to reach a maximal effort level and to avoid possible injuries. To calculate VO₂max, these tests take into account age, gender, body weight and other factors based on submaximal VO₂ and/or HR, speed and distance determined during the standardized test. Although the obtained result is less accurate than an actually measured VO₂max value, such predictive tests could be useful in the equine species where cooperation of the horse is sometimes difficult to obtain. This would require initial devising of VO₂max measurements in a sufficiently wide population of horses of varying age and training status. A recent study by Votion et al. has shown the potential application of this ergospirometry system in ridden horses in a submaximal field trial.

**Conclusion**

This study shows that indoor treadmill exercise tests were more physically demanding for horses than outdoor track tests. This difference in metabolic demand could be due to various factors such as stress, thermoregulation or participation of different groups of muscles in locomotion and/or respiration, for instance due to the slope on the treadmill and differences in surface resistance. As a result, even when similar exercise protocols are respected, treadmill and track tests will measure different elements of a horse’s athletic capacity. Since it seems to impede ventilation at high exercise levels, preliminary adaptation of the Cosmed K4b² system and Equimask is required before more studies are engaged. The tests have not allowed eliciting VO₂max; increased resistance to airflow due to mask design could have induced premature fatigue and precluded reaching maximal exertion. Furthermore, consistent VCO₂ measurements should be implemented by the manufacturers.

With minimal acclimatization and an adapted mask design, the K4b² could represent a useful and promising tool for the analysis of physiological and metabolic mechanisms of field exercise in equine sports medicine. Its suitability to track conditions can enable avoidance of the metabolic bias generated by working on the treadmill.

**Acknowledgements**

The authors wish to thank Mr M Grisez, Mr P Ronchain and Dr F Grisez for allowing their horses to participate in this study and for their preparation. They also wish...
to thank M Leblond for her help in preparing this manuscript and W Westergren for the photographs.

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