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Reproducibility and Validity of VLA4 in Standardbred Pacer Horses on Track

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ABSTRACT. The reproducibility of the speed producing a blood lactate concentration of 4 mmol l^{-1} (VLA4) was evaluated in 7 Standardbred pacer horses on track, using a 3 min stepwise increasing speed protocol. The validity of VLA4 was assessed using a 5 min exercise step protocol. The lactate concentration of arterial blood (AB) was used. The reproducibility of VLA4 based on a test/retest experiment was doubtful ($r = 0.38$, p-value = 0.62). There was no significant difference between mean values of VLA4. No horse reached a blood lactate steady state while running at VLA4. Fatigue time ranged from 10 to 40 min. It is concluded that the reproducibility of VLA4 on track is not acceptable, whereas mean values of VLA4 are very similar. Furthermore, the validity of VLA4 could not be confirmed with this exercise test protocol.

Key words. Horses; exercise test; blood lactate.

INTRODUCTION

It has been suggested that the intensity of continuous running in exercising horses can be best established using cardiorespiratory[3,5] and metabolic parameters.[11] However, the maximum intensity that can be used safely by a competent trainer, in terms of fatigue-related injuries, is referred to as the intensity just before the redline area[5] or that producing maximum blood lactate steady state (MLSS). As in the human,[4] it has been suggested that running a horse at a speed corresponding to a blood lactate concentration of 4 mmol l^{-1} (VLA4), determined using a stepwise increasing speed protocol, would produce MLSS.[11]

The validity of VLA4 has been the object of only one study.[11] They found VLA4 to provide a reasonable estimate of steady state conditions in the horse, although some questions regarding their materials and methods remain. The reproducibility of VLA4 has been reported to be less than for oxygen uptake variables and pulse/work relationships,[8] although the type of blood collected and other details of the exercise test protocol used, i.e. exercise step duration, pause period, running surface and exercise step progression, were not mentioned. How long a horse can exercise at VLA4 has not been studied.[10]

Even if some believe that horses running at VLA4 are at a blood lactate (La) steady state, we are not convinced, still less than they are at MLSS. Thus, the purpose of this study was to assess the validity of the concept of VLA4, i.e. running a horse at a speed corresponding to a La concentration of 4 mmol l^{-1}, determined using a stepwise increasing speed protocol, would produce MLSS. The study also assessed the reproducibility of VLA4 under the constraint of measuring it on track.

There is no standardized exercise test protocol established to determine VLA4. In this study, we arbitrarily selected a 3 min stepwise increasing speed protocol to assess the reproducibility of VLA4 in Standardbred pacer horses on track. The idea being that, at VLA4 we could predict MLSS during prolonged exercise. The validity of VLA4 was

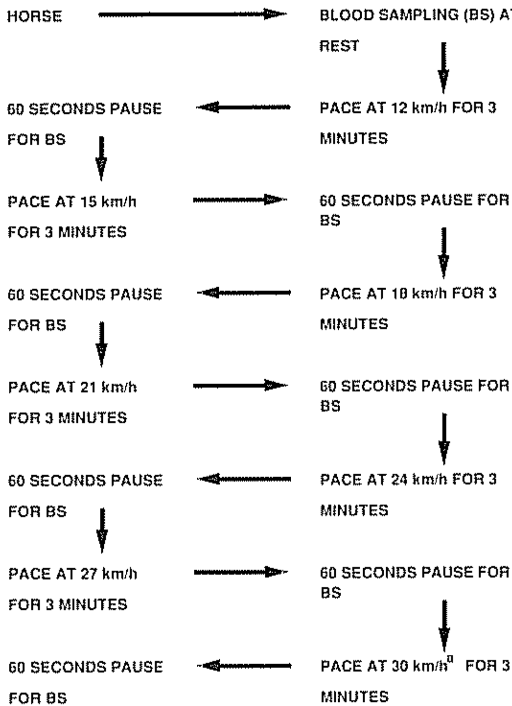

Fig 1 Schematic representation of the exercise protocol to determine VLA4. (*a*) Speed of pacing at the last step of exercise varied from 30 to 36 km h^{-1} depending on the horse used.

assessed by having each horse running at VLA4 as long as it could and measuring the arterial blood (AB) La concentration during a 60 second pause period every 5 min.

MATERIALS AND METHODS

Subjects

Seven healthy untrained Standardbred pacer horses were used in this study: 3 females and 4 males, aged 3 to 8 years, weighing 352 to 484 kg. Each horse had its left common carotid artery (CA) relocated subcutaneously while under general anesthesia. The day of the experiment, the horse selected had its left CA catheterized (Deseret, USA, Angiocath, 16 gauge, 13.3 cm) under local anesthesia. The catheter was secured with glue and sutures. Patency of the catheter was maintained by flushing it with heparinized saline (50 μ ml^{-1}) until the experiment began.

Experimental procedure

The experiment took place on an oval sand track 436 m in length at its center. Cones were positioned on the side of the track to indicate the stopping sites of the horse. At every site indicated, material for blood collection was placed on a cardboard sheet and protected from dust. Pacing speed of the horse was indicated by an electronic tachometer (Avocet, Cyclometer 20) fixed to the sulky, giving constant visual feedback on pacing speed to the driver. The tachometer, which was calibrated before every experiment, indicated the speed of pacing in km h^{-1}. The interpolated value of VLA4 had thus to be rounded toward the smallest integer. All experiments were done under similar track (dry surface) and weather (minimal wind, 22.9±8.3°C and 68.9±10.2% humidity) conditions. The exercise protocol to determine VLA4 (Fig. 1) was to fatigue and was repeated twice (T1 and T2). The minimum elapsed time between the two tests was 2 days. The exercise protocol to verify the evolution of the lactatemia with time at VLA4 (mean of T1 and T2) (Fig. 2), was carried out 1 or 2 weeks after T2. The horse was brought to VLA4 progressively to warm up the tissues and allow for any delay of the energy systems reaching a steady state. To establish a La steady state at VLA4, the La concentration must not fluctuate more than +1 mmol l^{-1} within the last 20 min of exercise.[4] If, at some point, the La concentration curve displayed a tendency toward the resting level (i.e. at least the last two exercise steps), the horse was considered to have not exceeded La steady state.

Blood samples collection and analysis

Blood samples from the CA were collected with 5 ml syringes, with their deadspace filled with sodium heparin (1 000 μ ml^{-1}), at rest and within 60 seconds after each exercise step. During exercise, between sampling times the catheter was flushed with saline

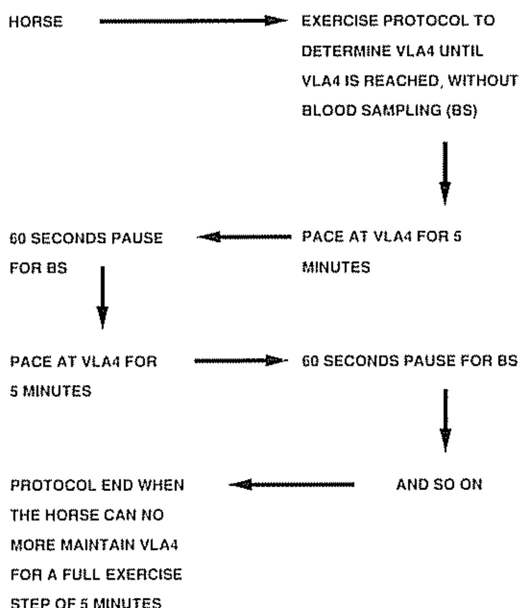

Fig 2. Schematic representation of the exercise protocol to verify the evolution of the lactatemia with time at VLA4.

Table I. *Individual values of VLA4 in kilometers per hour (km h^{-1}) determined from arterial blood (AB) samples for the first (T1) and second (T2) test of each horse and mean values of VLA4*

Horse	T1	T2
1	30	–[a]
6	27	25
8	24	28
9	30	30
10	–[a]	30
11	30	–[b]
12	30	28
Mean[c]	28.5	28.2

[a] Blood sampling has not been possible.
[b] Blood lactate concentration did not reach 4 mmol l^{-1} following the last step of exercise completed.
[c] No significant difference between mean values of VLA4 of T1 and T2.

only, to maintain patency. Prior to sampling, 10 ml of saline and blood were withdrawn and discarded. One ml of blood sample was mixed in a tube with 2 ml of ice cold 0.6 M perchloric acid for deproteinization. The tubes were stored on ice at the track and at 4°C in the laboratory until analysed. After 5 min of centrifugation at 3 000 rpm, the La concentration was measured automatically (Hitachi, Model 705 Analyser) in duplicate on the supernatant by an enzymatic method (Behring Diagnostics, Stat-Pack™ Rapid Lactate Test).

Statistical analysis

Two group paired *t*-test, arithmetic mean and simple regression which include the Pearson correlation coefficient (*r*), the standard error of estimate (SEE), which is the level of dispersion of the data around the regression line, were the statistics used. The SEE was expressed in kh h^{-1} and as percentage of the mean. $P<0.05$ was accepted as statistically significant.

RESULTS

Reproducibility of VLA4

The results ($r=0.38$, *p*-value = 0.62, SEE = 2.335 km h^{-1} or 8.41%) indicated that the reproducibility of VLA4 was doubtful. Two group paired *t*-test showed no significant difference between mean values of VLA4 of T1 and T2 (Table I).

Validity of VLA4

To assess the validity of VLA4, graphs of the La concentration vs time of pacing at VLA4 were established for horses number 1, 9, 10, 11 and 12 (Fig 3). No horse reached a La steady state nor did the La concentration curves display a clear tendency toward the resting level. Time to fatigue ranged from 10 to 40 min.

DISCUSSION

Reproducibility of VLA4

Individual values of VLA4 did not display an acceptable reproducibility, whereas mean

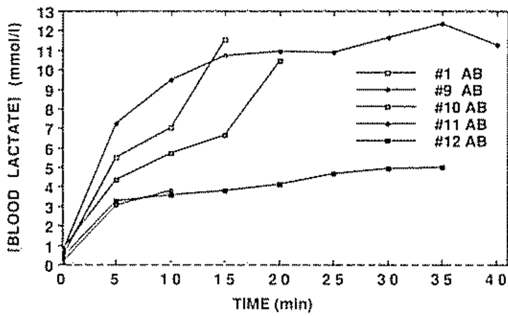

Fig 3 Arterial blood (AB) lactate concentration versus time of pacing at VLA4 curves for horses number 1, 9, 10, 11 and 12. No horse reached a blood lactate steady state nor did the curves display a clear tendency toward the resting level.

values of VLA4 were very similar. A correlation coefficient of 0.63 has been reported[8] for the reproducibility of individual values of VLA4 with a standard error of duplicate determination of 0.58 m s^{-1} or 7.1% expressed as a percentage of the mean, which support our finding, but the type of blood collected and other details of the exercise test protocol are not mentioned. Dahl et al.[2] found no significant difference between mean values of VLA4 determined under two different ambient temperatures in a climate chamber on a treadmill, which also supports our finding. In humans,[1] a continuous incremental exercise test/retest protocol on a bicycle ergometer has been used and a correlation coefficient of 0.86 was observed for the determination of the oxygen consumption at a venous La concentration of 4 mmol l^{-1}.

Several factors will influence the reproducibility of VLA4. Since it was determined outside on track, the surface adherence, wind condition and ambient temperature[2] could have changed from T1 to T2 as well as the air pressure of the sulky tires and the muscular substrate available.[12] However, these variables differed only minimally between T1 and T2. Even between sampling times, the catheter was flushed only with saline, instead of heparinized saline, to avoid extra lipolysis. The driver of the sulky, who was always the same, could not always maintain exactly the speed of pacing required for the full exercise step. The speed of pacing oscillated around the required speed. So, according to the amplitude of oscillation in both directions, the mean speed maintained for the full step of exercise could have varied from T1 to T2. However, the distances covered during the 3 min exercise steps were similar and the stopping sites corresponded closely to the designated cone. Probably a combination of all the variables mentioned, although controlled, played an important role in the non-acceptable reproducibility of VLA4 observed in this study.

Validity of VLA4

No horse reached a La steady state while running at VLA4. However, the La concentration vs time of pacing at VLA4 curve of horse number 9 indicated a tendency toward the resting level, but not enough to consider that the horse has not exceeded a La steady state. Wilson et al.[11] concluded VLA4 gives a reasonable estimate of steady state conditions in horses. However, in addition to having a very different exercise test protocol and procedure to determine VLA4, their materials and methods section raise certain questions. First, the blood samples were deproteinized with perchloric acid and analysed for lactate concentration with Boehringer Mannheim kit No. 149993, which is not made to work with deproteinized samples. Secondly, the testing procedure to verify the validity of VLA4 is simply a partial repetition of the test previously used to determine VLA4, except that one group of horses stopped running at an exercise step with speed equal to the calculated VLA4 and a second group began running at an exercise step with a speed higher than the calculated VLA4. This testing procedure does not allow the assessment of the La kinetics during prolonged exercise.

Humans exercising at the 4 mmol l^{-1} threshold demonstrated a mean La concentration vs time of exercise curve which indicated that they did not exceed a La steady state. Mean fatigue time was 38.2 min, al-

though some subjects exercised more than 60 min before being fatigued.[7] Full motivation of the subjects was ensured by according them a reward proportional to the time of exercise. In this study, motivation of horses came from the threat of a whip. So, we cannot be sure that they would not have tolerated a couple more exercise steps at VLA4, in particular horses number 9 and 12. Motivation has been reported as a problem with horses.[6]

In humans, Heck et al.[4] argue that VLA4 is bound to a defined testing procedure. When the 3 min exercise step protocol is used, the intensity of exercise producing MLSS is suggested to be at an arterialized La concentration of 3.5 mmol l^{-1}, whereas with a 5 min exercise step protocol 4 mmol l^{-1} is suggested. They demonstrated that the longer the duration of the exercise step, the steeper the exponential curve and the lower the speed at a La concentration of 4 mmol l^{-1}. So, if we had used exercise steps of 5 min duration, VLA4 would have been lower and possibly some horses would have reached a La steady state pacing at VLA4.

Even if in this study all horses paced at VLA4, it did not produce the same metabolic stress. The period of time horses paced at VLA4 was different as well as the La kinetics. It suggests that it may not be possible to determine the exercise intensity producing MLSS from a fixed La concentration. Perhaps testing procedure should be oriented toward individual determination of the speed and La concentration producing MLSS. Heck et al.[4] used a trial-and-error procedure to individually verify the exercise intensity producing MLSS. Stegmann et al.[9] proposed for human subjects a procedure taking into account the individual kinetics of the La concentration curve to determine the exercise intensity producing MLSS. Perhaps an adaptation to horses of this procedure is a solution.

Mean values of VLA4 are very similar on a test/retest and may be useful when comparison of aerobic fitness is made between several groups of horses or in the same group but at different phases of training. Individual values of VLA4 are not reproducible under the constraint of measuring it on track. The validity of VLA4 could not be confirmed with this exercise test protocol. A test to determine the exercise intensity producing MLSS in horses, should probably take into account the individuality of the physiological characteristics of each horse.

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Exercise-induced Changes in Muscle and Plasma Amino Acid Levels in the Standardbred Horse

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ABSTRACT. Concentrations of amino acids in the gluteus muscle and in venous plasma were analysed in six Standardbred horses that performed an incremental standardised exercise tolerance test (SET) and also a submaximal exercise test to fatigue (SEF) on a treadmill. The intramuscular concentration of phenylalanine increased after SET suggesting net protein breakdown during exercise. Alanine accumulated in the muscle in SET and an increasing tendency was observed in SEF. In both tests the increase in alanine concentration correlated with the decrease in glutamate concentration. Concentration of glutamine in the muscle increased only after SEF test. Concentration of alanine after both SET and SEF and glutamine after SEF also increased in plasma indicating that plasma concentrations reflect the changes in the muscle. The results support the view that amino acid metabolism in the working muscle is enhanced and related to both intensity and duration. It is suggested that the *de novo* synthesis of alanine may be a physiologically important pathway for the removal of the carbohydrate-derived pyruvate.

Key words. Alanine; glutamate; glutamine; branched-chain amino acids; phenylalanine; horses.

INTRODUCTION

Amino acid and protein metabolism during and after exercise have been extensively reviewed in man, dog and rat.[3,17] The rate of protein synthesis has been reported to decrease during exercise, whereas the breakdown of proteins increases. In the muscle, the oxidation of leucine and the synthesis of alanine increase and changes in the steady-state concentrations of many amino acids have been reported. These changes have been shown to depend mainly on intensity and duration of the exercise, but the dietary state, the training status and the species studied may also contribute.[3,17]

The concentration of alanine has been shown to increase in horse muscle[20] and plasma[18] during exercise, but the response of other amino acids is not known. The aim of this study was to investigate the amino acid concentrations in skeletal muscle of Standardbred horses after short intense exercise and prolonged submaximal exercise. Plasma amino acid concentrations were measured to evaluate their suitability as indicators of intracellular changes in protein and amino acid metabolism during exercise. Amino acids discussed here will be limited to the branched-chain amino acids (leucine, isoleucine and valine) that are readily metabolised in the muscle, aromatic amino acids (tyrosine and phenylalanine) that are not oxidised in the muscle, alanine and glutamine that are released by the muscle during exercise, and glutamate that is the key link between energy and amino acid metabolism.[3,17]

METHODS

Horses. Six clinically healthy Standardbred horses with the mean age of 8 years (range from 5 to 14 years) were studied.