



Original Research

Validation of the Lactate Plus Lactate Meter in the Horse and Its Use in a Conditioning Program



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ABSTRACT

The equine industry has a need for a convenient, rapid, and reliable method of measuring blood lactate concentrations ([LA]). We hypothesized that the handheld Lactate Plus lactate meter (LPlus), developed and tested for use in humans, would provide dependable results when used in horses undergoing an exercise conditioning program and that horse's fitness would improve following individualized conditioning based on each horse's velocity at which [LA] = 4 mmol/L (V_{LA4}) was reached. Five adult horses underwent a 4-week training program that consisted of 3 exercise bouts/wk. Horses were subjected to an incremental step standardized exercise test (SET) before starting (SET-1) and after the completion of the program (SET-2). Blood samples were collected before each increase in speed until [LA] reached ≥ 4 mmol/L, and then the SET was terminated. The [LA] sample range in our study was 0–8 mmol/L. Blood was analyzed at the time of collection using a calibrated LPlus, and plasma was collected for [LA] determination using the lactate dehydrogenase-based enzymatic colorimetric method. Although the LPlus tended to significantly underestimate [LA] by 0.39 mmol/L ($P < .001$), the LPlus proved to be a dependable device for use in horses based on good correlation with the biochemical analysis ($r = 0.978$) and Bland–Altman limits of agreement and 95% confidence intervals. All horses showed an increase in V_{LA4} from SET-1 to SET-2, consistent with improved fitness following our 3 exercise bout/wk training protocol. The LPlus can reliably be used in horses to determine [LA] ranging from 0–8 mmol/L. When determining serial [LA], analytical techniques should not be used interchangeably.

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1. Introduction

Blood lactate concentrations ([LA]) are commonly measured in horses to estimate the level of fitness of individual animals [1–7] and assess perfusion status in clinical

situations [8–10]. Measuring lactate accumulation in blood or plasma is a proven quantitative method of determining the level of exercise fitness in the horse and has been successfully used to develop effective conditioning programs [4–7]. Normal resting [LA] in healthy adult horses is <1 – 1.5 mmol/L [8]. During exercise, an exponential relationship occurs between [LA] and velocity, with timescale dependent on workload [4]. The deflection of the lactate–velocity curve represents the start of an imbalance between lactate production and removal and/or metabolism and represents a switch from predominantly aerobic to predominantly anaerobic metabolism. The deflection within the lactate–velocity curve has been seen to occur in horses at [LA] of 2–4 mmol/L, leading to the frequent use of an arbitrary unit of

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4 mmol/L for comparative purposes [4]. Numerous studies have specified the speed at which [LA] reaches 4 mmol/L (V_{LA4}) as a proven repeatable and reliable value with successful correlations to other performance parameters. It is believed to be the ideal exercise intensity to boost endurance, making it a beneficial criterion to conditioning programs [1–5,11–13]. The intensity of exercise at which a horse reaches V_{LA4} will increase as conditioning improves. Increases in [LA] are also seen in a number of clinical situations, and may denote cellular dysfunction, severe disease, and are associated with decreased survival rates in some conditions [9,10].

Lactate analysis is based either on standard photometric or biosensor-based techniques. Photometric techniques are believed to provide high accuracy and reliability; however, the turnaround time resulting from sample transport and preparation is too long to allow for rapid decision making. Biosensors are the integral part of blood gas analyzers, which have the advantage that they do not require preparation of the sample and can be operated easily at the point of care [14]. Currently, there is not a specific method of [LA] determination considered “gold standard”; however, the lactate dehydrogenase (LD)–based enzymatic colorimetric assay has been in use for decades and typically serves as a reference for studies comparing different lactate assays [14–17]. There is a need in the equine industry for a convenient, portable, and reliable method of measuring lactate that can provide rapid feedback. It is necessary to validate the use of new devices, such as the Lactate Plus lactate meter (LPlus), for use in horses because of the ongoing improvement of techniques, changes in available devices, and their typical design for human use. The LPlus is a small battery-operated handheld lactate meter by Nova Biomedical and uses 0.7 μ L of whole blood to read out [LA] in 13 seconds and has a lactate test range of 0.3–25 mmol/L. Other similar devices such as Accusport and Lactate Pro have been studied for use in horses as well [18–21]. In this study, we sought to validate the LPlus for use in healthy exercising horses and determine the effect on V_{LA4} of a three-times weekly conditioning protocol.

2. Materials and Methods

For this study, five untrained adult Arabian horses (9.6 ± 1.70 years; 969 ± 27.69 kg) were subjected to a 4-week conditioning program and underwent a standardized exercise test (SET) before (SET-1) and after (SET-2) the conditioning program to determine the velocity at which they achieved a [LA] of 4 mmol/L (V_{LA4}). Horses were housed in pasture with ad libitum grass and water and did not receive supplemental feed. Horses were not exercised outside of the conditioning program. Horses underwent a lameness examination and placement of an IV jugular vein catheter before each SET by a licensed veterinarian. Horses were fitted with Polar Heart Rate monitors (Polar Electro Inc, Lake Success, NY) attached to a surcingle to continuously monitor heart rates. For the SET, horses were placed on a SÄTO1 high-speed equine treadmill and allowed to warm-up for 5 minutes at 1.8 m/s and 5 minutes at 3.5 m/s at 0% incline. After this warm-up, the treadmill was set to an incline of 6% for the SET. The SET started at a speed of 4.0 m/s followed by increasing increments of 0.5 m/s every 5 minutes. Blood was

collected from the IV catheter after warm-up (baseline [BL]) and between each step of the SET until the [LA] reached >4 mmol/L. Although blood samples were taken between each SET step, the treadmill was stopped for approximately 45 seconds. Once [LA] reached ≥ 4 mmol/L, the SET was terminated and horses were taken off the treadmill. Heart rate, temperature, and body weight were monitored throughout conditioning and during the SETs. Blood samples were also collected after exercise at 15 and 30 minutes. This study was approved by the Animal Care and Use Committee of California State Polytechnic University, Pomona.

Once blood was collected, a whole blood sample (0.7 μ L) was immediately analyzed in duplicate using two calibrated Lactate Plus handheld lactate meters (Nova Biomedical, Waltham, MA). The remainder of the blood was divided into an EDTA-containing vacutainer (5 mL), for determination of packed cell volume (PCV), and a sodium fluoride- and potassium oxalate-containing vacutainer (5 mL) for further analysis. The PCV was measured within 4 hours of blood collection, whereas the sodium fluoride- and potassium oxalate-containing vacutainers were centrifuged within 15 minutes of collection and plasma stored at -80°C for later enzymatic biochemical analysis using Lactate kit 737-10 (Trinity Biotech, Jamestown, NY). This method uses the conversion of lactic acid to pyruvate and hydrogen peroxide (H_2O_2) by lactate oxidase. In the presence of the H_2O_2 formed, peroxidase catalyzes the oxidative condensation of chromogen precursors to produce a colored dye with an absorption maximum at 540 nm.

From the [LA] obtained via the LPlus, a [LA] versus speed curve was generated and used to calculate each individual horse's speed at which lactate reached 2.5 mmol/L ($V_{LA2.5}$) and V_{LA4} . The conditioning program consisted of exercise bouts that took place three times per week at a 6% incline on the high-speed equine treadmill. Horses exercised at their respective V_{LA4} for 25 minutes for two consecutive days. Horses were then rested 1 day before the third exercise bout was conducted at their $V_{LA2.5}$ for 45 minutes.

Bland–Altman analysis [22] was used to compare values from the LPlus to the LD-based enzymatic analytical method. Agreement between the two methods was assessed by calculating bias (mean difference between LD-based analysis and LPlus) and limits of agreement ($2 \times$ the standard deviation of the difference). The difference between the methods was tested for normal distribution, and 95% confidence interval (CI) was calculated for the mean bias and the limits of agreement. The formula used for calculating the CI for the mean bias was $\bar{d} \pm t \times \text{SD}/\sqrt{n}$. The formula for calculating CI for limits of agreement: CI for mean $-2\text{SD} = (\bar{d} - 2\text{SD}) \pm t \times (\sqrt{3\text{SD}^2/n})$ and CI for mean $+2\text{SD} = (\bar{d} + 2\text{SD}) \pm t \times (\sqrt{3\text{SD}^2/n})$. Data between the SETs were compared using a paired t test (SPSS, IBM Corporation, Armonk, NY). Values are presented as average $\pm$ standard error unless stated otherwise.

3. Results

Four of the five horses completed SET-2. A total of 78 blood samples were collected over the course of the study for validation of the LPlus. Lactate concentrations determined by LD-based enzymatic analysis ranged from 0.46 to 8.2 mmol/L, and [LA] determined by LPlus ranged from <1.5 to

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