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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.

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 μL 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 μL) 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 × 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 $đ \pm t \times SD/\sqrt{n}$. The formula for calculating CI for limits of agreement: CI for mean − 2SD = $(đ - 2SD) \pm t \times (\sqrt{3SD^2/n})$ and CI for mean + 2SD = $(đ + 2SD) \pm t \times (\sqrt{3SD^2/n})$. Data between the SETs were compared using a paired *t* test (SPSS, IBM Corporation, Armonk, NY). Values are presented as average ± 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

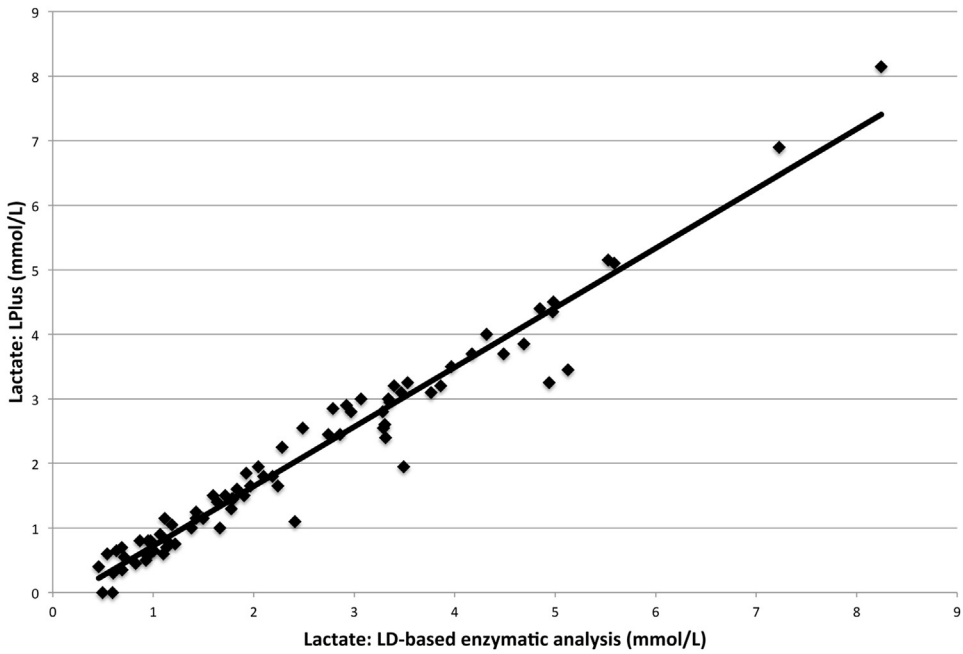

Fig. 1. Lactate values determined by the lactate dehydrogenase (LD)–based enzymatic colorimetric method plotted against lactate values determined by the portable Lactate Plus lactate meter (LPlus) (n = 78; $r = 0.978$).

8.2 mmol/L. Fig. 1 shows LPlus [LA] plotted against the LD-based enzymatic [LA]. The Pearson correlation coefficient was 0.978. A Bland and Altman plot for comparing the analytical methods is shown in Fig. 2. Lactate Plus lactate meter underestimated [LA] by 0.39 ± 0.08 mmol/L (bias ± 95% CI) compared with the LD-based enzymatic analysis; this bias was statistically significant ($P < .001$). The limits of agreement ± 95% CI were −0.29 ± 0.14 and 1.08 ± 0.14 (Fig. 2).

During the course of the SETs, [LA], PCV, Heart Rate, and rectal temperature increased. Lactate concentration was 0.41 ± 0.09% at the start of both SETs (after warm-up; BL)

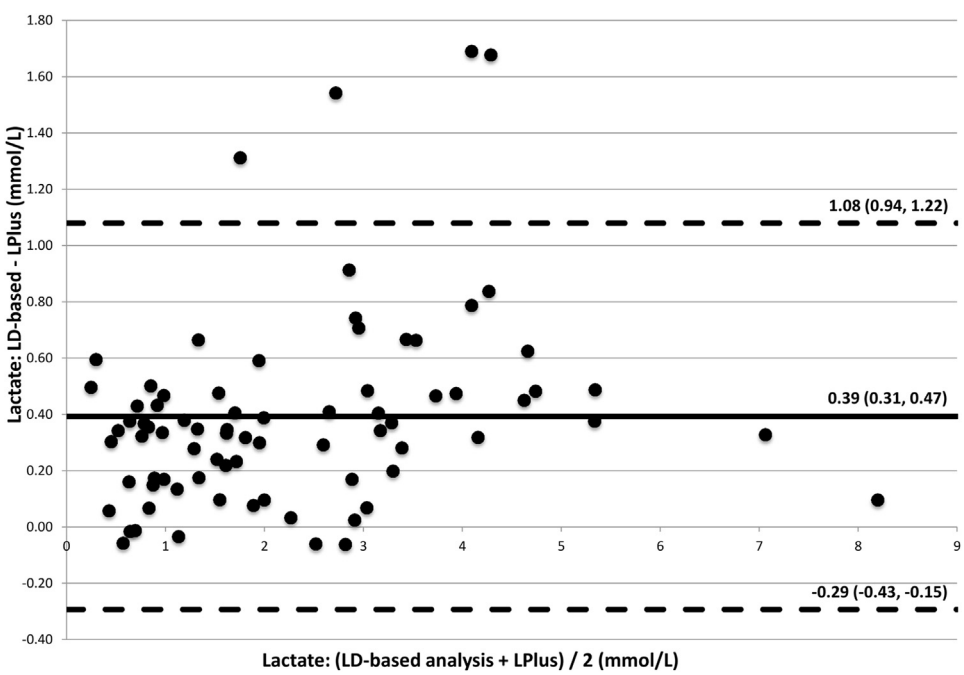

Fig. 2. Bland and Altman plot for the lactate values determined by the lactate dehydrogenase (LD)–based enzymatic colorimetric method or by the portable Lactate Plus lactate meter (LPlus). Bias and upper and lower limits of agreement (±95% confidence intervals) are indicated.

and 5.3 ± 0.5% at the last time point of the SETs. At 15 and 30 minutes after exercise, [LA] was 2.6 ± 0.3% and 2.2 ± 0.3%, respectively. Packed cell volume was 39 ± 0.9% at BL and 49 ± 1.6% at the last time point of the SET, and Heart Rate was 110 ± 5 beats/min at BL and 172 ± 5 beats/min at the last time point of the SET. Rectal temperature was 37.6 ± 0.1°C at BL and 39.6 ± 0.2°C at the last time point of the SET. V_{LA4} increased by 17.8% from SET-1 to SET-2. V_{LA4} was 5.6 ± 0.2 m/s at SET-1 and increased to 6.6 ± 0.2 m/s at SET-2, which was a significant change (Table 1; $P = .002$).

4. Discussion

This study showed a strong correlation between the two methods of [LA] analysis we used, the portable LPlus and the laboratory-based LD-based enzymatic analytical method, suggesting that the LPlus is a dependable lactate meter for use in horses. Although the LPlus is used in horses extensively, this is the first report to validate this device under exercise conditions, as far as the authors are aware. Bland–Altman analysis indicates that the [LA] values obtained from the LPlus were within acceptable limits of agreement, which further strengthens the dependability of this device. These results validate that the LPlus is a dependable device to use in horses with [LA] up to 8 mmol/L. However, the LPlus was not accurate and consistently underestimated [LA], shown by the significance of the bias. Because of this lack of accuracy, it should be noted that these methods not be used interchangeably. In particular for determination of lactate thresholds in exercise physiology, it is important to use one method.

Most lactate analyzers use whole blood samples, making their use less time consuming than other methods that require processing of blood, and easier to use for non-laboratory personnel. It is important to note, then, that although LPlus uses a whole blood sample, the LD-based enzymatic analytical method that it was compared with, used plasma to measure [LA]. The lack of accuracy and underestimation of values have been seen with other handheld devices (e.g., Accusport, Lactate Pro) used in horses [12,20], and contributors to this bias could include differences between blood components in humans and horses that may affect analytical methods, as well as the fact that there is a difference in blood versus plasma [LA] [9,14]. Studies have noted that there can be a large difference in [LA] between plasma and whole blood [9,14]. Lactate is found in both red blood cells and plasma, but lactate distribution between the two varies unevenly during and after exercise, especially in horses [16,23]. Rainger et al [23] noted that [LA] in plasma increase faster during exercise than they do in red blood cells and whole blood, possibly because of the horse's unique ability to release red blood cells via splenic contraction. This was found to be especially true when [LA] was >8 mmol/L. This is particularly important to note because we did not obtain values greater than 8 mmol/L. In human athletes, there is a high correlation between blood lactate and plasma [LA], which allows for the use of correction factors to compare the two [23]. However, Rainger et al [23] recommends that this should not be applied to the horse, particularly at values more than 8 mmol/L, as they could produce errors more than 2 mmol/L for whole blood [LA]. Interestingly, the Accusport handheld device, which saw more trouble with its results than Lactate Pro and our Lactate Plus, measures [LA] in plasma, from a whole blood sample, and then calculates the value out to whole blood using a correction factor [24]. This could very well be one of the reasons Accusport, in particular, was relatively inaccurate for use in horses. It would be beneficial to note whether other human-based handheld devices use this correction factor as well because it could be a factor in their underestimation and low accuracy when applied to horses. In general, low [LA] values tend to be underestimated, whereas high [LA] values tend to be overestimated, making comparisons between methods and devices more difficult [14]. Accuracy between devices and methods seems to be unpredictable and increasingly variable. The variability in lactate distribution between red blood cells and plasma is dependent on hematocrit or PCV, particularly at greater PCV values [23]. Although PCV was a crucial consideration in lactate distribution, the effect of different PCV values on lactate distribution was unpredictable and incredibly variable, even individually, during and after exercise [23]. The implications PCV seems to have on [LA] differing from plasma to whole blood, particularly in horses, certainly need further study to fully understand how and why it may affect the results found with these handheld analyzers, especially at greater (clinical) values.

When conditioning protocols are assessed and compared based on [LA], it should be noted that SET design and frequency could affect [LA], particularly V_{LA4}. A single SET protocol has been proposed [4,6] to allow accurate comparisons between studies. Considering how sensitive and variable [LA] seems to be to varying methods and techniques, it is important that future studies take this into account and try to produce data that are comparable across laboratories. The significant increase in V_{LA4} that occurred over the two SETs in our study is considered a strong indicator that our conditioning program was successful in using LPlus [LA] values to increase fitness in these horses. Although it is uncommon to use a three-times weekly conditioning protocol with two short high-intensity training days and one longer but lower intensity training day, in this study we show that such a protocol can be effective in increasing the level of fitness in horses based on [LA]. Although we did not achieve [LA] values greater than 8 mmol/L, which is a frequent occurrence in clinical situations, we were successful in obtaining sufficient samples to

Table 1
V_{LA4} (m/s) and the percentage change that occurred from SET-1 to SET-2 shown for the four horses that completed both SETs

Subject	V_{LA4} SET-1 (m/s)	V_{LA4} SET-2 (m/s)	%Δ
1	6.3	7.1	12.7
2	5.4	6.5	20.4
3	5.2	6.4	23.1
4	5.4	6.2	14.8
AVG ± SE	5.6 ± 0.2	6.6 ± 0.2	17.8 ± 2.4

AVG ± SE, average ± standard error; SET, standardized exercise test; SET-1, standardized exercise test before starting the completion of the program; SET-2, standardized exercise test after the completion of the program.

demonstrate the dependability of LPlus for use in exercise conditioning programs where [LA] >8 mmol/L is an infrequent event. The LPlus would be beneficial to horse owners and trainers looking to efficiently improve their horses' fitness. V_{LA4} has long been considered an indicator of fitness in the industry and by monitoring V_{LA4} values with an LPlus and adjusting an exercise program to those values as they increase over time, it is possible to condition horses more accurately and effectively.

Scientific evidence is showing an increasing importance placed on [LA] as a valuable parameter for use in exercise conditioning and clinical situations. Handheld lactate analyzers common to the human industry may be able to fulfill a need for a portable and rapid method for determining [LA] in horses, but only if they can be proven as accurate and reliable. The continued development of novel handheld analyzers warrants continued examination and validation of these devices, and furthering our understanding of the different factors that influence [LA], particularly in horses, and development of techniques that allow for data collection to be less invasive, will allow for the comparison of such devices to determine the most accurate and suitable product to use in horses.

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