

EDITORIAL

Science-in-brief: Wearable sweat lactate sensors in equine athletes: Science fiction or reality?

1 | INTRODUCTION

Wearable sensors are increasingly used in equestrian sports, for example to assess heart rate and rhythm during exercise, perform continuous temperature monitoring or evaluate stride characteristics.^{1,2} By measuring variables such as heart rate, respiratory rate and speed, wearables can aid in evaluating the training load. Establishing an optimal training schedule at an individual level is crucial for the prevention of injuries and metabolic problems. Individual training schedules for equine athletes are increasingly based on lactate measurements during an exercise test.^{3,4} Lactate accumulation serves as an indicator of metabolic stress and anaerobic activity, thereby evaluating muscle fatigue.⁵ However, lactate measurements in horses are traditionally performed through repeated intravenous blood sampling, an invasive and sometimes stressful method. Lactate measurement in sweat has been proposed as an alternative non-invasive technique in human athletes.^{6,7} This offers the additional advantage of continuous monitoring, which is important for more accurate evaluation of performance and physiological responses during training and competition. This editorial discusses the potential application and technical challenges associated with sweat lactate measurement in horses.

2 | LACTATE MEASUREMENT IN HORSES

Lactate is a product of anaerobic muscle metabolism and thus can provide information about the aerobic and anaerobic capacity of a horse. During exercise, an exponential relationship is found between blood lactate and speed,⁸ with the empirical value of 4 mmol/L often used as the anaerobic threshold.⁹ Peak blood lactate concentrations are significantly higher in horses compared to humans and can reach values of 25–30 mmol/L in racehorses after maximal exercise, while the resting blood lactate values are 0.5–1.0 mmol/L.¹⁰ The gold standard for measuring aerobic capacity in both human and equine athletes is the Maximum Lactate Steady State (MLSS).¹¹ The MLSS, also known as the anaerobic threshold, represents the highest constant intensity or speed at which the blood lactate concentration remains constant. This value is derived from multiple exercise test sessions at a constant velocity, each lasting 30 min and spread over multiple days. Due to practical constraints, alternative incremental exercise testing protocols have been investigated to determine the anaerobic threshold, both in humans and in horses.¹²

In a typical Standardised Exercise Test (SET) protocol, horses undergo a step-by-step test on a treadmill or track in which they run at a higher speed during successive phases of equal duration. Blood lactate is measured after each phase.^{8,13} However, the value of SET testing for predicting MLSS has been debated, as results are influenced by SET design and step duration. An arbitrary cut-off blood lactate concentration of 4 mmol/L is often used in a standard SET protocol, as the deflection in the lactate-velocity curve is seen to occur at values around 2–4 mmol/L. However, using VLa4 as the anaerobic threshold may overestimate the aerobic capacity of the horse.¹⁴ A modified SET protocol is the Lactate Minimum Speed test, which consists of three phases. In the first phase, high-intensity exercise is performed for a short period of time, so that hyperlactatemia occurs.^{3,12,14,15} This is followed by a recovery phase. In the third phase, the intensity is increased in a step-by-step manner. This protocol typically results in a U-shaped blood lactate curve. The lowest point on the curve indicates a balance between lactate production and excretion and corresponds to the MLSS. As one exercise testing session is sufficient, this is a simple and practical method to determine the MLSS in horses. Another advantage of this protocol is that maximal exhaustion is not required, which reduces the risk for injuries.

Currently, all lactate testing in horses is based on venous or arterial blood lactate, with venous blood sampling most performed.¹⁶ Lactate is preferentially determined on whole blood as this results in a weighted average of plasma and intra-erythrocytic lactate concentration. Analysis is ideally performed within 5–10 min after blood sampling.^{17,18} This can be done using a laboratory-based device such as a blood gas analyser, or with a portable point-of-care device.^{17,19} Venous blood sampling can be painful and stressful, which may affect performance during an exercise test. In addition, continuous blood lactate measurement is not possible.

3 | WEARABLE SENSORS FOR LACTATE MEASUREMENT

The need for non-invasive continuous monitoring of lactate levels during exercise has sparked interest in alternative methods for monitoring lactate, such as sweat. In human athletes, recent advancements have focused on the development and application of wearable sensor technologies for non-invasive monitoring of sweat lactate concentrations.^{6,20–22} A wearable sensor must meet several

conditions. First and foremost, the sampling method must adequately capture sweat and prevent both evaporation of sweat and possible contamination. The device must be secured to the skin with sufficient pressure to drive sweat through a microfluidic channel while preventing any exposure to the environment, but without obstructing the sweat glands. In addition, the sweat must be transported through the microfluidic system in a stable and fast way to minimise delay time. Second, the device must be biocompatible and safe with respect to cytotoxicity. Third, the detection range should include the sweat lactate concentrations expected during exercise. Sweat typically contains higher lactate concentrations than blood, with human sweat lactate concentrations ranging from 16 to 30 mmol/L.²³ In addition, the sensor must have a fast response time and a high sensitivity and selectivity for lactate. Calibration must be kept to a minimum and must be accessible and understandable to the user.^{6,24}

Among the different approaches being explored for lactate detection in sweat, enzyme-based sensors using an amperometric readout currently appear to take the lead.⁷ In these devices, an enzyme layer of lactate oxidase converts lactate into pyruvate and hydrogen peroxide. This hydrogen peroxide is then detected by its reaction with the redox character of Prussian Blue, which produces current changes proportional to the amount of hydrogen peroxide formed at the electrode surface. This detection mechanism is known as amperometry and has already been successfully applied in various wearables. The most successful method of meeting the expected concentration range is to add an outer layer that can regulate the diffusion of lactate from the sweat to the enzyme. This can be done, for example, by adding a plasticised polymer as an outer membrane. This allows a response ranging from 1 to 30 mmol lactate during continuous measurements. The most recently studied sensors combine microfluidics and electronics and consist of an electronic reader, a microfluidic channel sampling cell and sensor electrodes. The sensor electrodes are placed on the microfluidic channels for contact with the sweat. The chemical reaction of the electrodes with sweat, as described above, creates an electrical signal which the electronic reader translates into a lactate value. This value is visualised in real-time on a smartphone, giving users direct access to sweat lactate values.²⁵

In human research, several studies show that the changes in sweat lactate levels usually evolve in a similar way to those in blood, especially with increasing exertion.^{26–29} However, correlations between sweat and blood lactate during exercise are inconsistent and the absolute lactate values differ, for example due to individual variation in sweat production and composition. The measured lactate values in sweat are often higher than the blood lactate values, both at rest and during exercise. While blood lactate at rest is typically between 1 and 2 mmol/L and can reach 20 mmol/L during intense exercise, sweat values of up to 50 mmol/L have been reported.³⁰ The exact cause of these differences has not yet been fully scientifically explained, although it is assumed that sweat lactate does not originate from blood filtration but is produced locally within the sweat gland.^{23,31} In addition, a physiological lag is observed in sweat

biomarker response to exercise.³² Nevertheless, sweat lactate can be a promising indicator of physiological stress during exercise. As an indirect parameter for determining the lactate threshold, sweat lactate measurement is a promising tool for tuning training intensity and evaluating performance in athletes.

There are some important caveats to be made when interpreting sweat lactate measurements. The reliability of these measurements can be influenced by both internal and external factors. A first factor is the position of the sensor on the body as the lactate concentration in sweat might vary depending on location on an active or latent muscle.³³ The athlete's hydration status also plays a role: with reduced hydration, the composition and amount of sweat are altered. In addition, environmental factors are also important. For example, the relative humidity and ambient temperature will influence sweating and consequently the quality and interpretation of the results.³⁴ This may explain why inconsistent results were found regarding the association between sweat lactate and blood lactate or exercise level. The variation in measurement and collection methods of sweat must be taken into account when analysing data.^{6,20,25} One approach to addressing these issues involves quantifying sweat rate, a key parameter for flow-dependent electrochemical sensors designed for sweat analysis.³⁰ The magnitude of sweat rate serves as a valuable metric, enabling normalisation of lactate concentrations.³⁵ Alternatively, intradermal lactate monitoring based on a microneedle sensor patch has also been described.^{32,36} Interstitial fluid lactate is in dynamic equilibrium with blood and therefore provides a minimally invasive approach to detect systemic lactate trends. In addition, other sensors are now also used to estimate training zones and lactate thresholds. For example, oxygen saturation in muscle tissue (SmO_2) can be measured locally by near-infrared spectroscopy. This can provide local information about oxidative muscle metabolism in a continuous and non-invasive way. Examples of commercially available sensors are the Humon and the Moxy Monitor.^{37,38} These devices can calculate lactate thresholds based on the oxygen saturation in the active muscles. Despite the promising results of these devices, there are still large variations in measurements during high-intensity efforts. In addition, these values are obtained by indirect calculation of lactate thresholds.

4 | SWEAT LACTATE IN HORSES

Although sweat lactate measurements in human athletes show promising results, the use of this method in horses appears to be more complex. To date, limited research has been conducted on sweat lactate measurements in horses. Lactate is present and measurable in the sweat of horses, although no correlation with blood lactate could be demonstrated during a SET.^{39,40} Sweat collection in horses is a major technical challenge. In human studies, different methods are used for sweat collection. Many of these techniques, for example rinsing sweat from the skin and collecting sweat in absorbent gauze, have also been studied in horses.^{41–44} Often, these methods require more than 10 min to obtain a sufficient volume of sweat for analysis to be



FIGURE 1 Sensor applied directly to the equine skin behind the left olecranon and fixed with a 9 × 15 cm adhesive foam bandage.

possible. In addition, most techniques are unsuitable for performing repeatable measurements without interfering with the exercise test. A technique in which sweat is scraped from the horse's skin into a container may be efficient for collecting large volumes of sweat, but this requires centrifugation of the samples for accurate lactate measurement, as large soiling occurs during collection.

When evaluating the feasibility of sweat lactate measurements using a wearable sensor (Figures 1 and 2),⁴⁵ we have found that several obstacles for sweat lactate measurements remain and must be systematically tackled. The IDRO sensor we have used was originally developed for human use and may not have been optimised for application in horses. Horses produce significantly more sweat than humans, which is in line with their larger body mass and higher heat production during physical exertion. In addition, they also rely heavily on sweating for thermoregulation. In addition, the composition of horse sweat differs considerably: it contains relatively higher concentrations of proteins and enzymes, as well as latherin, a substance that contributes to foaming.^{42,46,47} The presence of these components could interfere with the operation of the sensor, resulting in erroneous readings. In addition, the higher degree of contamination in horse sweat increases the risk of clogging the sensor.

5 | CONCLUSION

Monitoring physical exertion in horses non-invasively through sweat remains interesting, especially in the context of animal welfare and practical applicability in the field. In clinical practice and field settings, it is not always feasible to acquire blood samples, especially during or just after a training session. Further development of sensors specifically tailored to the physiology and sweat profile of horses may lead to more reliable results. In the future, this technology could be a valuable addition to the existing methods for monitoring and optimising training load and condition in equine athletes.



FIGURE 2 Schematic representation demonstrating real-time sweat lactate monitoring on a smartphone, showing a lactate calibration curve measured by the sensor.

CONFLICT OF INTEREST STATEMENT

Maarten Gijssels was employed by IDRO B.V. at the time of writing this editorial which contains information about a wearable lactate sensor owned and patented by IDRO B.V. IDRO B.V. provided wearable sweat lactate sensors for pilot testing at the faculty of Veterinary Medicine, Ghent University (Prof. Annelies Declodeet).

AUTHOR CONTRIBUTIONS

Annelies Declodeet: Conceptualization; writing – original draft; writing – review and editing; supervision; investigation; methodology; data curation; visualization; resources. **Maarten Gijssels:** Conceptualization;

writing – review and editing; data curation; resources. **Maria Cuartero:** Conceptualization; writing – review and editing. **Gastón A. Crespo:** Conceptualization; writing – review and editing; visualization.

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